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## **Biochemical study for Amoebic dysentery in patients with *Entamoeba histolytica* in Thi-Qar province/southern Iraq**

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### **Abstract**

*Entamoeba histolytica* is a parasitic protozoan that caused amoebiasis or amoebic dysentery, it is an intestinal disease which infects colon and liver. Amoebic dysentery is spread all over the world. *E. histolytica* is causing the death of human cells and leading to develop invasion, and ulceration of the intestine. However, amoebic liver abscess (ALA) considers as major common extraintestinal disease caused by *E. histolytica*. The current study was designed to estimate the effect of *E. histolytica* trophozoite on liver functions through the evaluation of some bio-chemical parameters for patients with amoebiasis. Blood sample were collected from patients with amoebic dysentery after diagnosed about 100 from stool sample from them microscopically and detected by PCR technique to confirm the infection with amoebiasis, all sample with amoebiasis were subjected to biochemical study after the separation of serum. Biochemical parameters that included during this study were AST, ALT and ALP. Lipid profile included Triglyceride and Cholesterol as well as some minerals like Iron, Zinc, and Potassium. The results for the level of AST and ALT were showed a high significant difference ( $P < 0.01$ ) where AST was (22.84 $\pm$ 7.83) in patients' compared with (55.36 $\pm$ 16.8) in control groups and ALT level Was (20.4 $\pm$ 9.37) in patients compared with (47 $\pm$ 20.1) in control groups. Additionally, there is no significant difference ( $p > 0.05$ ) in ALP level where it was (351.7 $\pm$ 235.2) in patients with amoebic dysentery compared with (336.13 $\pm$ 204.08) in controls group. Triglyceride were also checked in patients with amoebic dysentery and the results showed that a significant difference ( $p < 0.05$ ) between (166.72 $\pm$ 62.4) in patients and (135.43 $\pm$ 45.2) in control groups. Cholesterol level was also checked in patients and the results showed there is no significant difference ( $P > 0.05$ ) between (185.8 $\pm$ 77.2) in patients and (160.19 $\pm$ 51.3) in controls groups. In addition, some minerals were studied during the following study like Iron, Zinc, and Potassium for patients with and the results were not showed a significant difference ( $P > 0.05$ ) in zinc, potassium and iron where the level of iron were (85.24 $\pm$ 16.58) in patients compared with (81.22 $\pm$ 15.5) in control groups, while the level of zinc were (108.09 $\pm$ 24.4) in patients and (101.76 $\pm$ 24.4) in control groups, the level of potassium were (3.73 $\pm$ 1.89) in patients and compared with (3.84 $\pm$ 1.14) in control groups.

**Key words:** *E. histolytica*, Amoebic dysentery, liver function, biochemical parameters.

## **Introduction**

Amoebiasis is an intestinal disease caused by unicellular parasite called *E. histolytica*. Studies have shown that amoebiasis is third most important diseases that caused of death from parasitic diseases after malaria and schistosome according to the world health organization (WHO) (1). This parasite caused 40,000 -100,000 deaths annually (1). The spread of the parasite is recorded up to 10% of the world's population, it is recorded approximately 90% of the infected individuals with amoebiasis are asymptomatic, but 10% of them developed into invasive infection (2). This type of infection is spread in many regions of the world, especially in developing countries, and in the tropics, sub tropics, the India subcontinent and areas of poor health (3).

Intestinal parasites lead many health problems including diarrhea which caused dehydration, death and anemia especially for children under five years of age (4). The causes of health problems worldwide, particularly countries that lack or weakness in the surveillance and workers inspection systems, which increased the spread and trans-mission of parasitic pathogens ( 5, 6). The health and environmental conditions

play a major role in the spread of intestinal parasites and revealed many studies about amoebiasis in the fourth of the twentieth century (7).

Many studies and the research have recorded that the human being is the natural host for the parasite, and there is no common storing be-tween humans and animals. The parasite can move from one with help of the vital vector as it is found in some other parasites (8).

The amoebiasis is divided into two main types: intestinal amoebiasis or so-called primary amoebiasis and secondary type extraintestinal amoebic this occurs after 1-4 weeks after the occurrence of infection. As for people who carry the disease without symptoms, it occurs after several months. The clinical manifestation of amoeba including diarrhea to mucous-bloody diarrhea, the erosion of the mucous layer, which increases by increasing the depth of the area of infection and then attacking tissues by the amoeba and its degradation by protein enzyme (9).

