

Impact of Entamoeba Histolytica and Giardia Lamblia Infections on Key Physiological and Biochemical Parameters in Human Hosts

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ABSTRACT

This study investigates the impact of *Entamoeba histolytica* and *Giardia lamblia* infections on various physiological and biochemical parameters in patients from Tikrit, Iraq. Blood and fecal samples were collected from male and female visitors of different age groups at Tikrit Teaching Hospital and private laboratories in Salah al-Din Governorate from December to April. The findings revealed no significant differences in cholesterol concentrations among individuals infected with *E. histolytica* or *G. lamblia* compared to healthy controls. Similarly, no significant differences were observed in triglyceride levels between infected and healthy groups, with values of 163.93 ± 13.05 mg/dl for *E. histolytica*-infected individuals, 158.40 ± 11.97 mg/dl for *G. lamblia*-infected individuals, and 172.57 ± 19.04 mg/dl for healthy individuals. However, high-density lipoprotein (HDL) concentrations showed a significant decrease in *E. histolytica*-infected individuals (34.59 ± 4.61 mg/dl) and *G. lamblia*-infected individuals (35.66 ± 4.92 mg/dl) compared to healthy controls (46.31 ± 5.89 mg/dl). Low-density lipoprotein (LDL) levels did not significantly differ for *E. histolytica* infections (89.02 ± 18.29 mg/dl) but showed a significant increase in *G. lamblia* infections (110.60 ± 20.00 mg/dl) compared to healthy controls (75.62 ± 16.86 mg/dl). Very-low-density lipoprotein (VLDL) levels were similar across all groups, with no significant differences observed. A significant increase in coronary artery risk index was recorded in both *E. histolytica* (4.995 ± 1.080) and *G. lamblia* (4.668 ± 1.228) infections compared to healthy individuals (3.581 ± 0.646). Total protein levels showed no significant differences among *E. histolytica* (6.150 ± 1.294 g/dl), *G. lamblia* (5.664 ± 0.941 g/dl), and healthy groups (6.906 ± 2.004 g/dl). Notably, a significant decrease in C-reactive protein (CRP) levels was observed in *E. histolytica*-infected individuals (11.43 ± 3.04), while *G. lamblia*-infected individuals exhibited no significant change (12.79 ± 3.07) compared to healthy controls (17.06 ± 4.86). These findings provide insights into the physiological effects of protozoan infections and their implications for disease management.

Keywords: Intestinal Parasites, Physiological Parameters, *Entamoeba Histolytica*, *Giardia Lamblia*.

INTRODUCTION

Intestinal parasites *E. histolytica* and *G. lamblia* are common causes of diarrhea and malabsorption in humans. The 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 [1]. Infection with these parasites is important from both an economic and health standpoint. Humans discovered them a long time ago because of their connection to the diseases they cause to both themselves and their animals, which may lead to death. Parasites in general, and intestinal ones in particular, appear in human societies. Tropical and subtropical regions are among the most common parasites in the world. The most suitable areas for living and breeding intestinal parasites because of their climatic conditions, which are more suitable for the survival of cysts, the development of eggs, and the larval stages [2]. 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 [1]. The *E. histolytica* is a single-celled organism,

resembling an amoeba, with a length of about 20 micrometres. The parasite is spread through the feces of an infected person and can enter the body orally through eating contaminated food or water, or through direct contact with contaminated feces. Symptoms of infection vary. *E. histolytica* depends 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 the infection can be prevented by avoiding eating food or water. From unreliable sources, wash fruits and vegetables well before eating them [3]. *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 [4]. 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 with giardiasis at any given time. Infection rates vary widely by geographic region, being highest in areas with poor sanitation [5]. 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 [4]. Physiological variables play an

