

The Effect of Infection with some Intestinal Parasites on some Immune Parameters

Haneen Muneef Saab¹, Dr. Ashraf Jamal Mahmoud Zangana², Dr. Maksood Adel Mahmoud Al-Doori³

^{1,2}Coll. Of Edu. For women/Bio. Dep./ Tikrit univ./Iraq

³Al-Dour Technical Institute/ Immediat Ambulance Dep. / Northern Tech. Univ.

ABSTRACT

275 fecal samples were collected from visitors to Tikrit Teaching Hospital and some private laboratories in Tikrit city/Salah al-Din Governorate. The collection period lasted from December 2022 until April 2023. The study included examining samples of both males and females of different ages.

The study showed a significant decrease in level of IgG among people infected with *Entamoeba histolytica* by 92.00 ± 19.1 , as well as for people infected with *Giardia lamblia*, as a significant decrease was recorded by 76.43 ± 15.1 compared to healthy people by a percentage of 130.60 ± 29.5 .

No significant difference was recorded between people infected with *E.histolytica* in level of IgM, as the percentage reached 83.4 ± 13.6 . Likewise, for people infected with *G.lamblia*, no significant difference was recorded, as the percentage was (106.0 ± 21.7) compared to healthy people, as the percentage reached (84.6 ± 18.0).

There was a significant decrease in the level of the CD4 differentiation complex among people infected with *E. histolytica* by a rate of 160.8 ± 23.9 . Likewise, for people infected with *G. lamblia*, a significant decrease was recorded, reaching a rate of 187.5 ± 31.8 compared to healthy people by a rate of 475.2 ± 28.1 .

A significant decrease was recorded among people infected with *E. histolytica* in the level of CD8 differentiation complex, as the percentage reached 9.00 ± 2.51 . Likewise, for people infected with *G. lamblia*, a significant decrease was recorded by 10.18 ± 2.60 compared to healthy people, with a percentage of 14.50 ± 3.40 .

There was a significant decrease in the level of IL6 in people infected with *E. histolytica*, as the percentage reached 85.02 ± 13.20 , while in people infected with *G. lamblia*, no significant difference was recorded, as the percentage reached 196.30 ± 17.10 , compared to healthy people, with a percentage of 220.20 ± 23.20 .

KEYWORDS: intestinal parasites, *Giardia lamblia*, *Entamoeba histolytica*, immunological parameters.

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INTRODUCTION

Intestinal parasites *Entamoeba histolytica* and *Giardia lamblia* are common causes of diarrhea and malabsorption in humans. Infection leads to severe diarrhea in children, especially under five years of age, and is accompanied by chronic infections that lead to weight loss and delayed growth (Cook, 1995).

Intestinal parasites are tiny organisms that can live in a person's small intestine, and are transmitted to humans through ingestion of contaminated food or water. Intestinal

parasites cause a variety of symptoms, including diarrhea, abdominal pain, nausea, and vomiting. In some cases, intestinal parasites can lead to serious complications, such as dehydration, weight loss, and malnutrition. Intestinal parasites are an important health problem worldwide, with estimates indicating that more than 1.5 billion people are infected with intestinal parasites, which leads to millions of deaths every year (Paniker and Ghosh, 2018).

Most of the primary parasites that infect humans and are most widespread throughout the world are *E. histolytica*,

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which is one of the primary intestinal parasites that causes what is known as Amoebiasis, which is the main cause of diarrhea, especially in children. The parasite is spread through the feces of an infected person and can enter the body through the mouth. While eating or drinking contaminated food or water, or through direct contact with contaminated stool, the symptoms of *E. histolytica* infection vary depending on the severity of the infection. In mild cases, no symptoms may appear, and in more severe cases, the infected person may suffer from serious complications such as intestinal perforation or liver abscess. *E. histolytica* infection is treated with antibiotics, and infection can be prevented by avoiding eating food or water from unreliable sources. Wash fruits and vegetables well before eating them (Peter *et al.*, 2022). *G. lamblia* is one of the eukaryotic parasites and causes Giardiasis, which is a common disease between humans and animals. The parasite infects the small intestine (jejunum and ileum) of humans (Rudolph *et al.*, 2022).

