



Article

Analysis of Liver Function in Patients with Intestinal Parasitic Infections: A Comparative Study of AST, ALT, and ALP Levels

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Abstract: This study aimed to evaluate the levels of certain liver function markers in individuals infected with *Entamoeba histolytica* in Kirkuk City, Iraq. Samples were collected from hospitals in Kirkuk (Kirkuk General Hospital and Children's Hospital) and private laboratories between October and December 2024. Out of 220 stool samples examined for cyst and trophozoite stages of *Entamoeba*, 130 samples were excluded due to chronic infections or other diseases. Participants were divided into two groups: 70 infected individuals and 20 healthy individuals as a control group. Serum samples were analyzed to measure the levels of certain liver enzymes (ALT, AST, ALP) in the infected individuals. Statistical analysis (using SPSS software) revealed no significant increase ($p \leq 0.05$) in the levels of liver enzymes (ALT, AST, ALP) in the infected individuals, indicating that the infection did not affect liver function or enzyme levels in transient or non-chronic amoebic infections. Based on the findings, this study highlights the impact of *Entamoeba histolytica* infection on liver function and provides scientific data that can help develop more effective diagnostic and therapeutic strategies for infected individuals in endemic areas.

Keywords: Amoebic Dysentery, ALT, AST, ALP

1. Introduction

Entamoeba histolytica, a tissue-invasive protozoan parasite, is one of the anaerobic intestinal parasites responsible for approximately 50 million infections annually worldwide, with a mortality rate exceeding 100,000 cases per year [1]. This parasite colonizes and invades the intestines, causing a condition known as amoebiasis, which leads to colitis and may also result in the formation of amoebic liver abscesses [2]. Amoebic dysentery is the third leading cause of parasite-related deaths globally and in Iraq, after malaria and schistosomiasis [3]. The infection is widespread in Kirkuk[4] and in developing countries such as Bangladesh, India, tropical African nations, and some regions of Mexico and Brazil. An increase in cases has also been observed in both developed and developing countries, including European countries and the United States, due to migration and international travel from endemic areas [5].

Most parasitic infections are asymptomatic. However, common symptoms associated with the infection include abdominal pain, vomiting, discomfort, and bloody diarrhea [6]. In severe cases, the infection can lead to complications such as gastroenteritis, malnutrition, and malabsorption. Children are more susceptible to primary intestinal parasitic infections, primarily due to poor socioeconomic conditions, which are the main factor in the spread of intestinal parasites [7]. When *Entamoeba histolytica* infects the host,

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the parasite binds to the epithelial cell layer of the colon and the mucous layer by secreting the Gal/GalNAc lectin protein through its trophozoite cells. After attachment, the parasite begins to destroy epithelial cells using lytic enzymes and surface proteins, particularly cysteine proteases [8]. This results in intestinal cell damage and the destruction of extracellular matrix components. In this process, the parasite bypasses the first line of defense against parasitic infection, which is the intestinal mucous layer [9]. Amoebic dysentery caused by *Entamoeba histolytica* can lead to significant liver complications, particularly amoebic liver abscesses. These abscesses are associated with elevated liver enzymes, such as alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST), indicating liver inflammation or damage in cases of concurrent intestinal and hepatic infections. Patients often exhibit higher levels of bilirubin and liver enzymes, necessitating interventions such as percutaneous drainage. Furthermore, liver enzyme levels are critical for assessing the severity and management of liver conditions associated with amoebic dysentery. Study Aim:

This study aims to evaluate the impact of *Entamoeba histolytica* infection on liver function by measuring the levels of liver enzymes (ALT, AST, ALP) in infected individuals in Kirkuk City, Iraq, and comparing them with a control group of healthy individuals. The study also seeks to determine whether intestinal parasitic infections lead to significant changes in liver function, thereby contributing to a better understanding of the mechanisms by which the infection affects other organs and aiding in the development of more effective diagnostic and therapeutic strategies.