important role in maintaining human health, and infection with some parasites due to changes in these variables can lead to a variety of symptoms, including inflammation, immune deficiency, changes in cholesterol levels, etc., and some parasites infect the digestive system, including *E. histolytica* and *G. lamblia*. Cholesterol homeostasis is vital for proper cellular and systemic function, and disturbed cholesterol not only leads to cardiovascular disease but can lead to an increasing number of other diseases such as neurodegenerative diseases and cancers [6]. It has many roles that contribute to the normal functioning of cells. For example, cholesterol is an important component of the cell membrane. It also contributes to the structural composition of the membrane. Cholesterol acts as an essential molecule in the synthesis of vitamin D, as well as the synthesis of steroid hormones such as cortisol, aldosterone, adrenal androgens, and sex hormones such as testosterone, estrogen, and progesterone. Cholesterol is also a component of the bile salt used in digestion to facilitate the absorption of fat-soluble vitamins such as (K, E, D, and A). Since cholesterol is mostly lipophilic, it travels through the blood along with triglycerides within lipoprotein molecules. (HDL, LDL, IDL, VLDL Chylomicrons) [7]. Triglycerides are the main carrier of dietary fats in the bloodstream, along with the transport of other fats. They also work as stored fats in adipose tissue, providing the body's cells with thermal energy. This is the primary function of triglycerides, as they produce twice the energy generated by carbohydrates. A high blood triglyceride level of more than (500) mg/dL is a biological marker for cardiovascular disease, Alzheimer's disease, pancreatitis, and diabetes [8]. Lipoproteins are smaller molecules than other types of lipoproteins and contain equal amounts of cholesterol, phosphorylated lipids, large amounts of protein and smaller amounts of triglycerides. The potential of high-density lipoprotein (HDL-C) as natural delivery carriers has also enhanced the production of (HDL-C) in the field of genetic engineering aimed at improving the effectiveness of (HDL-C) or as drug delivery agents with therapeutic potential. There are many studies on the effect of HDL-C on heart disease, as it has been found to have an inverse relationship with disease events, as low HDL-C plays an essential role in the development of atherosclerosis in the heart arteries [9]. HDL-C levels decrease in patients with chronic liver disease and are inversely associated with disease severity. During acute conditions, HDL-C levels decrease rapidly, and HDL-C molecules undergo profound changes in their composition and function. HDL-C works to prevent atherosclerosis by transferring antioxidants to HDL-C, thus making HDL -C) is less affected by oxidation within lining of blood vessels. Estrogen works to increase levels of (HDL-C) in blood serum, while its levels decrease in cases of obesity, smoking, and stress [10]. The presence of chronic kidney disease is associated with disorders in lipoprotein metabolism, the consequences of which are dyslipidemia and the accumulation of atherosclerotic particles [11]. Low density lipoprotein are small molecules that originate in the liver and contain large amounts of cholesterol and a small amount of protein and triglycerides. These molecules are the main carrier of cholesterol. High levels of low-density lipoprotein cholesterol (LDL-C) are the main cause of atherosclerosis and cardiovascular disease. LDL-C is called bad cholesterol because it deposits cholesterol in the heart arteries, causing narrowing and blockage of the arteries and the occurrence of atherosclerosis [12]. Very low density lipoprotein, which are large fatty proteins that are mainly made in the liver and contain small amounts of cholesterol and protein. These proteins play a role in the process of transferring neutral fats formed in the liver to tissues,

as it is observed when the level of (VLDL-C) is high in the blood plasma with The presence of a low level of (HDL-C) is an important factor in indicating the occurrence of atherosclerosis due to the formation of fatty deposits on the internal surfaces of blood vessels and delayed blood flow to the heart [13]. Coronary risk index is a measure used to evaluate blood flow to the heart muscle. It is calculated by dividing the diameter of the coronary artery by the diameter of the left ventricle. There are several possible reasons for the increased risk of coronary heart disease in people infected with *E. histolytica* and *G. lamblia*. One of these reasons is that parasites may cause inflammation in the coronary arteries. Inflammation can lead to the accumulation of fatty plaques in the arteries, which increases the risk of coronary artery blockage. Atherosclerotic index is a measure of level of arterial stiffness in the body that is calculated by measuring the thickness of the arterial walls in different areas of the body. A study conducted by Daniel and colleagues in 2022 found that people infected with intestinal parasites, such as *Giardia* and *E. histolytica*, had a higher atherosclerosis index compared to uninfected people. The increased atherosclerosis index in people infected with intestinal parasites can be explained as it leads to increased inflammation in the arteries by increasing the production of substances that can It damages the walls of the arteries, leading to the accumulation of fatty deposits, and infection with intestinal parasites increases the production of harmful cholesterol, which leads to the accumulation of fatty deposits in the arteries, meaning that infection with intestinal parasites increases the risk of atherosclerosis [14].