E. histolytica belongs to the phylum Sarcomastigophora, class Gymnamebia

(Paniker and Ghosh, 2018). And *G. lamblia*. It is classified within the Sarcomastigophora phylum, Zoomastigophora phylum (Plutzer *et al.*, 2010).

Giardiasis is a common parasitic disease affecting humans worldwide. It is caused by *G. lamblia*, a single-celled organism transmitted by ingestion of water or food contaminated with the feces of infected people. It is estimated that up to 2 billion people worldwide are infected. Giardiasis begins at any given time. Infection rates vary widely by geographic region, being highest in areas with poor sanitation and lack of access to clean water. In the United States, it is estimated that up to 20 million people are infected with giardiasis (Robertson *et al.*, 2023). In a study conducted by researchers in 2022, it was found that the epidemic prevalence of giardia ranges between 2% and 50% in developing countries (Rudolph *et al.*, 2022). Another study conducted in 2020 found that the epidemic prevalence of *Giardia* ranges between 5% and 20%, with the highest rates in countries with hot and humid climates (Machadol *et al.*, 2020).

IgG is the most abundant type of antibody in plasma, as it represents 80-70% of all antibodies. It is characterized by its ease and speed of movement in cells because it is smaller in size and more abundant. It protects various types of disease causes such as bacteria, viruses, fungi, and toxins, and is present in plasma, lymph, and external body fluids. other (Dekkers *et al.*, 2018). It is the main antibody generated during the primary and secondary immune response. IgG specific to allergens directly affects the production of IgE antibody by memory B cells, in addition to causing inhibition and activation of the complement system. It has the ability to cross the placenta towards the fetus, which gives the fetus negative immunity and provides protection. in the first months of pregnancy (Parker *et al.*, 2017).

IgM constitutes about 10% of all antibodies. It is found alone on the surface of plasma B cells, as it is produced automatically by a subset of B cells. It is the first type of antibody secreted during the primary immune response, and therefore its presence in plasma is considered a diagnostic method. It indicates the presence of a recent infection, and due to the multiple binding sites for antigens, it has a high ability to coagulate antigens. Hence, it is useful in examining blood groups. Likewise, like IgG, it is able to activate and inhibit the complement system (Michaud *et al.*, 2020).

Immunoglobulin IgM is initially produced as a molecule attached to the surface of B cells during their early differentiation. Then, when an immune response occurs, it is produced by plasma cells in the form of soluble pentagons containing 10 antigen-binding sites, or in the form of hexagons containing 12 antigen-binding sites. IgM has a molecular weight of about 900 and 1050 kDa for penta or hexa, respectively, and due to its multivalent nature, IgM is also very effective in participating in defending the body as it causes cell lysis and pathogens (Keyt *et al.*, 2020).

Immune variables play an important role in maintaining human health. Infection with some parasites and changes in these variables can lead to a variety of symptoms, including inflammation, immunodeficiency, and some parasites infect the digestive system, including the *E. histolytica* parasite. *G. lamblia* parasites can stimulate the immune system, leading to increased production of immune cells such as B and T lymphocytes. This also leads to increased levels of antibodies such as IgG and IgM.

The study aimed to evaluate the effect of intestinal parasite infection on some immune parameters: IgM, CD4, CD8, IL-6, and IgG.

MATERIALS AND METHODS

1- Collection of samples

A - Stool samples: 275 stool samples were collected from visitors to Tikrit Teaching Hospital and some private laboratories in Tikrit in Salah al-Din Governorate. The collection period lasted from December until April. The study included examining samples of both males and females of different ages. The collected faecal samples were stored in sterile, dry plastic bottles with airtight seals prepared for this purpose. The patients' information was recorded (gender, age, residence, water sources, educational level, etc.) according to the questionnaire form.