2. Materials and Methods

2.1 Sample Collection

Stool samples were collected from 220 individuals between October 2024 and December 2024 from hospitals in Kirkuk City (Kirkuk General Hospital and Children's Hospital) and private laboratories in Kirkuk. The samples included individuals with gastrointestinal symptoms as well as a group of healthy individuals. A total of 150 samples were excluded from the study due to chronic diseases such as kidney failure or diabetes, as well as individuals with bacterial or viral infections. Ultimately, 90 valid samples were retained for analysis. The samples were collected in sterile, tightly sealed containers to preserve sample integrity and prevent moisture loss. Additionally, 90 blood samples were drawn from both infected and healthy groups using a medical syringe (5 ml) and placed in test tubes containing gel. The samples were then centrifuged for 10 minutes at 3000 rpm. Serum was collected using Eppendorf tubes and stored frozen at -20°C until analysis.

2.2 Microscopic Examination

The samples were examined macroscopically and microscopically to diagnose cyst and trophozoite stages in the stool samples. The samples were divided into two groups: 20 samples from healthy individuals and 70 samples from individuals infected with the parasite. Samples were collected from government hospitals and private laboratories to ensure participant diversity and better representation of the disease's local prevalence in the region.

A stool sample was taken using a wooden stick and placed on a glass slide with a drop of normal saline solution. A coverslip was then placed at an angle to avoid air bubbles. The slide was examined under a microscope, first at 10X magnification and then at 40X magnification. A second slide was prepared using the same steps, with the addition of one drop of Lugol's iodine stain, which was prepared in advance according to [10]. This was done by dissolving 5 grams of potassium iodide in 1000 ml of distilled water, then adding 10 grams of iodine crystals. The mixture was stirred slowly until dissolved, filtered, and stored in sterile, tightly sealed containers. The stain was used to color the cyst stage of the tissue-invasive amoeba parasite. Samples were taken from different areas of the specimen to increase the likelihood of detecting the parasite.

2.3 Biochemical Tests (Liver Function Tests)

2.3.1 Determination of Alkaline Phosphatase (ALP) in Blood Serum

The level of alkaline phosphatase in blood serum was determined using ready-made analysis kits designed for measuring ALP concentration, produced by the Spanish company Linear [11].

Basic-Principle:

Alkaline phosphatase (ALP) catalyzes the hydrolysis of p-nitrophenyl phosphate, forming p-nitrophenol and inorganic phosphate, with the alkaline buffer acting as a phosphate acceptor. The reaction is monitored kinetically at 405 nm based on the rate of p-nitrophenol formation, which is proportional to the ALP activity in the sample.

2.3.2 Determination of Alanine Aminotransferase (ALT/GPT) in Blood Serum

The level of ALT/GPT in blood serum was measured using a ready-made analysis kit produced by the Spanish company Linear, following the IFCC protocol [12].

Basic-Principle:

This method measures the activity of alanine aminotransferase (ALT/GPT), which catalyzes the transfer of an amino group from L-alanine to 2-oxoglutarate, forming L-glutamate and pyruvate. Pyruvate is then reduced to lactate by lactate dehydrogenase (LDH), consuming NADH and converting it to NAD⁺. The reaction is monitored kinetically by measuring the decrease in absorbance at 340 nm, which is proportional to the ALT activity in the sample.

2.3.3 Determination of Aspartate Aminotransferase (AST/GOT) in Blood Serum

The level of AST/GOT in blood serum was measured using a ready-made analysis kit produced by the Spanish company Linear, following the IFCC protocol [12].

Basic-Principle:

This test measures the activity of aspartate aminotransferase (AST/GOT), which catalyzes the transfer of an amino group from L-aspartate to 2-oxoglutarate, forming L-glutamate and oxaloacetate. Oxaloacetate is then reduced to L-malate by malate dehydrogenase (MDH), consuming NADH and converting it to NAD⁺. The reaction is monitored kinetically by measuring the decrease in absorbance at 340 nm, which is proportional to the AST activity in the sample.

2.4 Statistical Analysis

The results were statistically analyzed using SPSS software to determine significant differences between groups using an independent t-test. Subsequently, the strength and type of correlation between variables were assessed using Pearson's correlation coefficient [13].

