



## RESEARCH ARTICLE

## The Effect of Amoebic Dysentery on Some Physiological Parameters

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ARTICLE INFO	ABSTRACT
Received: May 22, 2024 Accepted: Jul 3, 2024	The incidence of amoebic dysentery was recorded at a percentage of 26.66%. Stool samples were collected from the Balad General Hospital laboratory for age groups that ranged between (1-60) years and for both sexes for the period from December 2022 until May.
<b>Keywords</b> Entamoeba histolytica Amoebic dysentery Some physiological criteria	The results of the current study showed high significant differences for the blood parameters PLT, Granulocytes, Monocytes, for those infected with the Entamoeba histolytica parasite between males and females compared to healthy ones. As for Ferritin, it showed a high significant difference between infected females and healthy males, and there was no significant difference between males. Infected people And healthy females. As for Lymphocytes, HGB, and HCT, no significant differences appeared between affected males and females compared to healthy ones. A significant increase was recorded in those infected with the parasite compared to healthy people, and with regard to the blood standard, platelet elevation in those infected with amoebic dysentery, and there appeared to be a significant difference in the rate of mononuclear cells between the group infected with the parasite compared to healthy people. Regarding lymphocytes, it was recorded that there was a high significant decrease in the level of ferritin between females infected with the parasite and healthy males.
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### INTRODUCTION

Entamoeba histolytica is one of the intestinal protozoan parasites that causes what is known as amoebiasis (Ali, 2015). The main cause of diarrhea, especially in children, infection begins with the ingestion of infective stages, represented by the cystic form found in contaminated drinks and food. Dysentery is known as an infection resulting from tissue decomposition and may be symptomatic or asymptomatic (Pereira et al., 2014). The infection spreads throughout the world, and poses a serious threat to health in tropical and subtropical developing regions (Gupta et al., 2022) as well as in developed countries (Gwairgi and Ghildyal, 2018).

The amoeba parasite E. histolytica infects up to 90 million people each year, with a mortality rate exceeding 100,000 people annually in all countries of the world (Wesel et al., 2021). This parasite causes amoebiasis and can develop into liver abscess and amoebic colitis, which is listed among the 15 most important causes of diarrhea in the first two years of life of children living in the developing world (Gupta et al., 2022). The life cycle of the parasite passes through four stages, which is the vegetative stage or what is called the active Trophozoite, the precyst phase, the cyst phase, and the post-cyst phase (Mitchell et al., 2023). This type of amoeba is distinguished from the rest by its ability to infect tissues and decompose them, and for this reason it is called histolytic amoeba. In most

infections, damage occurs at the level of the mucous layer, and symptoms may be mild or non-existent, Asymptomatic, it is possible for the infected person to be a carrier of the disease only without showing symptoms, The infection here is known as a non-invasive infection, and the infected individuals do not have symptoms. Asymptomatic carriers, such that the excretion of cysts with feces is not accompanied by disease symptoms, and these individuals are an important cause of the epidemiology and spread of the parasite, and most of the infections caused by this parasite are from Without symptoms, but in cases of severe infection, it causes diarrhea, vomiting, pain, and weight loss, especially in children. The variation in infections is due to the different virulence factors of this parasite. When the feeding phase of the parasite is present in the lumen of the large intestine, it begins to adhere to the mucous tissue, decompose it, then penetrates it and destroys the epithelial tissue, which provokes an inflammatory condition. During this process, the parasite analyzes and devours immune cells and red blood cells and degrades them, thus causing a decrease in the size of PCV (Al- Mansup et al., 2022).

The infection is transmitted when eating contaminated food and drink. The infection occurs by swallowing the infectious cysts with contaminated food and water or from contaminated hands. It passes through the stomach without being affected and does not show any activity in the acidic environment. When it reaches the basic environment in the small intestine, it is stimulated and escape from the excystation cyst occurs and division begins. The cytoplasm and nuclear form are composed of eight small amoebulas, and the post-cystic trophic stages are similar to the mature trophic stages but differ in size (Ebert et al., 2020).