B - Blood samples: 5 ml of blood was drawn from patients who were confirmed to be infected with amoeba dysentery and *G. lamblia*, in addition to patients who were not infected within the control groups. It was placed in disposable tubes 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 rpm and for 3 minutes. The blood serum was separated. The serum was withdrawn using a micropipette and placed in small eppendorf tubes and kept in the refrigerator for immunological examinations. .

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2- Laboratory diagnosis of infection

Microscopic examination is divided into the Direct Smear Method. Normal saline solution was used to determine the movement of the cyst and the phase of the activator. Two slides were prepared for each sample using clean glass slides. A drop of local iodine solution was placed on the slide with the addition of a small amount of... The feces were mixed using stick wood, and then the sample was examined using an optical microscope under 40X magnification and then under 100X magnification (WHO, 1991).

As for the indirect method, it includes the floatation method. A solution of zinc sulphate ZnSO₄ was used at a concentration of 33%. The bags and eggs float on the surface of these solutions while the feces gradually settle to the bottom. We take a portion of the floating materials after 1-5 minutes, because the bags tend to deteriorate. After a period of 30 minutes and examined microscopically (Souza et al., 2003). This is to determine the presence of the type of infection with amoeba dysentery or Giardia lamblia.

3- Immunological tests:

-1.3 Estimation of *E. histolytica* (IgG) concentration

The ELISA kit used the sandwich-ELISA system manufactured by the Chinese company Sunlong.

2.3- Estimation of IgM concentration (*E. histolytica*).

The ELISA kit used the sandwich-ELISA system manufactured by the Chinese company Sunlong.

3-3: - Estimating the concentration of *G. lamblia* (IgG).

The IgG level of *G. lamblia* in the sample is tested using a kit produced by the Chinese company Sunlong.

4. 3- Estimation of *G. lamblia* (IgM) concentration:

The IgM level of *G. lamblia* in the sample was tested using an ELISA kit manufactured by the Chinese company Sunlong.

5. 3- Estimating the concentration of interleukin (IL-6) in blood serum:

The ELISA kit used the sandwich-ELISA system manufactured by the Chinese company Sunlong.

3-6 – Differentiation complex tests:

1.6.3- Estimation of CD4 concentration

The ELISA kit used the sandwich-ELISA system manufactured by the Chinese company Sunlong.

2.6.3- Estimation of CD8 concentration

The ELISA kit used the sandwich-ELISA system manufactured by the Chinese company Sunlong.

4- Statistical analysis: we analyzed the results statistically using the (Minitab) program and calculated the significant levels of the variables between two different sets of data using T-Test and Chi-Square test to determine the significant differences at the level of probability ($P \leq 0.05$).

RESULTS AND DISCUSSION

The results of the current study indicate that there is a significant decrease in the level of IgG among people infected with *E. histolytica* by 92.00 ± 19.1 compared to healthy people, as well as for people infected with *G. lamblia*, as a

significant decrease was recorded by 76.43 ± 15.1 compared to healthy people by 130.60 ± 29.5 .

The low level of IgG immunoglobulin in people infected with *E. histolytica* is the result of several factors, including: chronic infection with the parasite: chronic infection leads to an increase in the body's consumption of IgG immunoglobulin to fight infection. The reason for the decrease may be damage to the immune system, as the parasite causes damage to the immune cells that produce IgG. The immune system of a person infected with the parasite can attack the body's own proteins, including IgG antibodies. *E. histolytica* also produces chemicals that suppress the immune system, leading to decreased production of antibodies (Al-Saadi et al., 2017).