Total Proteins are essential biological components and are in the form of complex nitrogenous compounds with high molecular weights consisting of amino acid units linked together by peptide bonds. The body of a living organism contains thousands of different proteins, and most of these proteins are structural elements in the body, while other proteins are soluble. They exist as free-moving molecules in fluids inside and outside the cell. There are three types of these proteins in plasma: albumin, globulin, and fibrinogen. The liver is the main source of albumin and fibrinogen formation, while globulins are formed in the lymph nodes [15]: reactive protein-C is the first acute phase protein and is a class of immune proteins. This protein is manufactured by hepatocytes as a main source, in addition to the presence of other cells located outside the liver that are responsible for its production, such as monocytes, lymphocytes, and adipocytes [16]. The study aimed to evaluate the effect of intestinal parasite infection on some physiological parameters such as Cholesterol, Triglycerides, HDL-C, LDL-C, VLDL-C, Coronary risk index, Atherogenic index, Total protein, C-reactive protein.

MATERIALS AND METHODS

Collection of Samples

A- 85 fecal 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 (gender, age, residence, water sources, educational level, disease symptoms, etc.) was recorded according to the questionnaire form. B- 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.

The blood was separated into two parts, and the first part of blood, with a size of 2 ml, was placed in a test tube containing a substance that works to prevent blood clotting, Ethylene diamine tetra acetic acid (EDTA), in order to conduct complete blood count tests. The CBC Complete Blood Count device was used for this purpose. As for the second part of the blood, which is 3 ml in size, 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 some physiological tests.

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 [17]. As for the indirect method, it includes the floatation method. A solution of zinc sulphate $ZnSO_4$ 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, it is examined microscopically, in order to determine the presence of the type of infection with amoeba dysentery or *G. lamblia* [18].

Physiological Tests

1. Estimation of cholesterol in blood serum: An ELISA kit manufactured by the Spanish company Bio-Science Medica was used.
2. Estimation of triglycerides concentration in blood serum: An ELISA kit manufactured by the French company Biolabo was used.
3. Estimation of HDL-C concentration in blood serum: The concentration of high-density lipoproteins in blood serum was estimated using several special solutions.
4. Calculation of LDL-C concentration in blood serum
5. Calculation of VLDL-C concentration in blood serum
6. Calculation of Coronary risk index: The coronary artery index was

calculated with the following formula: $CRI = LDL/HDL$. 7. Calculation of the Atherosclerotic Index: The arterial stiffness index was calculated with the following formula: $AI = TG/HDL$. 8. Estimation of total protein concentration in blood serum: An ELISA kit manufactured by the Spanish company Bio-Science Medica was used. 9. Estimating the concentration of C-reactive protein (CRP): An ELISA kit manufactured by the Spanish company Bio-Science Medica was used.

Statistical Analysis

I analyzed the results statistically using the (Minitab) program and calculated the significant levels of the variables between two different sets of data using the T-Test and the 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, as shown in Figure (1), indicate that there are no significant differences in cholesterol concentrations in people infected with *E. histolytica* and people infected with *G. lamblia* compared with healthy people. The results of the current study agree with the findings of Al-Saadi et al. [19], that there is no significant difference between those infected with parasites compared to healthy people. Al-Mahdawy [20] did not agree in Baghdad, and Anwar [21] in Kirkuk. Both are considered *G. lamblia* and *E. histolytica* are intestinal parasites that can cause various symptoms, including diarrhea, abdominal pain, and fever. However, the relationship between these parasites and blood cholesterol concentrations is unclear. There are a number of other studies that have found no association Between infection with *G. lamblia* and *E. histolytica* and the concentration of cholesterol in the blood, there may be a number of reasons for the lack of a significant difference in the cholesterol concentration in those infected with *G. lamblia* and *E. histolytica* compared to healthy people. The reason for this may be that these parasites They do not directly affect cholesterol metabolism. Instead, the increase in cholesterol concentration in some people infected with these parasites may be caused by other factors, such as obesity or an unhealthy diet [19].