3. Results and Discussion

3.1 Microscopic Examination

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

Table 1. Infection rate of *E. histolytica* based on microscopic examination in the study groups.

Microscopic Examination	Positive Samples	Percentage	Negative Samples	Percentage
	70	31.81%	150	68.18%

Cyst Stage: Characterized by a transparent, round, or oval body containing 3-4 nuclei and a chromatoid body, as shown in Figure 1.

Trophozoite Stage: Characterized by an irregular protoplasmic mass with projections extending in all directions. It contains a single nucleus with a central nucleolus that regulates and controls cell functions, as shown in Figure 2.

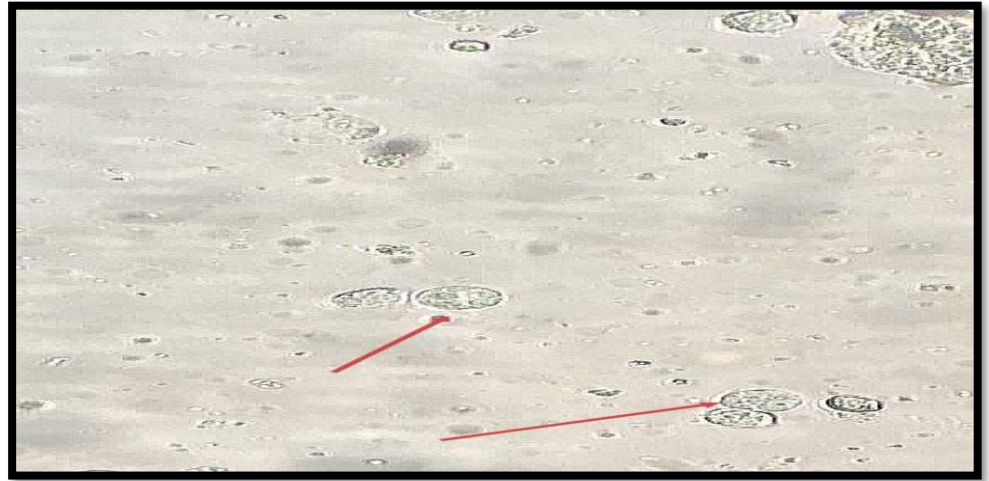


Figure 1. Cyst stage of *Entamoeba histolytica* stained with Lugol's iodine under 40X magnification.

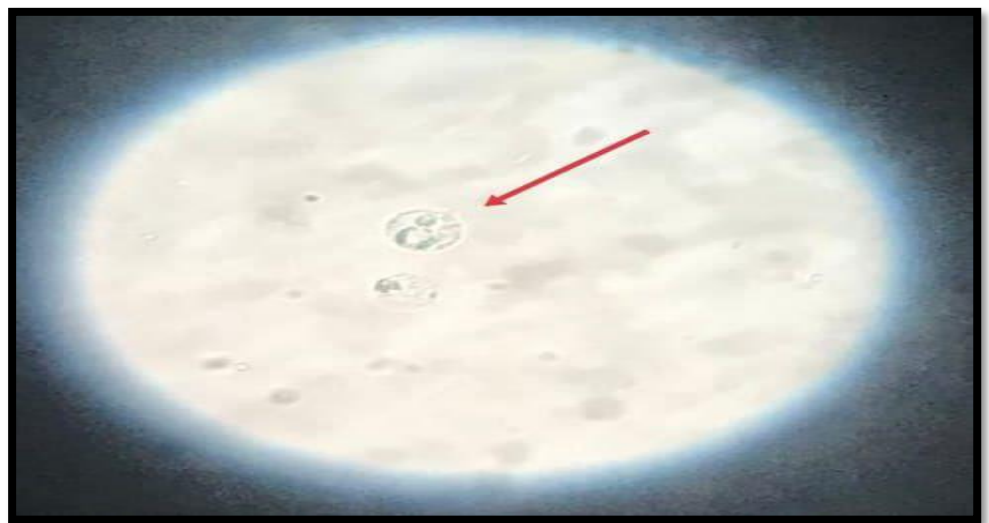


Figure 2. Trophozoite stage of *Entamoeba histolytica* under 40X magnification.