Low IgG concentration is one of the common symptoms of *G. lamblia* infection, and there are several reasons for low IgG concentration in people infected with the *G. lamblia*, including: Chronic infection: Chronic infection with the *G. lamblia* can lead to a decrease in IgG concentration, because the immune system is making an effort It is important to fight infection, depleting IgG stores. The Giardia parasite can suppress the immune system, leading to a decrease in IgG concentration. The results of the current study are consistent with the findings of (Al-Omari et al., 2019).

The results of the current study indicate that there is no significant difference in the level of IgM between people infected with *E. histolytica*, as the percentage was 83.4 ± 13.6 compared to healthy people. Likewise, for people infected with *G. lamblia*, no significant difference was recorded, as the percentage was 106.0 ± 21.7 compared to healthy people. The percentage was 84.6 ± 18.0 .

In a study conducted to measure the concentration of IgM in people infected with *E. histolytica*, Abd-All et al., (1992) and Medel et al., (2002) reported an increase in the level of antibodies during the duration of infection. This contradicts our current study, which showed the absence of significant differences,

The results of the current study indicate that there is a significant decrease in the level of CD4 among people infected with *E. histolytica* by a rate of 160.8 ± 23.9 compared to healthy people. Likewise, for people infected with *G. lamblia*, there was a significant decrease of 187.5 ± 31.8 compared to healthy people by a rate of 475.2 ± 28.1 .

E. histolytica and *G. lamblia* are two of the most common intestinal parasites in the world, infecting millions of people every year. Both parasites cause a variety of symptoms, including diarrhea, abdominal pain, and weight loss. They can also cause inflammation of the colon and intestines, and in severe cases, they can lead to ulcerative colitis. The immune system plays an important role in protecting the body from infection with parasites. One of the most important immune cells involved in fighting parasitic infections are CD4+ T cells. These cells produce a variety of hormones and chemicals that help activate other immune cells and kill parasites. Studies have shown that people infected with

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E.histolytica and *G.lamblia* have lower CD4 concentrations than healthy people and these decreases in CD4 levels are associated with an increased risk of severe infections, including ulcerative colitis. It may explain the reason for low CD4 levels in people infected with *E.histolytica*. The parasite can attack CD4+ T cells and kill them by releasing toxic chemicals or by attaching to the cells and inhibiting their activity. These chemicals prevent CD4+ T cells from multiplying or maturing. Decreased production of new CD4+ T cells due to other factors Chronic colitis caused by *E. histolytica* can lead to decreased production of new CD4+ T cells. *G.lamblia* infection can damage the immune system, leading to decreased production of new CD4+ T cells (Alghamdi *et al.*, 2023).

The results of the current study indicate a significant decrease in CD8 levels among people infected with *E. histolytica*, as the percentage reached 9.00 ± 2.51 . Likewise, for people infected with *G. lamblia*, a significant decrease was recorded by 10.18 ± 2.60 compared to healthy people, with a percentage of 14.50 ± 3.40 .

The results of the current study are consistent with the findings of (Al-Alyawi *et al.*, 2023) that there is a significant decrease in CD8 concentration in those infected with *E. histolytica* compared to healthy controls, as well as the findings of (Al-Nasser *et al.*, 2023). (Al-Dulaimi *et al.*, 2023) showed a decrease in CD8 levels in people infected with *G. lamblia* compared to healthy people.

CD8+ T cells play an important role in the immune system, as they target and destroy infected cells. The low CD8 concentration in people infected with *E.histolytica* is due to several factors, including: immunosuppression, as *E.histolytica* produces proteins that suppress the immune system, including CD8+ T cells. These proteins prevent CD8+ T cells from recognizing and destroying cells infected with the parasite. Another reason that leads to a decrease in differentiation complex concentration is oxidative stress: the parasite *E.histolytica* causes an increase in oxidative stress, which leads to damage to CD8+ T cells.