## **Materials and Methods**

**Collection of Blood Sample:** Five ml of blood was taken from each patient with

amoebiasis whom entered Mohamed Al-Mosawi and Al-Habobi teaching hospitals in Nasiriyah city from Thi-Qar province in Iraq during the period extended from 2020-2021, it was drawn by vein-puncture using 5 ml disposable plastic syringes under sterile conditions. blood was collected in the gel tube and left until clotted at room temperature for one hour. After blood clotting, it was centrifuged at 4000 rpm for 10 minutes, and then the serum was divided into two equal parts in Eppendorf tubes for biochemical parameter test (AST, ALT, ALP, cholesterol, triglyceride, zinc, Iron and potassium) then stored in  $-20^{\circ}\text{C}$ . Each part of sera was used once to avoid repeated thawing and freezing. All materials (i.e., reagents and sera) were allowed to stand at room temperature before use (10, 11, 12).

**Biochemical Parameters tests:** Liver function test like (AST, ALT, and ALP), lipid profile test like (cholesterol, triglyceride), and minerals test like zinc. Iron and potassium were examined during the current study based on (13,14) methods

### **Result:**

The current study was deal with some of biochemical parameter among patient with amoebic dysentery in Nasiriyah city including AST, ALT, ALP, Cholesterol, Triglyceride and some Minerals like Zinc, potassium as well as Iron concentration. High decrease significant difference was

noted during the current study in the level of AST for patient with amoebic dysentery when it was  $22.84 \pm 7.83$  compared with control group. Through examining 22 number of infected males and 28 females as showed in table (1).

High decrease significant differences were showed during the current study in the concentration of ALT for patient with amoebic dysentery when it was reported  $20.4 \pm 9.37$  in ALT concentration for patients with amoebic dysentery compared with control group when it was reported  $47 \pm 20.1$  in ALT concentration, 22 number of males were examined and 28 of females for testing ALT, as illustrated in table (2). The concentration of ALP was also checked during the following study when involved the patients with amoebic dysentery, the concentration of ALP were  $351.7 \pm 235.2$  in serum of patients' compared with  $336.13 \pm 204.08$  of ALP concentration in control group. The results were not explained a significant differences in ALP concentration among patients with amoebic dysentery in comparison with control group for each of males and females as showed in table (3).

The concentration of cholesterol was also measured during the following study when involved the patients with amoebic dysentery, the concentration of cholesterol was  $185.8 \pm 77.2$  in serum of patients compared with  $160.19 \pm 058$  of cholesterol concentration in control group. There was no a significant difference in cholesterol concentration between the patients with amoebic dysentery and the control group for

each of males and females as showed in table (4).

There was a significant difference in the concentration of Triglyceride between patients with amoebic dysentery (166,72±62.4) p value (p<0.05) and the control group (135.43±45.2) including males and females as the following table (5). The concentration of Zinc was also test during the current study when involved the patients with amoebic dysentery, the concentration of Zinc was 108.09±24.4 in serum of patients compared with 101.76±24.4 in the control group.

There was no a significant difference in concentration of Zinc between patients' group with amoebic dysentery in comparison with control group including males and females as showed in table (6). No significant differences were showed during the current study in the concentration of potassium for patient with amoebic dysentery when it was reported 3.73±1.89 in potassium concentration for patients with amoebic dysentery compared with control group when it was reported 3.84±1.14 in potassium concentration, including males and females as showed in table (7).

No significant differences were showed during the current study in the concentration of Iron for patient with amoebic dysentery when it was reported 85.24±16.58 in Iron concentration for patients with amoebic dysentery compared with control group when it was reported 81.22±15.5 for each male and females included and as explained in table (8).

Statistical analysis shows no significant differences (P>0.05) between age of patients and infection with amoebic dysentery as explained in table (9). Statistical analysis shows a significant difference (P<0.05) in IL-25 of patients with high show rate for rural, but low rate in urban, no significant difference (P>0.05) for IL-35 in the patient with *E.histolytica* for urban and rural. Table (10) show the results of geo-graphic distribution for patients with *E.histolytica* infections, in different biochemical and immunological parameters

**Table (1): AST concentration in patients with amoebic dysentery and control.**

	AST Concentration	No. Male (%)	No. female (%)	No. of total sample
<b>Patients</b>	22.84±7.83	22(44%)	28(56%)	50
<b>Control</b>	55.36±16.8	14	16	30
<b>T test</b>	9.924			