The current study recorded an infection rate of 31.81%. These results are consistent with those reported by [14] in Dhi Qar and Qadir and [15] in Kalar, who reported infection rates of 29.9% and 31.6%, respectively. These results are higher than those reported by [16] in Kirkuk and Obaid in Kirkuk, who reported an infection rate of 27.7%.

3.2 Effect of *Entamoeba histolytica* Infection on Liver Enzymes (ALT, ALP, AST)

3.2.1 ALP Level

In the current study, the level of the liver enzyme ALP was examined, and no significant differences were observed between the patient and control groups. The levels of this enzyme in the study group were similar to those in the control group in all tests, as shown in Figure 3.

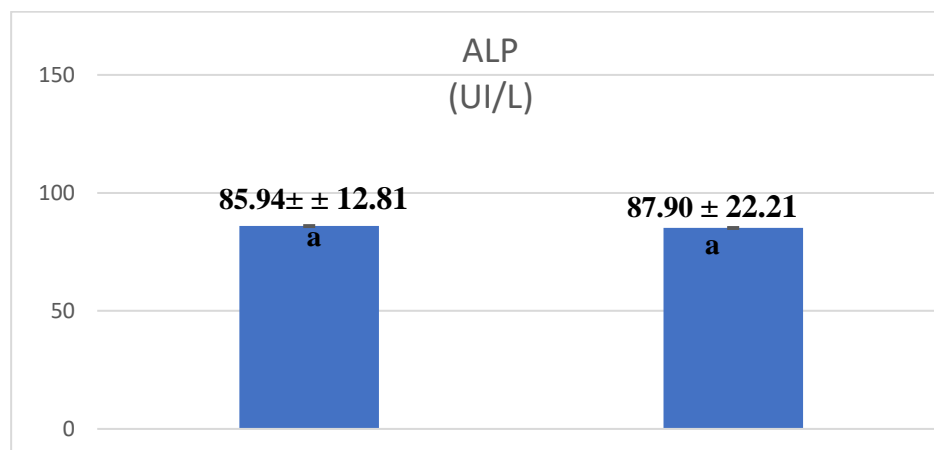


Figure 3. Shows the ALP level in the study groups.

Similar letters on the columns indicate no significant differences at a probability level of ($P \leq 0.05$).

This study agrees with the findings of [17] and [18], who did not record any increase in enzyme levels during amoebic dysentery infection. These results contradict those reported by [19] and [20]. In contrast, [21] reported a significant increase in enzyme levels in infected samples compared to healthy ones [22].

3.2.2 ALT Level

In the current study, the level of the liver enzyme ALT was examined, and no significant differences were observed between the patient and control groups. The levels of this enzyme in the study group were similar to those in the control group in all tests, as shown in Figure 4.

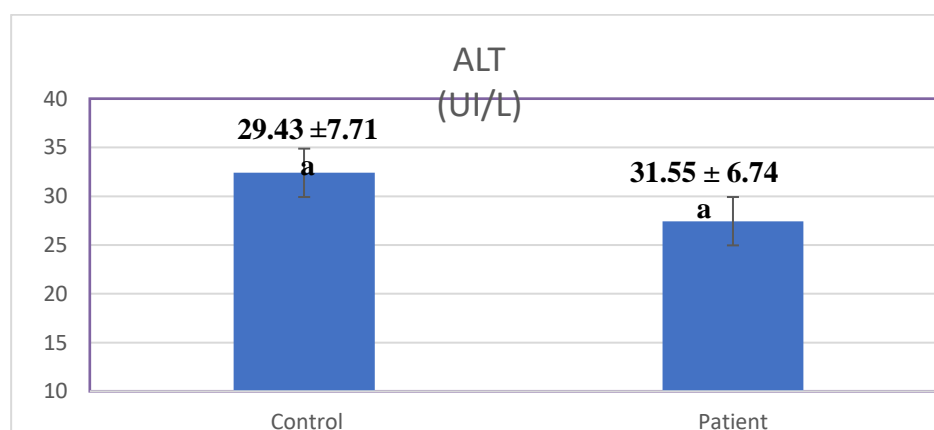


Figure 4. Shows the ALT level in the study groups.