The autotrophs live and multiply in the crypts of the mucous layer of the large intestine and feed on the mucous secretions. There is also a metabolic interaction or interaction with the intestinal bacteria. The autotrophs usually begin tissue invasion when they decompose the mucous cells with hydrolytic enzymes and absorb the decomposed product, and ulcers occur in the intestinal wall. It may continue to penetrate until it reaches beneath the superficial lining, and may spread sideways into the submucosal layer, forming a flask-shaped ulcer. The ulcer may develop in the cecum, appendix, ascending colon, ileocecal valve, terminal ileum, sigmoid colon, and rectum, and may reach the lower blood vessels, and from here. It may travel with the blood through the hepatic portal vein, forming systemic amoebiasis or extraintestinal amoebiasis, and it continues to grow and multiply in the liver cells, causing amoebic liver abscess. It may reach the lung when it penetrates the diaphragm after infecting the liver, and it may be transmitted from the liver to the brain or skin via Through the bloodstream, the active stage may remain in the lumen of the large intestine, and when unsuitable environmental conditions are available, it begins to multiply and divide, and the mature cysts are thrown out with the feces to infect another new host, and so the life cycle continues (Selberherr et al., 2022).

Some cases of nutritive phases remain in the intestinal lumen for a limited period and are called non-invasive infection, the rate of infection reaches 90%. Individuals infected with this condition are called asymptomatic carriers of the disease, the cysts emerge with the feces without showing any symptoms, and these individuals represent an important source of epidemiology, the parasite (Guillen and Nancy, 2021). The remaining 10% of pathogenic infections attack the mucous layer of the intestine and may penetrate it and pass through it into the bloodstream to infect sites outside the intestine, such as the liver, spleen, brain, skin, heart, and lung (Guillen and Nancy, 2023).

The success of parasite infection depends on many factors, including: the amount of dose, the virulence of the parasite, intestinal motility, the host's diet, the presence or absence of intestinal bacteria normal flora, the ability of the amoeba to adhere to colonic mucosal cells, and the activators penetrate the epithelial cells in the colon by means of their movement and enzymes that It possesses a histolysin enzyme and a lytic enzyme that causes damage to the mucous membranes, and Lactin

mediates the adhesion of the active phase to host cells such as mucous cells in the colon (Guillen and Nancy, 2023).

*Histolytica amoebiasis* releases the enzyme (EhCPs) cysteine proteinase E. *histolytica* around itself. This enzyme works to decompose intercellular proteins such as Collagen and Fibronectin, which leads to the separation of epithelial cells from each other and their attachment to the enzyme (Miller et al., 2022). The hydrolytic enzymes secreted by the parasite disrupt the mucous membranes. The intestine and the barriers formed by epithelial cells, thus facilitating the process of penetrating tissues, and decomposing the intestinal mucosal cells down to the submucosal layer and other layers underneath it, as they work to decompose the host cells, and form separate pin-like ulcers with raised edges and are more numerous in the colon and mucous membrane area (Guillen and Nancy., 2023).

The disease may develop to affect the liver, and a liver abscess arises from the spread of pathogens through the blood vessels, the center of the abscess contains brown pus, which is liquefied liver tissue free of amoebae. The liver abscess may be single or multiple and is usually located in the right upper lobe of the liver. Then, it puts pressure on the bile ducts and is accompanied by an increase in the number of white blood cells to 20,000 cells/cm<sup>3</sup> and an increase in the rate of red blood cell sedimentation. The amoebiasis may reach the lungs or the brain, causing a pulmonary or brain abscess, or it may reach any other organ, and the pulmonary abscess may explode. Directly in the lungs or explodes outward under the skin to lead to amoebic dermatitis (Mehlhorn, 2016).