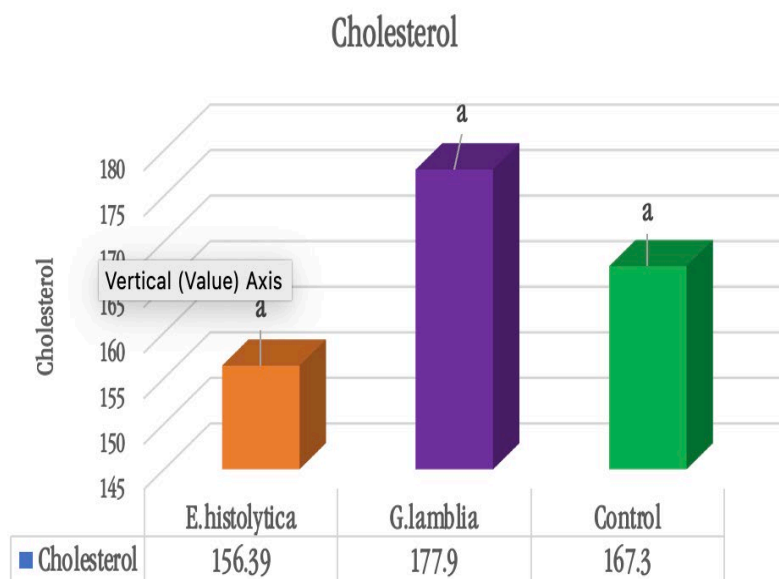


Figure 1: Cholesterol Concentration in Affected People in the Study Groups

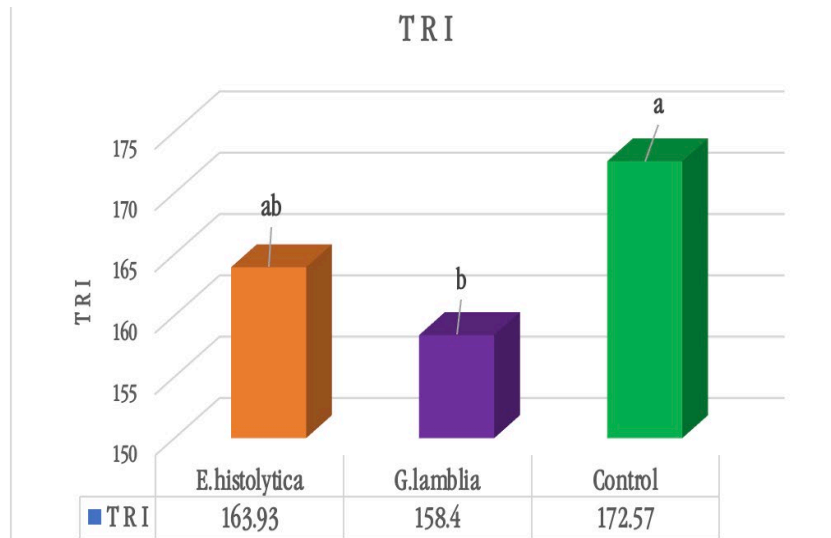


FIGURE 2: Concentration of Triglycerides in the Study Groups

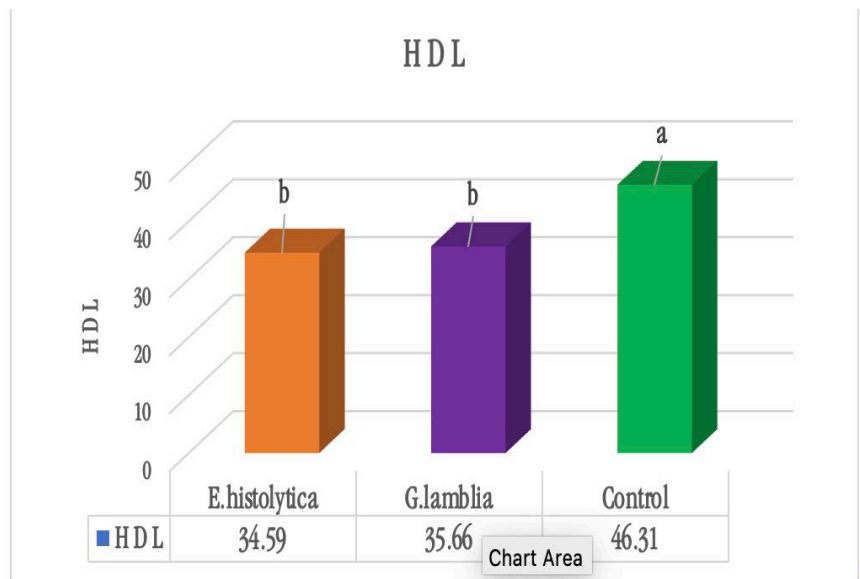


FIGURE 3: High-Density Fat Concentration in the Study Groups

The results of the current study indicate that there is no significant difference between the infected and healthy groups in the concentration of triglycerides, as the percentage of people infected with *E. histolytica* mg/dl was 163.93 ± 13.05 compared to healthy people, as well as for the people infected with *G. lamblia*, as the percentage of infection was mg/dl. dl 158.40 ± 11.97 compared to healthy controls, where the mg/dl ratio was 172.57 ± 19.04 . The results of the current study are consistent with the findings of Alves et al [22]. The reason for the lack of a significant difference in the concentration of triglycerides in people infected with the *Giardia* parasite compared to healthy people is that these parasites do not directly affect fat metabolism in the body. Blood triglyceride concentrations depend on a variety of factors, including diet, physical activity, genetics, and general health. In the case of *G. lamblia*, the primary mechanism that may lead to high triglyceride concentrations is fat malabsorption. *Giardia* secretes chemical compounds that can damage cells of the small intestine, which may lead to a decreased ability of the intestine to absorb fat from food. However, this effect is usually mild and does not cause a significant rise in triglyceride concentration. This is because the

amount of fat that is improperly absorbed from the small intestine is usually relatively small. Additionally, the body has compensatory mechanisms to process fats that are not absorbed properly, such as Increasing the production of good cholesterol (HDL), which is a type of cholesterol that helps remove triglycerides from the blood [23].