Oxidative stress results from the buildup of free radicals, which are unstable molecules that can damage cells. As well as the rapid reproduction of the parasite, as *E. histolytica* reproduces very quickly, which requires the immune system to make a great effort to eliminate it, which leads to the exhaustion of CD8+ T cells (Al-Alyawi *et al.*, 2023).

Low CD8 concentration in people infected with *G.lamblia* results in a weak immune system response to infection. This weakness increases the risk of developing complications, such as persistent diarrhea and weight loss. In addition to the three factors mentioned, other factors may also contribute to the decrease in CD8 concentration in people infected with *G. lamblia*, such as: Nutritional deficiency. Nutritional deficiency may lead to a weak immune system.

Including CD8+ T cells. Likewise, chronic diseases such as diabetes and kidney disease weaken the immune system, including CD8+ T cells. Some medications, such as

immunosuppressive drugs, may affect the concentration of CD8+ T cells (Al-Nasser *et al.*, 2023). The reason for the low CD8 concentration in people infected with *G.lamblia* can vary from person to person, based on various factors, Such as the severity of the infection and the person's general health (Al-Dulaimi *et al.*, 2023).

The results of the current study indicate a significant decrease in the level of IL6 in people infected with *E. histolytica*, as the percentage reached 85.02 ± 13.20 , while in people infected with *G. lamblia*, there was no significant difference, as the percentage reached 196.30 ± 17.10 , compared with healthy people, with a percentage of 220.20 ± 23.20 .

The results of the current study are consistent with the findings of Sheikh *et al.* (2022). and Yusuf *et al.*(2021) and Al-Azzawi *et al.*,(2020) indicated that there was a significant decrease among those infected with *E. histolytica* compared to healthy people.

Interleukin-6 is an important cytokine in the immune response to inflammation. It is mainly produced by immune cells such as T lymphocytes and mast cells. IL-6 plays a role in many immune functions, including stimulating the production of anti-inflammatory proteins, activating other immune cells, and regulating the response. Immunology (Sheikh *et al.*,2022).

The lower concentration of IL-6 in people infected with *E. histolytica* compared to healthy people has several reasons, including: *E. histolytica* can inhibit the production of IL-6, as researchers found that *E. histolytica* produces a protein called AMA-1, which can bind to IL-6 receptors on cells. This association leads to inhibition of IL-6 production (Sheikh *et al.*, 2022).

Intestinal amoebiasis can lead to a decrease in the number of immune cells that produce IL-6, such as T lymphocytes and mast cells. A decrease in the number of immune cells that produce IL-6 is caused by damage or death of immune cells. (Yusuf *et al.*,2021) Also, intestinal amoebiasis leads to the release of enzymes that lead to increased breakdown of IL-6, thus decreasing IL-6 levels in the blood (Al-Azzawi *et al.*, 2020).

The reason is that there is no significant difference in the concentration of interleukin 6 in people infected with *G.lamblia* compared to healthy people, and that *G.lamblia* does not cause a significant inflammatory response in the body. Therefore, the concentrations of interleukin 6, a protein that plays a major role in the inflammatory response, do not increase significantly in people infected with the parasite (El-Khodary *et al.*, 2023).

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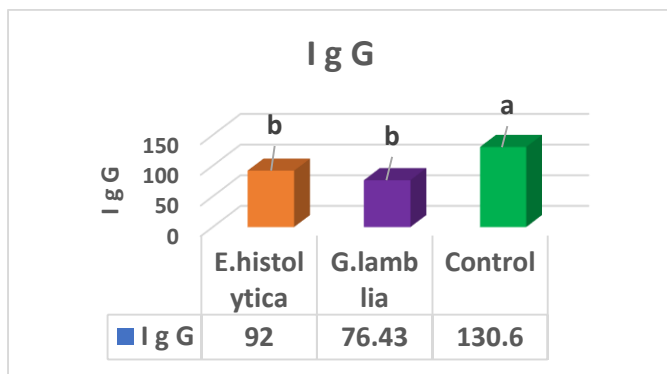