**S: significant differences (P<0.05).**

**Table (2); ALT concentration in patients with amoebic dysentery and control.**

	<b>ALT Concentration</b>	<b>No. Male (%)</b>	<b>No. female (%)</b>	<b>No. of total sample</b>
Patients	20.4+/-9.37	22(44%)	28 (56%)	50
Control	47+/-20.1	14	16	30
T test	6.812			

**Hs: High significant differences (P<0.01).**

**Table (3): ALP concentration in patients with amoebic dysentery and control.**

	<b>ALP concentration</b>	<b>No. Male (%)</b>	<b>No. female (%)</b>	<b>No of total sample</b>
<b>Patients</b>	351.7±235.2	22(44%)	28(56%)	50
<b>Control</b>	336.13±204.08	14	16	30
<b>T test</b>	0.302			

**NS: No significant differences (p<0.05).**

**Table (4): Cholesterol concentration in patients with amoebic dysentery and control.**

	<b>Cholesterol Concentration</b>	<b>No. Male (%)</b>	<b>No. female (%)</b>	<b>No. of total sample</b>
<b>Patients</b>	185.8±77.2	22(44%)	28(56%)	50
<b>Control</b>	160.19±51.3	14	16	30
<b>T test</b>	1.781			

**NS: No significant difference (P<0.05).**

**Table (5): Triglyceride concentration in patients with amoebic dysentery and control.**

	<b>Triglyceride Concentration</b>	<b>No. Male (%)</b>	<b>No. female (%)</b>	<b>No of total sample</b>
<b>Patients</b>	166.72±62.4	22(44%)	28(56%)	50
<b>Control</b>	135.43±45.2	14	16	30
<b>T test</b>	2.588			

**S: significant difference (p<0.05).**

**Table (6): Zinc concentration in patients with amoebic dysentery and control.**

	<b>Zinc Concentration</b>	<b>No. Male (%)</b>	<b>No. female (%)</b>	<b>No. of total sample</b>
<b>Patients</b>	108.09±24.4	22(44%)	28(56%)	50
<b>Control</b>	101.76±24.4	14	16	30
<b>T- test</b>	1.121			

**NS: no significant difference (P>0.05).**

**Table (7): Potassium concentration in patients with amoebic dysentery and control.**

	<b>Potassium Concentration</b>	<b>No. Male (%)</b>	<b>No. female (%)</b>	<b>No. of total sample</b>
<b>Patients</b>	3.73±1.89	22(44%)	28(56%)	50
<b>Control</b>	3.84±1.14	14	16	30
<b>T test</b>	0.288			

**NS: no significant difference (P<0.05).**

**Table (8): Iron concentration in patients with amoebic dysentery and control.**

	Iron Concentration	No. Male (%)	No. female (%)	No of total sample
<b>Patients</b>	85.24±16.58	22(44%)	28(56%)	50
<b>Control</b>	81.22±15.5	14	16	30
<b>T test</b>	1.074			

NS: no significant difference (P>0.05).

**Table (9): Biochemical parameters based on the age of patients with amoebic dysentery.**

Parameter	Lest than 8y	8-17	18-27	28-37	38-47	48-57	T test	P value
<b>AST</b>	23.18±8.8	20.36±6.62	25.4±9.44	19.33±2.88	21±4.24	25.85±7.5	0.661	0.655 (NS)
<b>ALT</b>	20.54±9.99	18.54±9.7	25.4±7.23	15±7	17.5±13.4	22.42±8.97	0.650	0.663 (NS)
<b>ALP</b>	350.2±173	261.09±124.7	344.4±320.2	537.6±555.6	443±111.7	398.5±330.9	0.802	0.554 (NS)
<b>Cholesterol</b>	101.2±27.1	118.6±20.1	106.6±16.4	122.8±12.4	98.5±42.5	110.4±23.8	1.060	0.395 (NS)
<b>Triglycerid e</b>	181.9±78.5	211.05±72.6	197.4±90.8	95.8±26.5	167.1±99.4	194.1±73.4	1.138	0.355 (NS)
<b>Zinc</b>	164.09±47.07	188.1±80.7	130±48.9	132±93.5	175±49.4	180±73.9	0.857	0.517 (NS)
<b>Potassium</b>	3.24±1.07	3.42±1.61	4.3±2.02	2.96±0.5	4.9±2.4	5.32±3.46	1.834	0.126 (NS)
<b>Iron</b>	86.27±13.7	80±13.2	77.16±22.4	92.56±11.2	92.7±32.9	90.7±23.4	0.804	0.551 (NS)