The incubation period varies from several days to several months or years, and generally ranges between 1-4 weeks. The parasite may exist in some people for several years without any disease appearing in the host, but suddenly symptoms of the disease appear, such as amoebic colitis. Amoebic colitis occurs gradually, with symptoms appearing and increasing from one to two weeks. What distinguishes amoebic colitis from bacterial dysentery is diarrhea, which is one of the most common symptoms, as bacterial diarrhea is watery. Patients with amoebic colitis usually suffer from Watery or bloody diarrhea, abdominal pain, cramps, and weight loss (Slater et al., 2019). Healing occurs spontaneously if tissue erosion is minimal and regeneration processes are faster than destruction, and the nutritional phases are destroyed or stopped in the muscular layer of the intestine. The damage may develop to include parts close to the ascending colon and the cecum. In other cases, it may develop into an amoebic granulomatous tumor, which is a mass. A tumor-like disease known as amoeboma. It forms on the intestinal wall and sometimes grows very efficiently, leading to blockage of the lumen. Other symptoms include amoebic appendicitis and fulminant colitis. The symptoms resulting from the infection vary, and the reason is due to the nature of the parasite strain that is resistant to natural or acquired immunity. of the host, the interactions that occur with the bacterial flora, the psychological state as well as the functional state of the host, as well as the genetic predisposition of the host, in addition to other factors represented by malnutrition, age, immune competence, and gender (Roberts et al., 2013)

The spread of infection varies greatly because some settings appear to be more susceptible than others, such as mental hospitals, prisons, and asylums. Acutely ill patients with invasive amoebiasis represent an important source of transmission of the disease because they pass through the infective phase, which is able to survive outside the environment. Intestinal environment (Bogitsh et al., 2018).

Hemoglobin Hb, where Hb is in the form of a red pigment present inside the red blood cell, is a protein consisting of two main parts: the first is represented by the iron-containing protein called haem, while the second part is globin. The functions of hemoglobin include transporting oxygen O<sub>2</sub> from the lungs to the tissues and transporting carbon dioxide CO<sub>2</sub> from the tissues to the lungs (Kabir Tamanna et al., 2023). The Hb percentage increases in the case of dehydration resulting from acute diarrhea, continuous vomiting, and in the case of severe burns. Also, an increase in the percentage of red blood cells in smokers leads to an increase in the hemoglobin rate, while the Hb percentage

decreases in the case of anemia and also in cases of bleeding. Hemorrhage, continuous blood loss, leukemia, and hypothyroidism (Charles, 2020).

(P.C.V) Packed Cells Volume, which is a process to measure the percentage of the volume of compressed red blood cells to the total volume of blood, as it is related to the number and size of red blood cells. A decrease in PCV may occur in many cases, including bleeding, destruction of red blood cells, bone marrow failure, and poor blood circulation. Absorption and malnutrition (Yoshida et al., 2019). During studies conducted by (Roberts et al., 2019), a decrease in PCV was observed in patients with amoebic dysentery, and anemia in patients with amoebic liver abscess (Alaboudi et al., 2023).

**Total Leucocytes Count:** White blood cells are cells with nuclei found in the blood to provide defense against germs, parasites and tumors. In general, they have defensive functions against foreign bodies that invade the body. Their number in adults ranges from 10 to 4 thousand cells/cubic ml (Al-Shaibani et al. 2020).

In some physiological cases, an increase in the number of white blood cells (leucocytosis) occurs during exercise, emotional reactions, and the birth of recent children, pregnant women, and stress. In other cases, including splenectomy, there are some medications that increase the number of these cells, including Epinephrine, Heparin, Corticosteroid, and Aspirin. There are other medications that reduce the number of these cells, including Antihistamine, antithyroid drugs. The number of white blood cells also increases in some diseases, including leukemia, anemia, burns, and bleeding. There is also a decrease in white blood cells (leukopenia) in the event of a failure in bone marrow function, tumors, fibrosis, blood poisoning, malaria, typhoid fever, and exposure to radiation (Cheng et al., 2019).

**Differential Leucocyte Count** There are 5 types of white blood cells that differ in their sizes, proportions, functions, and contents:

**A - Neutrophils:** The diameter of these cells ranges between 40-70 microns. Their number increases during bacterial infections, acute infections, burns, and stress, and decreases when exposed to radiation and vitamin B12 deficiency, as well as taking some medications (Hammad et al., 2022).

**B - Eosinophils:** The shape of these cells is circular, their diameter is 12-15 microns, their color is orange to red, and their nucleus has two lobes. The percentage of these cells is 1-4%, and their percentage may sometimes reach 1-5%. The numbers of these Eosinophilia cells increase in cases of allergic diseases and parasitic worm infections, skin diseases and autoimmune diseases (HEGDE, 2019; Hammad et al., 2022; Cheng, 2019).