In the case of *E.histolytica*, it causes inflammation of liver, which may lead to damage to the liver cells responsible for removing triglycerides from the blood. However, this effect is also usually mild and does not cause a significant increase in the concentration of triglycerides. The results of the current study indicate a significant decrease in the concentration of high-density fat among people infected with *E. histolytica* parasite, at a ratio of 34.59 ± 4.61 mg/dl, compared with healthy people, as well as for people infected with the *G. lamblia* parasite, as the percentage reached. The reason for a decrease in the concentration of high-density proteins in people infected with the parasite is due to several reasons, including that *E. histolytica* infection leads to chronic inflammation in the body, and this inflammation leads to an increase in the production of pro-inflammatory proteins, such as C-reactive protein. Which leads to

decreased production of high-density proteins. It also leads to increased production of bad cholesterol (LDL), which leads to a lower ratio of HDL to total cholesterol (HDL/TC). The results of the current study indicate that there is no significant difference in the concentration of low-density fats among people infected with

E.histolytica parasite, as the percentage was 89.02 ± 18.29 mg/dl compared with healthy people, while people infected with *G.lambliia* recorded a significant increase, as the percentage reached 110.60 ± 20.00 . Compared with healthy people, the mg/dl ratio was 75.62 ± 16.86 (Figure 4).

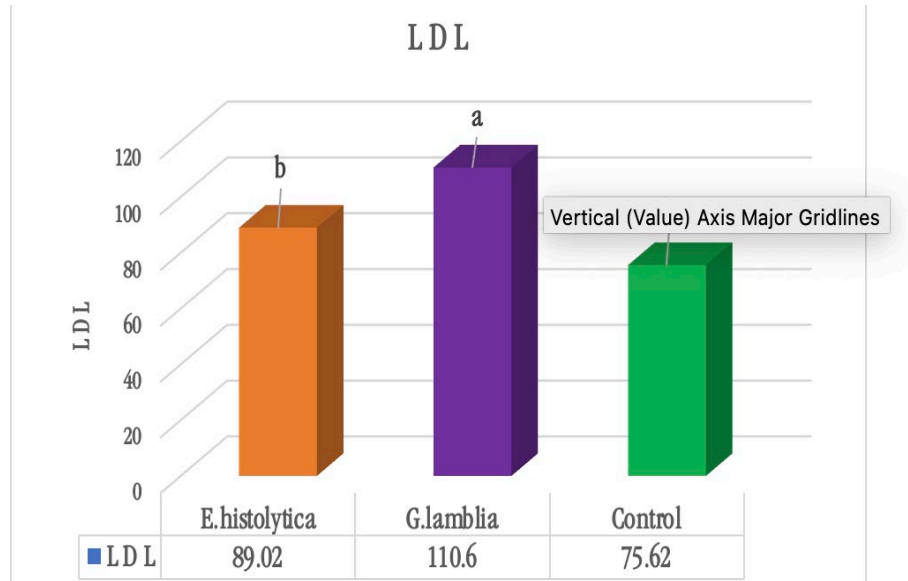


FIGURE 4: Low-Density Fat Concentration in the Study Groups

The results of the current study indicate that there is no significant difference in the concentration of very low-density fats between people infected with *E. histolytica*, with a ratio of 32.787 ± 2.611 mg/dl

compared with healthy people. Likewise, for people infected with *G. lamblia*, as the ratio reached 31.679 ± 2.391 mg/dl compared with Healthy people with a mg/dl ratio of 33.108 ± 2.849 (Figure 5).