NS: No significant differences (P>0.05)

**Table (10): Biochemical parameters for patients based on the geographic distribution.**

Parameter	urban	rural	T test	P value
AST	23.57±7.8	21.41±7.92	0.924	0.360(NS)
ALT	20.06±8.64	21.05±10.91	0.353	0.725(NS)
ALP	340.8±213.8	372.8±277.9	0.452	0.653(NS)
Cholesterol	104.7±24.5	114.5±23.4	1.357	0.181(NS)
Triglyceride	187.08±78.1	183.4±77.8	0.157	0.876(NS)
Zinc	156.2±56.1	187.05±70.51	1.684	0.099(NS)
Pota	3.63±1.79	3.92±2.11	0.515	0.609(NS)
Iron	83.92±17.38	87.7±15.07	0.778	0.440(NS)

S: Significant differences (P<0.05); NS: No significant differences (P>0.05)

## Discussion:

*E. histolytica* is so far considered as one the main health problem and it is the main reason to amoebiasis. Symptoms of this disease consists of several diagnostic sings, namely, fever, dysentery or diarrhea, dehydration and abdominal pain (15).

Amoebic liver abscess (ALA) is second of enteric infection in human, ALA is generally mentioned an single, major and distinct lesion, as well as, hepatomegaly occur in mostly peoples with ALA (15). The current study, show results in parameters of ALT, AST, and ALP where ALT showed highly significant different (P<0.01) when gave positive results in patients. AST showed

highly significant different (P<0.01) when gave positive results in patients, while ALP were showed no significant difference (P>0.05) in patients. The results were showed a significant difference in AST concentration (P<0.01) in patients infected with *E. histolytica* compared with control, the AST enzyme was high in total patients (22.84+/-7.83) when compared with healthy controls (55.36+/-16.8).

Cytotoxicity can occur due to secretion of amoebic molecules that cause toxic effects at a distance, even if there is no close contact between the stimuli and liver cells. Increased apoptotic cells were observed with

incubation time with the presence of weigh nuclei and / or nuclear fragmentation, which are important characteristics of apoptosis. There was a gradual increased in the programmed cells in the infected tissue section during the incubation period, which increased in number with time. Amoebic stimulates apoptosis of hepatocytes, and it's showed that the number increased during infection progresses. The development of ALA causes severe destruction of liver tissue (16).

These enzymes are mostly included during hepatic cells with lower concentration in the muscle cells. When the liver is infected or damaged, the liver cells secrete their enzymes into the blood, leading to increasing the aspartate aminotransferase (AST) and alanine aminotransferase (ALT) enzymes in blood concentrations and observation of hepatic disease, while ALP present at the bile ducts of the liver, enteric and the bone. Damage or barrier from the bile ducts can produce high concentrations of ALP. Analytical capabilities provide host observation a rate from disease produces (17).

Similar results identical the detecting by (18) which very high concentrations of liver enzymes in the serum from patients infected with *E. histolytica*.

*E. histolytica* can be grow in high-fat media in absence of serum, the relationship of cholesterol concentrations in serum from human with *E. histolytica* have dragged notice of the studies (13). Result of lipid profile as cholesterol are showed highly significant difference ( $P < 0.01$ ) when gave

positive in patients, triglyceride showed no significant difference ( $P > 0.05$ ) in patients with amoebiasis.

The results of cholesterol were showed no significant differences ( $P > 0.05$ ) in patients infected with amoebic dysentery compared with healthy controls, where cholesterol showed (185.8 $\pm$ 77.2) in patients compared with controls (160.19 $\pm$ 51.3). As well as, tri-glyceride results were showed a significant difference ( $p < 0.05$ ) in patients infected with *E. histolytica* recorded (166.72 $\pm$ 62.4) in patients infected compared with control where recorded (135.43 $\pm$ 45.2).

cholesterol showed little absorption in the concentration from lipid during *E. histolytica* infection. These results were agreed with (13) when indicates parasite utilize lipid for grow inside the host and recorded no significant difference in the concentration cholesterol and recorded significant difference in triglyceride in amoebiasis patients. *E. histolytica* obtain lipid and cholesterol from the upper part from intestine and also the mechanisms direct by *E. histolytica* for work are indistinct and numbers indicates has been showed a change in the lipid profile in most patients with *E. histolytica* especially in them inflammations (19), because cholesterol is used by the parasite due to inability of *E. histolytica* synthesis by itself (13) Triglyceride concentration showed significant differences in the same patient because depend on cholesterol concentration, this results of this study agreed (19, 20).