**C - Basophiles:** They are circular in shape, light purple in color, and their diameter is 11-13 microns. Their nuclei are S-shaped, their percentage is 0.5-1%. These cells increase in response to allergic responses, leukocyte cancer, and hypothyroidism, and their numbers decrease in pregnant women and stress. When thyroid secretions increase Hyperthyroidism (HEGDE, 2019; Cheng, 2019).

**D- Lymphocytes:** These cells are 7-10 microns in diameter and are bright red in color. Their nuclei occupy most of the pellet and their percentage is 20-40% of the total number of white blood cells (HEGDE, 2019). The numbers of these lymphocytes increase in some pathological conditions, including chronic infections and tuberculosis infections. Viral infections, whooping cough, immune diseases, and some types of leukemia, and a decrease in these cells occurs during severe and chronic infection with some diseases (Cheng et al., 2019).

**E - Monocytes:** The shape of these cells is somewhat irregular and they are the largest of the white cells, with a diameter of 10-20 microns, and their nuclei are shaped like a kidney or a bean. The average number of these cells is 2-8 (HEGDE, 2019). These cells increase during viral and fungal infections. Tuberculosis and some types of cancer, and the number of these cells rarely decreases (Russell et al., 2019).

Ferritin is a blood protein that contains iron that is stored in the body. It is called the “store” of iron in the body, but an imbalance occurs in the iron stores in the body, whether increased or decreased; it can cause several annoying symptoms that lead to serious diseases such as heart failure (Shin et al., 2023). It has also been documented that it is reduced in the case of intestinal infection with dysentery amoeba (Lee et al., 2022).

The aim of the study is to evaluate the extent of the effect of amoebic dysentery on some physiological parameters such as WBCS count, Hb, PCV, and Ferritin.

## MATERIALS AND METHODS

**1- Collection of stool samples:** The study included collecting 225 feces samples from people lying in bed and attending Balad General Hospital and some private laboratories in Salah al-Din Governorate who were suffering from illness symptoms such as fever, diarrhea, loss of appetite, and others, during the period from December 2022 until May 2023 and for different age groups. The samples were placed in dry, clean plastic containers with a capacity of 30 ml, with a wide opening and a tight lid to prevent the sample from drying out and to maintain its moisture. A paper sticker was placed on one side of it to record the patient's name and sample number. Other information was taken from the patients, such as age, gender, residential location, and educational level.

**2- Collection of blood samples:** Blood samples were drawn from patients suffering from amoebic dysentery, as the number of those infected reached 38, and 10 samples were drawn from people who do not suffer from amoebic dysentery. They were used as a control group and from different age groups. To draw blood, clean, sterile 5ml medical syringes, drawing 5ml of venous blood using a tourniquet. It was placed in a disposable tube free of any substance, the blood was left in it for 10 minutes, then it was placed in a centrifuge at a speed of 3000 r/min and for 3 minutes the blood serum was observed to separate. The serum was withdrawn using a micropipette and placed in an eppendorf and stored at -20°C for the purpose of performing serological tests.

**3- Examination of stool samples:** Stool samples were examined by preparing a direct wet swab to detect the presence of cystic or nutritive stages of the amoebic dysentery parasite. Two slides were prepared for each sample using clean glass slides. I placed a small drop of 1% local iodine solution or 0.85% saline solution on the slide and mixed well, adding a small amount of feces and mixing using stick wood. Then the cover slide was placed well, and the sample was examined using a microscope. Optical imaging under 40x magnification power and then under 100x power (WHO, 1991).

**4- Blood tests:** Complete Blood Count (CBC): I use this device to give a complete picture of the components of the blood. I use blood tubes containing the anticoagulant EDTA, which works to prevent blood cells and other blood components from colliding with each other and contributes to giving correct results, as well as to ensure the safety of the device's operation. Obstruction of its ducts occurs if clotted masses of blood components pass through. It is used to count red blood cells, white blood cells, and platelets. The device has two chambers, the first of which hemoglobin and white blood cells are measured by analyzing red blood cells with the help of a substance that breaks down the red blood cells and produces hemoglobin, which is measured by a spectrophotometer, while the second chamber is in which platelets and red blood cells are measured. The passage of an electric current leads to a distinction between the components. According to the volume of blood passed through it, the results are recorded on a printer connected to the device. It used to measure the percentage of Hb, PCV, and WBC (Buttarelo.2008).