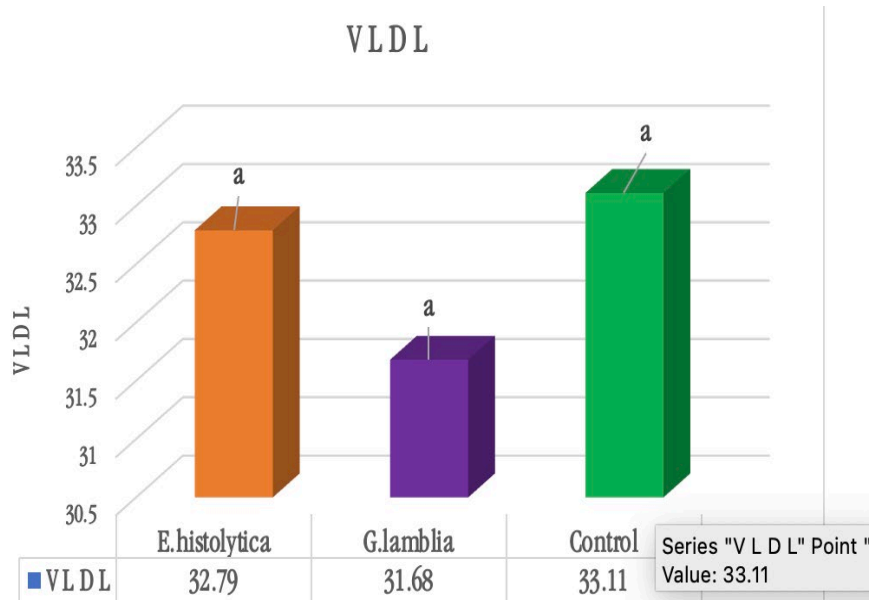


FIGURE 5: Very Low-density Fat Concentration in the Study Groups

The results of the current study are consistent with the findings of Al-Sabban et al., [24] that there is no significant difference between people infected with *E. histolytica* and healthy people, as well as with the findings of Al-Shammari et al., [25] that There is no significant difference between those infected with the *G. lamblia* parasite and healthy people. Very low-density lipoprotein (VLDL) is a type of lipoprotein that plays an important role in transporting cholesterol from the liver to other tissues. They are produced in the liver, then travel to the blood where they are converted into LDL (low-density

lipoprotein) and HDL (high-density lipoprotein). *E.histolytica* can affect cholesterol production indirectly by causing inflammation. Inflammation can lead to increased cholesterol production in the liver, which may lead to higher LDL and VLDL concentrations. However, this increase in cholesterol production is generally minor. It does not lead to a significant difference in the level of VLDL between those infected with the parasite and healthy people [24]. *G. lamblia* can affect fat absorption from the intestine. This effect leads to decreased LDL and VLDL concentrations. However, this effect is generally short-term, as fat

absorption generally returns to normal after the infection is treated. It can be said that *G. lamblia* does not significantly affect the levels of very low-density proteins. However, there can be a mild indirect effect by causing inflammation or decreased fat absorption [25]. The results of the current study indicate that there is a significant increase in the

coronary artery risk index among people infected with *E. histolytica* by 4.995 ± 1.080 compared to healthy people, as well as for people infected with *G. lamblia*, as a significant increase was recorded by 4.668 ± 1.228 compared to healthy people. By 3.581 ± 0.646 (Figure no 6).

