



THE ACTIVITY OF LIPASE, ALKALINE PHOSPHATASE AND LACTATE DEHYDROGENASE IN PATIENTS WITH *ENTAMOEBAHISTOLYTICA*

*Hwaida SH. AL-Mahdawy, *Dr.FarhanAboodRisan,**Dr. Khalil I. Abd Mohammed

* College of Health and Medical Technology/ Middle Technical University

**Clinical Communicable Diseases Research Unit/ College of Medicine / Baghdad University

ABSTRACT

The study was carried out during the period from the beginning of (November / 2013 - November / 2014) for detection of Entamoebahistolytica in patients with age range from (3-60) year who attended to AL-Yarmouk teaching hospital and AL-Tifil central hospital. The diagnosis done by microscopic examination. A total of 200 suspected patient there was 120 infected with the parasite diagnosed by the direct examination method, a blood sample was taken from each one, as well as (60) healthy controls were involved in the study, which included: Lipase, Alkaline phosphatase and Lactate dehydrogenase activity measurement by spectrophotometric method. The results indicated: The prevalence of Entamoebahistolytica by using microscopic examination was 145 (72.5%). The level of Lipase, ALP and LDH increased significantly ($P < 0.05$) in patients sera in comparison with healthy control, but there is no-significant ($P > 0.05$) differences between the gender in both groups.

Key words: *Entamoebahistolytica* ,Lipase , ALP and LDH

Introduction:

Entamoebahistolytica is a protozoan parasite that causes amoebic dysentery and liver abscess. The disease is still one of the major health problems and predominantly affects individuals of lower socioeconomic status who live in developing countries [1, 2, 3]. Infections can be intestinal, extra intestinal, or both. Most cases are intestinal and asymptomatic. Symptoms, when

occur, are multiple and varied, ranging from mild abdominal discomfort and diarrhea (often with blood and mucous) alternating with periods of remission or constipation, to severe illness with fever, chills, and significant bloody or mucoid diarrhea ("amoebic dysentery"). Amoebic colitis may be confused with inflammatory bowel disease such as ulcerative colitis [4]. A lipase is an enzyme that catalyzes the hydrolysis of fats lipids[5]. Lipases are a subclass of the esterases.

Lipases perform essential roles in the digestion, transport and processing of dietary lipids (e.g. triglycerides, fats, oils) in most, if not all, living organisms [6].

Most lipases act at a specific position on the glycerol backbone of lipid substrate (A1, A2 or A3) (small intestine). For example, human pancreatic lipase (HPL) [7], which is the main enzyme that breaks down dietary fats in the human digestive system, converts triglyceridesubstrates found in ingested oils to mono glycerides and two fatty acids [8]. Some lipases are expressed and secreted by pathogenic organisms during the infection. In particular, *Candida albicans* has a large number of different lipases, possibly reflecting broad lipolytic activity, which may contribute to the persistence and virulence of *C. albicans* in human tissue [9]. Lactate dehydrogenase is of medical significance because it is found extensively in body tissues, such as blood cells and heart muscle. Because it is released during tissue damage, it is a marker of common injuries and disease.

A dehydrogenase is an enzyme that transfers a hydride from one molecule to another. Lactate dehydrogenase catalyzes the conversion of pyruvate to lactate and back, as it converts NADH to NAD⁺ and back [10]. Alkaline phosphatase is a hydrolase enzyme responsible for removing phosphate groups from many types of molecules, including nucleotides, proteins, and alkaloids. The process of removing the phosphate group is called dephosphorylation. As the name suggests, alkaline phosphatases are most effective in an alkaline environment. It is sometimes used synonymously as basic phosphatase [11].

Aims of the study:

To evaluate the effect of *Entamoeba histolytica* infection on the level of lipase, Alkaline phosphatase and Lactate dehydrogenase.

Materials & Methods

Studied groups

The study carried out during the period from (November 2013- November 2014), the age of patients extended from (3 – 60) years, two studied groups were involved:

- Suspected patients: Blood and stool samples were obtained from a total of 200 patients clinically suspected with amoebic dysentery that had been examined and defined as suspected cases by specialized physician; the samples were collected from (Al-Yarmouk teaching hospital & Al-Tifil central hospital) in Baghdad.
- Healthy Control: Blood and stool samples from a total 60 healthy control group were involved from Al-Yarmouk teaching hospital staff and from different places in Baghdad; they were examined and defined as healthy, with no history of amoebic dysentery.