Figure (1) IgG concentration in study groups

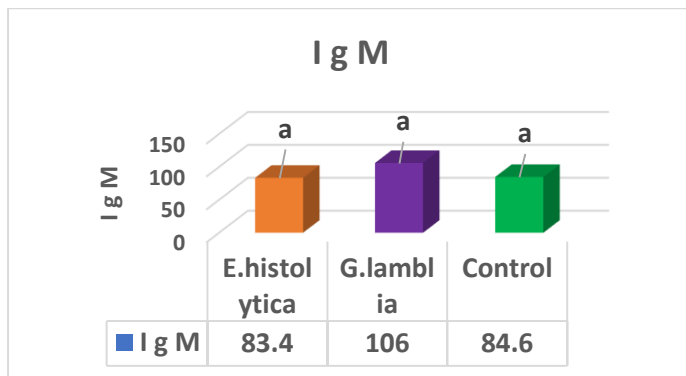


Figure (2) IgM concentration in study groups

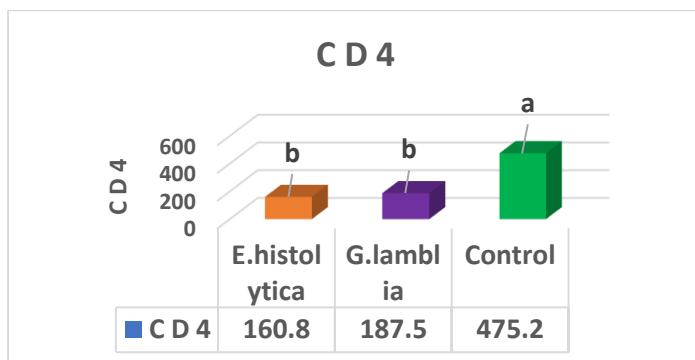


Figure (3) CD4 concentration in study groups

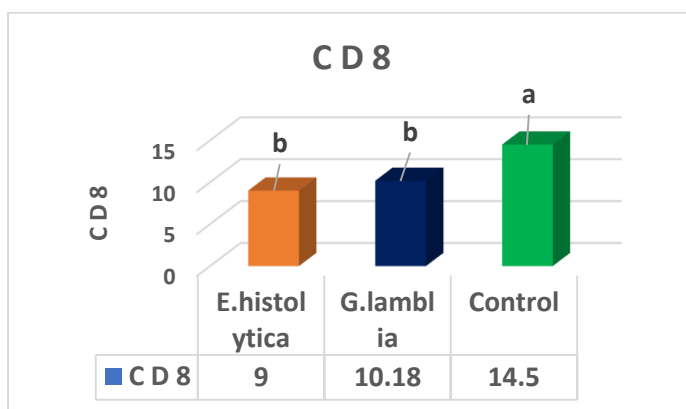


Figure (4) CD8 concentration in study groups

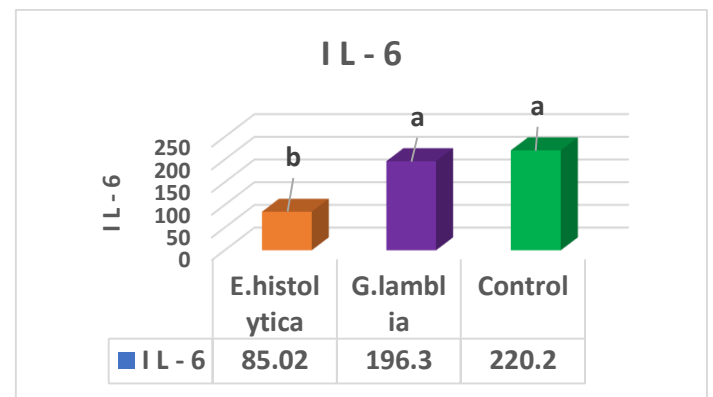


Figure (5) IL₆ concentration in study groups

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