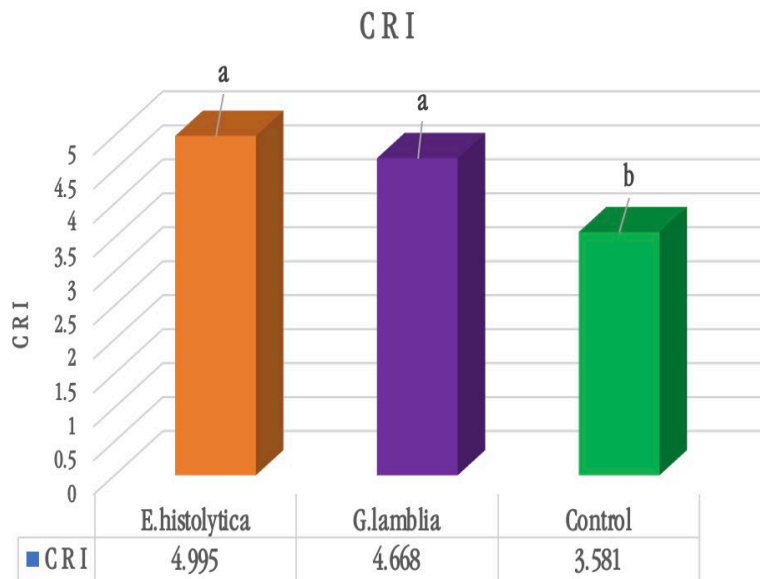


FIGURE 6: Coronary Artery Risk Index in the Study Groups

Infection with intestinal parasites, such as *E. histolytica* and *G. lamblia*, causes inflammation of the intestinal wall. This inflammation can lead to the release of chemicals called cytokines, which can affect blood vessels throughout the body, including the coronary arteries.

The results of the current study indicate that there is a significant increase in the arterial stiffness index among people infected with *E. histolytica*, as well as for people infected with *G. lamblia*, compared to healthy people, where the ratios were 2.795 ± 1.266 (3.269 ± 1.638) (1.721 ± 0.480) over respectively (Figure 7).

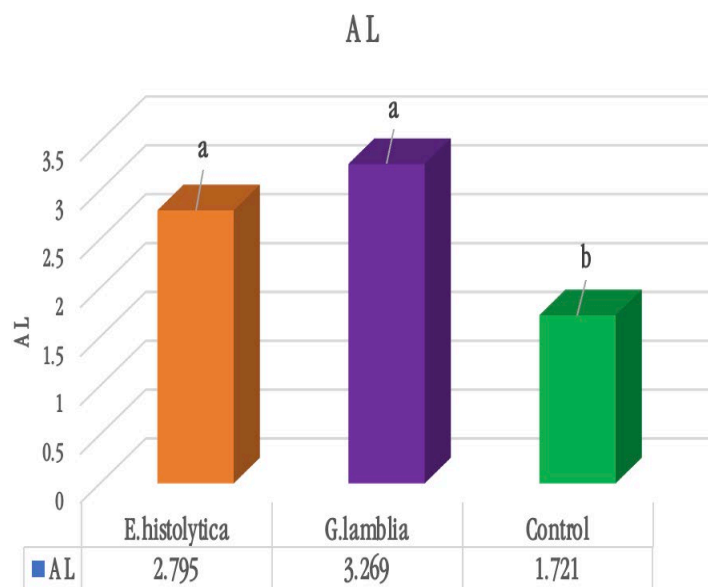


FIGURE 7: Arterial stiffness index in the study groups

The risk of atherosclerosis occurs when fatty plaques build up on the walls of the arteries, narrowing the arteries and increasing the risk of heart attacks and strokes. There are many factors that can contribute to atherosclerosis, including high blood pressure and high cholesterol. In the case of infection with *E. histolytica* or *G. lamblia*, the parasite can increase the risk of atherosclerosis through a range of mechanisms. The parasite can damage the inner lining of the arteries,

allowing fat to accumulate on the walls. The results of the current study indicate that there is no significant difference in the concentration of total protein between people infected with *E. histolytica*, as the percentage was 6.150 ± 1.294 (g/dl) compared to healthy people, as well as for people infected with *G. lamblia*, where the percentage was 5.664 ± 0.941 (g./dl) compared to healthy people at a rate of 6.906 ± 2.004 (g/dl) (Figure 8).