Samples collection

Stool sample from each patient was collected in a clean, dry tight cover container and examined with a half an hour. The samples were examined for the presence of *E. histolytica*.

Stool sample examination

Macroscopic examination

It was performed by observing the consistency of stool, presence of blood, mucous and other substances.

Microscopic examination

For each stool sample, wet mount preparation slide was examined by clean, dry slides by obtaining one drop of normal saline and small amount of stool from different places of stool by using clean wooden stick, especially when blood or mucous were noticed, then mixed gently with normal saline and covered with cover slip, the slide was examined under the low (10x) and high power (40x) of microscope. [12].

Blood samples

Five mL of Venous blood was obtained from each patient and collected in sterilized screw cap plastic tube, blood samples were left for 30 min. at room temperature, then centrifuge at 3000 rpm for five minute, then the serum for each sample was collected in Eppendorf tubes and stored in deep freeze at -20°C until the time for using. The current study included some Immunological aspects:

One hundred twenty clinical patients of *E.histolytica* and (60) healthy control involved in the study. The level of lipase, LDH and ALP which examined by spectrophotometric method according to [13,13, 14] respectively.

Statistical analysis: The statistical Analysis (T – test) was used to compare between means in studied groups according to [15].

Results & Discussion

Alkaline Phosphatase activity

The level of ALP increased significantly ($P < 0.05$) in patients sera with *E.histolytica* in comparison with healthy control. While the results showed no-significant difference ($P > 0.05$) between the genders in both groups. The level was (196.39 ± 0.24), (197.05 ± 0.25) in males and females in patients groups respectively. In comparison with (112.58 ± 0.88), (113.96 ± 0.50) in males and females of healthy control groups respectively. Table (1).

These results are agree with another study [16] which illustrated that ALP enzyme elevated in liver diseases, the blood concentration rises when excretion of this enzyme as a result to obstruction of the biliary tract. A study done by [17] which demonstrated that the elevated ALP level as a result to the infection with amoebiasis. These results are expressed by the affecting of infection with *E.histolytica* of ALP in the serum as a result to damage of liver cells and other cells and released of the enzymes to blood stream.

Table (1): Alkaline phosphatase activity (IU /mL) in patients with *E.histolytica* and healthy control

Group	Mean \pm S.D Gender	
	Males	Females
Patients	196.39 \pm 0.24	197.05 \pm 0.25
Control	112.58 \pm 0.88	113.96 \pm 0.50
T-test value	1.45 *	1.012 *

* (P<0.05)

Lactate dehydrogenase activity

The activity of Lactate dehydrogenase increase significantly (P<0.05) in patients sera with *E.histolytica* in comparison to healthy control .While the results show no-significant difference (P>0.05) between gender in both groups. The level was (245.09 \pm 1.24), (248.22 \pm 0.32) in males and females of patients group respectively, in comparison with (167.44 \pm 0.14), (167.09 \pm 0.24) in males and females of healthy control groups respectively. Table (2).

The present study is in agree with another study done by [16] which illustrated that LDH enzyme level was increased as an indicator to cellular death and leakage of the enzyme from the cells.

High levels of LDH indicate some form of tissue damage. High levels of more than one isoenzyme may indicate more than one cause of tissue damage, for example, a patient with pneumonia could also have a heart attack. When levels of all five LDH enzymes are high, it could indicate multiple organ failure, because LDH is found in so many tissues throughout the body, complete LDH levels alone will not be enough to determine the location and cause of your tissue damage, also the levels of LDH isoenzymes will also need to be measured in order to reach a diagnosis. For example, high LDH-4 and LDH-5 may mean either liver damage or muscle damage, but liver disease cannot be confirmed without a full liver panel [18].