**5- Statistical Analysis:** The program Statistical analysis system-SAS (2010) was used in the statistical analysis of the studied data to study the effect of various factors on the studied traits. The significant differences between the means were compared with the least significant difference (LSD) test, and the percentage differences between them were compared with a square test. Chi-square).

## RESULTS AND DISCUSSION

Faecal samples were collected from the Balad General Hospital laboratory for age groups that ranged between (1-60) years and for both sexes for the period from the beginning of December 2022 until the end of May 2023, and the *E. histolytica* parasite was diagnosed through microscopic examination of the samples. Faeces were found in 60 samples out of 225 samples, with a percentage of 26.66% Table 1.

The results of this study are consistent with what was recorded by (ALYassaree, 2004) in Babil Governorate, where he recorded an infection rate of 29.5%, while they did not agree with what (Kadir et al., 2018) recorded in Tikrit, and with what (Ibraheem, 2008) recorded in the city of Kirkuk, where the infection rate reached 41.1% when examining 1,250 samples for children under 5 years of age, and what was recorded by (Ismail, 2006), where he recorded in his study an infection rate of 47.7% in the city of Kirkuk, and what was recorded by (Ibraheem, 2018) in Baghdad, where the rate reached 19.5%. And what Al-Masoudi (2009) found in Babylon, he recorded an infection rate of 34.3%.

The difference in the rate of parasite infection in this study and the studies mentioned may be due to the difference in the level of sanitation, population density, personal hygiene, climatic conditions, geographical location, the number of samples examined, the extent of the study and the age groups in the population on which the study was conducted. As for the similarity in the rates. The incidence may be due to similarity in cultural, social, and health levels (Kurt et al., 2007).

The results of the current study showed high significant differences for the blood parameters PLT, Gran, Mono, for those infected with the *E. histolytica* parasite between males and females compared to healthy ones. As for Ferritin, it showed a high significant difference between infected females and healthy males, and there was no significant difference between males. Infected people and healthy females. As for Lympho, HGB, and HCT, no significant differences appeared between affected males and females compared to healthy ones, as in Table 2.

The current study showed that there was a significant increase in blood platelet levels in people infected with the parasite compared to healthy people, because amoebic dysentery is considered an inflammatory disease, where inflammation leads to an increase in the production of platelets from the bone marrow. Amoebic dysentery can cause rupture of the affected area. In the mucous membrane of the intestine, where the body responds to this rupture by increasing the production of platelets to help in the blood pooling process, as high platelets are a compensatory response to the blood loss that occurs as a result of amoebic dysentery, as the body tries to compensate for the blood by increasing the production of platelets, and it agreed with (AI). Salehy and Mohammad, 2020),

The current study showed that there is a significant difference in the rate of mononuclear cells between the groups infected with the parasite compared to healthy people. The increase in mononuclear cells is due to the body's inflammatory response to confront the parasite infection. Their increase indicates the body's attempt to overcome the parasitic infection. (Duttaroy et al., 2021) The current study recorded a significant difference with regard to the blood standard (Russell et al., 2019) in those infected with the parasite compared to healthy people. The reason why the hemoglobin level is not affected depends on the period of infection, as if the infection is acute and short-term, it may not affect the hemoglobin level, as it depends on the patient's health condition in general and also depends on good nutrition (Ahmed et al., 2020).

The reason why the agglutinated cells were not affected may be due to the ability of the agglutinated cells to reproduce and replace damaged cells more quickly than dysentery. The percentage of these cells is not affected except in the case of severe inflammation or the presence of other digestive problems in the healthy group. Our results showed that there was no significant difference between the groups of infected people to the parasite compared (Yoshida et al., 2019).