Similar letters on the columns indicate no significant differences at a probability level of ($P \leq 0.05$).

This study agrees with the findings of [17] and [18], who did not record any increase in enzyme levels during amoebic dysentery infection. These results contradict those reported by [19] and [20]. In contrast, [21] reported a significant increase in enzyme levels in infected samples compared to healthy ones.

3.2.3 AST Level

In the current study, the level of the liver enzyme AST was examined, and no significant differences were observed between the patient and control groups. The levels

of this enzyme in the study group were similar to those in the control group in all tests, as shown in Figure 5.

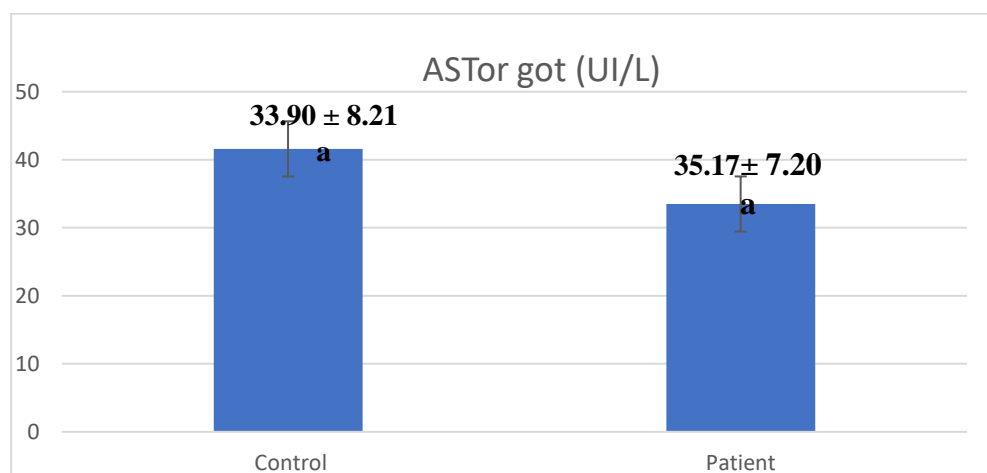


Figure 5. Shows the AST level in the study groups.

Similar letters on the columns indicate no significant differences at a probability level of ($P \leq 0.05$).

This study agrees with the findings of [17] and [18], who did not record any increase in enzyme levels during amoebic dysentery infection. These results contradict those reported by [19] and [20]. In contrast, [21] reported a significant increase in enzyme levels in infected samples compared to healthy ones [22].

In severe infections caused by *Entamoeba histolytica*, the parasite migrates to other organs, such as the liver, causing liver abscesses and affecting the secretion of liver enzymes such as alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST). ALT is primarily specific to liver cell activity, while AST is found in many body tissues, such as the heart, kidneys, liver, and skeletal muscles. ALP is found in various organs, such as the liver, kidneys, bones, and intestines, but is primarily located in the bile duct lining. Any damage to liver cells due to the invasion of the parasite, which contains tissue-lysing enzymes and a phagocytosis mechanism, leads to the release of these enzymes into the blood, causing an increase in their concentration. In liver diseases, such as necrosis or cirrhosis, the levels of ALT, AST, and ALP rise before the appearance of clinical symptoms such as jaundice. Therefore, elevated levels of these enzymes are observed in patients with liver diseases and cardiovascular diseases. Some studies have shown that any dysfunction in various organs can lead to increased levels of these enzymes in the blood. Generally, under normal conditions, the levels of ALT, AST, and ALP in tissues are higher than their levels in the blood [23].

4. Conclusion

The study concluded that there was no significant increase in liver function markers (AST, ALT, ALP) between the two groups (the group of patients with intestinal parasitic infections and the control group). This indicates that intestinal parasitic infections in these non-severe or acute cases did not significantly affect liver function.

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