The results showed that, there was no significant difference in the concentration of zinc between the patients group ( $p>0.05$ ) (108.09 $\pm$ 24.4) and control group (101.76 $\pm$ 24.4). Results were agreed (21), which showed that the significant low concentration zinc during the infection with *E. histolytica*. This may be due to the impact of diarrhea that lead to low concentration in minerals in the patient (22). While potassium concentration showed no significant difference ( $p>0.05$ ) in infected patients, (3.73 $\pm$ 1.89) when compared with healthy controls (3.84 $\pm$ 1.14). This determined for ex-amine potassium channels such as relation mediators from host cell killing by *E. histolytica* the present which barrier of potassium evasion inhibited *E. histolytica* cytotoxicity. The action of human potassium ducts shows and decreased intercellular potassium concentration in host cell when together increased extracellular potassium concentration, this refer to direct action parasite of potassium evasion, following its present which *E. histolytica* produce potassium has been needed to inflammation action and cell death in human. The importance from potassium ion carry to *E. histolytica* cytotoxicity with inflammation is appropriate with diarrhea symptoms from amoebic colitis (20, 21).

In the current study, the concentration of iron was showed no significant difference ( $p>0.05$ ) in patients infected with *E. histolytica* recording (85.24 $\pm$ 16.58) compared with health control group recorded (81.22 $\pm$ 15.5).

The human iron stored mainly in the liver (23). In patients with liver abscesses, liver

iron shows no more. The concentration of binding iron in the liver plays an important role in the disease development. This research Provides protection iron hypothesis is the stripe research (23). Compared Masaai tribe in Africa, it shows that nomads use low-iron milk and *E. histolytica* are lower. The iron between them indicates the nomads. To be precise, these iron deficiencies significantly increase the risk of amoeba dysentery (24, 25).

### conflict of Interest

The author(s) declared that there is no conflict of interest.

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دراسة كيموحيوية لمرضى الزحار الاميبي في محافظة ذي قار / جنوب العراق

فواز عبد الله صبيح وامل خضير خلف

فرع الاحياء المجهرية / كلية الطب / جامعة ذي قار

## الخلاصة

يعتبر طفيلي الإنتاميبيا مسببا مرضيا للزحار الاميبي. وهو مرض معوي يصيب القولون والكبد وينتشر في معظم أنحاء العالم. يتسبب طفيلي الإنتاميبيا في موت الخلايا موديا الى حدوث نخر وتقرح في الأمعاء ومن ثم خراج في الكبد او ما يسمى بخراج الكبد الاميبي. صممت الدراسة الحالية لمتابعة تأثير طفيلي الزحار الاميبي على وظائف الكبد من خلال اختبار بعض المعايير الكيميوحيوية للمرضى المصابين بالزحار الاميبي. جمعت عينات الدم من مرضى الزحار الاميبي وبعد فحص ١٠٠ عينه من براز المرضى بطريقة الفحص المجهرى المباشر وتقنية تفاعل البلمرة المتسلسل للتأكد من وجود الاصابه ومن ثم اجراء الاختبارات الكيميوحيوية مثل اختبارات وظائف الكبد ونسبة الدهون اضافة الى بعض العناصر النزهره في أمصال المرضى المصابين بالزحار الاميبي. اظهرت نتائج الدراسة الحالية فروقا معنويه للاختبارات الخاصة بوظائف الكبد مثل AST و ALT عند مستوى احتماليه ( $P < 0.01$ ) في حين لم تكن الفروق معنويه مع اختبار ALP حيث كان مستوى الأنزيم  $351.7 \pm$  في مصلى المريض مقارنة ( $204.08 \pm 13.336$ ) لدى مجموعة السيطرة. كما واطهر مستوى الدهون الثلاثية في مصلى مرضى الزحار الاميبي فروقا معنوية حيث كان ( $62.4 \pm 166.72$ ) بين المرضى مقارنة بمجموعة السيطرة ( $135 \pm 43$ ). ولم تظهر نتائج الكوليسترول فروقا معنويه بين المرضى والاصحاء قيد الدراسة. كما لم تظهر مستويات العناصر النزهره في مصول المرضى المصابين بالزحار الاميبي لكل من مستوى الحديد والزنك واليوتاسيوم. **الكلمات المفتاحية: الأميبا الحالة للنسيج، الزحار الاميبي، وظائف الكبد، المعايير الكيميوحيوية.**