Regarding lymphocytes, the current study showed a high significant difference in the level of ferritin between females infected with the parasite and healthy males. The current study recorded a significant decrease in ferritin for females compared to healthy males. The results of our study agree with (Zeki et al., 2019). The reason for the decrease in ferritin is that infection with the parasite leads to damage to the intestinal lining, which negatively affects the absorption of nutrients, especially iron. Infection with the parasite may also cause bleeding in the intestine, whether apparent or hidden. This continuous bleeding leads to blood loss and thus Loss of iron present in the blood, which leads to a decrease in the level of ferritin. In some cases, chronic inflammation may occur in the intestine as a result of repeated infection with the parasite. This chronic inflammation may lead to a decrease in the level of ferritin. The results showed that both gender and age are significantly related to the decrease in levels. Iron in the blood in both infected and control groups. Some studies describe dysentery amoeba as breaking down blood cells by... (Al-Hadraawy, 2019) The parasite releases hydrolytic enzymes in large quantities into the intestinal lumen, where these enzymes break down tissues to obtain proteins and iron, which it adopts as a source of food (Teixeira et al., 2008). Infection with dysentery amoeba is accompanied by the presence of blood in the feces, and this can be explained. This is on the basis that the parasite causes disturbance in the digestive system, as its mobile feeding phase adheres to the villi of the intestine and absorbs nutrients from them (Satoskar et al., 2009). The parasite also secretes histolytic proteins that decompose the host's tissues. Thus, the parasite is able to destroy the intestinal mucous layer to penetrate the tissues and destroy them, causing blood bleeding and loss of red blood cells (Chimire & Mishra, 2005; Ankri, 2002). The parasite has the ability to devour human red blood cells (Erythrophagocytosis) and analyze their plasma membrane. The ability of the feeding phases of the parasite to degrade red blood cells is due to the mechanism of adhesion to cell surfaces, and inhibiting adhesion leads to a reduction in the pathogenicity of the parasite (Mora-Galindo et al., 2004; Nozaki & Nakada, 2006). Parasitic infection stimulates a number of immune defense mechanisms that it includes the production of both antibodies and immune cells, and the type of response that will prevail depends on the type of parasite and the stage of infection. The increase in the number of white blood cells may be due to the occurrence of an inflammatory immune response that occurred in the mucosal layer of the intestine and the submucosal layer. White blood cells are cells that have an immune function in addition to their other functions, so an increase in the number of white blood cells is associated with an increase in the immune response during Parasitic infestation (MacDonald et al., 2002; Figuerola et al.; 1999;). The white blood cells secrete toxic products that decompose the feeding phase of the dysentery amoeba through adhesion and lysis mechanisms of the pathogen (Salata et al., 1985). T-cells participate in the cellular immune response, as well as secreting cytokines and their role in stimulating B-cells to produce antibodies. Cytotoxic T cells also produce toxins that are destructive to tumor cells and cells infected with viruses and foreign microorganisms in the body (Cheesbrough, 2006; Bain, 2017).

**Table 1: Percentage of the number of samples positive for E. histolytica parasite**

Total number of samples tested	Number of infected samples	Percentage percentage
225	60	26.66 %

**Table 2: Average blood image parameters for infected and healthy patients by gender**

		WBC						
		Ferritin Mg/L	PLT 10 <sup>9</sup> /L	HCT %	HGB g/dL	Gran 10 <sup>9</sup> /L	Mono 10 <sup>9</sup> /L	Lympho 10 <sup>9</sup> /L
Patients	Males	65.53 ± 18.06 b	315.3 ± 32.9 a	37.11 ± 7.03 a	11.594 ± 2.236 a	7.847 ± 2.630 a	0.9824 ± 0.3592 a	2.976 ± 0.887 a
	Females	46.89 ±15.04 c	313.9 ± 31.5 a	35.43 ± 4.66 a	10.894 ± 1.416 a	7.278 ± 1.786 a	0.9944 ± 0.3115 a	2.450 ± 0.802 a
Healthy people	Males	120.00 ± 16.02 a	252.8 ± 33.0 b	34.74 ± 2.01 a	10.940 ± 0.358 a	3.540 ± 1.526 b	0.5400 ± 0.4280 b	1.940 ± 0.288 a
	Females	74.20 ± 5.85 b	246.2 ± 37.8 b	34.98 ± 2.43 a	11.000 ± 0.515 a	4.060 ± 1.024 b	0.4800 ± 0.0837 b	1.760 ± 0.666 a

\*Similar letters indicate that there are no significant differences between the rates of the different groups.  $P \geq$  at the probability level of 0.05.

\*Different letters indicate significant differences between the rates of the different groups at  $P \geq$  0.05 probability level

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