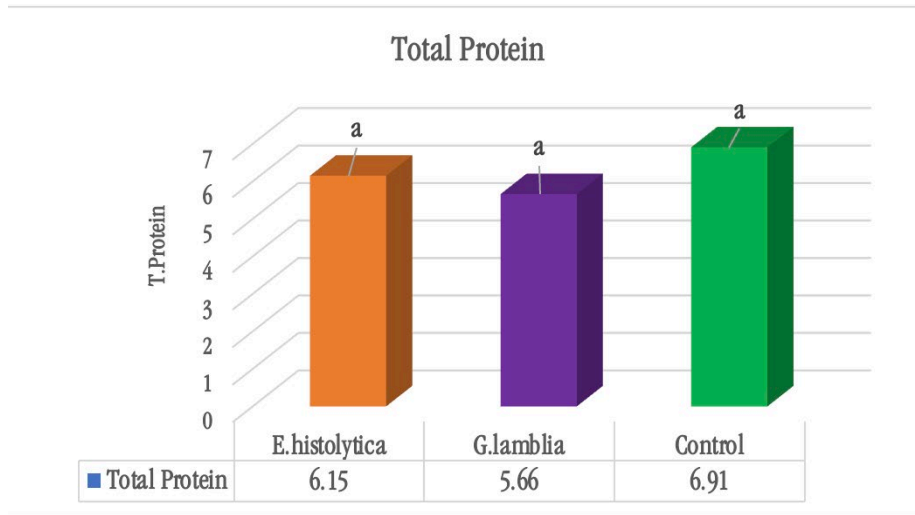


Figure 8: Total Protein Concentration in the Study Groups

The results of the current study are consistent with what was stated by Al-Sabban [24] and Al-Shammari [25], as they concluded in their study that there is no significant difference between infected and healthy people. The reason for the lack of a significant difference between infected and healthy people is that the *E.histolytica* does not affect the concentrations of all other proteins in the blood. CRP-interacting proteins have an important role in defending against infection by stimulating the immune system and other proteins such as albumin and globulin et al., [24]. *G. lamblia* can affect total protein production indirectly by causing inflammation or malabsorption.

Inflammation can lead to increased protein production in the liver, which may lead to higher total protein concentration. Malabsorption leads to lower protein concentration. Kidney, where protein is lost in the stool rather than absorbed into the blood [25]. The results of the current study indicate that there was a significant decrease in the concentration of C-reactive protein among those infected with *E. histolytica*, as the percentage reached 11.43 ± 3.04 compared to healthy people. Likewise, for people infected with *G. lamblia*, no significant difference was recorded, as the percentage was 12.79 ± 3.07 compared to with healthy people by 17.06 ± 4.86 (Figure 9).

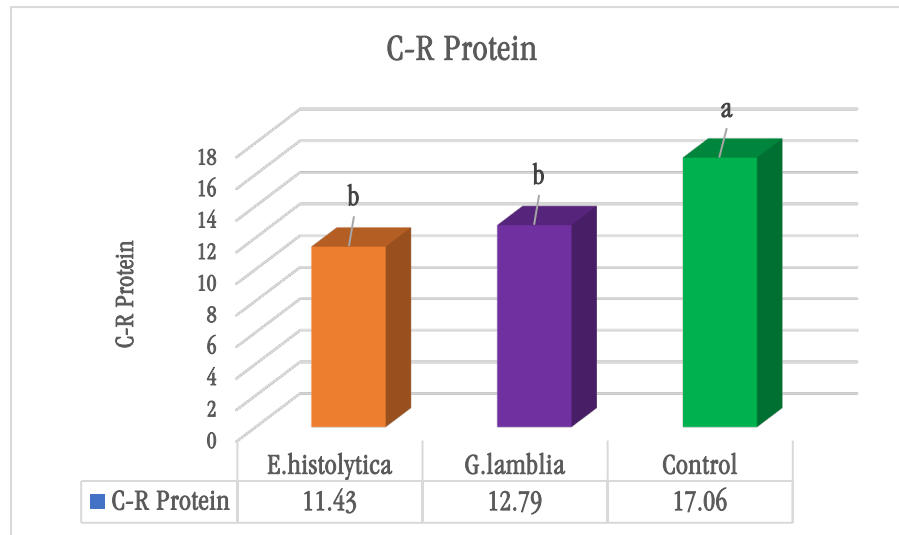


Figure 9: C-Reactive Protein Concentration in the Study Groups

The results of the current study are consistent with the findings of Al-Sabban et al., [24] and Al-Shammari et al., [25], as they concluded that there was a significant decrease between infected and healthy people, while they did not agree with the findings of AL-Ezzy et al., [26]. Inflammation caused by the parasite can lead to liver injury.

This injury leads to damage to liver cells, which play an important role in the production of C-reactive protein. In addition, inflammation caused by the parasite can lead to increased production of other proteins called cytokines that play a role in inhibiting the production of C-reactive protein [24].

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