Table (2): Lactate dehydrogenase activity (IU /mL) in patients with *E.histolytica* and healthy control

Group	Mean ± S.D Gender	
	Male	Female
Patients	245.09 ± 1.24	248.22 ± 0.32
Control	167.44 ± 0.14	167.09 ± 0.24
T-test value	3.30 *	1.013 *

*(P<0.05)

Lipase activity

The level of lipase activity in patients with *E.histolytica* increased significantly (P<0.05) in comparison with healthy control. While the activity level of enzyme show no significant difference between the genders in both groups. The activity was (43.59 ± 0.57), (41.92 ± 0.41) in males and females of patients group respectively in comparison to (35.98 ± 0.28), (36.69 ± 0.23) in males and females of healthy control group respectively Table (3). These results are in agree with another studies done by [19] which illustrated that lipase concentration in the serum may be increased in pancreatitis disease, hepatitis, intestinal obstruction. Also the results is disagree with other study [16] who proved that serum level of lipase is normal in patients with elevated amylase, who have inflammation bowel disease, intestinal obstruction.

Table (3): Lipase activity (IU/L) in patients with *E.histolytica* and healthy control

Group	Mean ± S.D Gender	
	Males	Females
Patients	43.59 ± 0.57	41.92 ± 0.41
Control	35.98 ± 0.28	36.69 ± 0.23
T-test value	1.58 *	1.30 *

*(P<0.05)

References:

1. Simonetta, G.; Giovanni, S.; Francico, R. and Mariella, A. (2002). Amebic infections due to the *Entamoebahistolytica* – *Entamoebadispar* complex. A study of the incidence in a remote rural area of Ecuador. *Am. J. Trop. Med. Hyg.* 67: 123-127.
2. ElBakri, A.; Samie, A.; Ezzedine, S. and Abu Odeh, R. (2013). Differential detection of *Entamoebahistolytica*, *Entamoebadispar* and *Entamoebamoshkovskii* in fecal samples by nested PCR in the United Arab Emirates (UAE). *J. Sprig. Intern. Pub.* 58(2):185-190.
3. Zahida, T.; Mushtaq, H.; Lashari, A. and Fariha, A. (2013). Institute of Pure and Applied Biology, BahauddinZakariya University, Multan. *J. Cell Anim. Biol.* 7 (6):73-76.
4. Guide to Surveillance, Reporting and control (2006). Amoebiasis. Massachusetts Department of public Health, Bureau of Communicable disease Control. Pp 29-35.
5. Svendsen, A. (2000). Lipase protein engineering. *BiochimBiophysActa* 1543 (2): 223–228.
6. Girod, A.; Wobus, C.; Zádori, Z.; Ried, M.; Leike, K.; Tijssen, P.; Kleinschmidt, J. and Hallek, M. (2002). The VP1 capsid protein of adeno-associated virus type 2 is carrying a phospholipase A2 domain required for virus infectivity. *J. Gen. Virol.* 83 (5): 973–8.
7. Winkler, F. K.; Darcy, A. and Hunziker, W. (1990). Structure of human pancreatic lipase. *Nature* 343 (6260): 771–774.
8. Diaz, B.L. and Arm, J. p. (2003). Phospholipase A. (2). Prostaglandins LeukotrensEssent. *Fatty Acids* 2(3): 87–97.
9. Hube, B.; Stehr, F.; Bossenz, M.; Mazur, A.; Kretschmar, M. and Schafer, W. (2000). Secreted lipases of *Candida albicans*: cloning, characterisation and expression analysis of a new gene family with at least ten members. *Arch. Microbiol.* 174 (5): 362–374.
10. Madern, D. (2002). Molecular evolution within the L-malate and L-lactate dehydrogenase super-family . *J. Mol. Evol.* 54 (6): 825–40.
11. Tamás, L.; Huttová, J.; Mistrk, I. and Kogan, G. (2002). Effect of Carboxymethyl Chitin-Glucan on the Activity of Some Hydrolytic Enzymes in Maize Plants (PDF). *Chem. Pap.* 56(5): 326–329.
12. Frances, F. and Marshall B. D. (2009). *A Manual of Laboratory and Diagnostic Tests.* Lippincott William & Wilkins. 8th ed. 286-290.
13. Klin, Z. (1974). Lactate Dehydrogenase liqui UV test. *Chem. Biochem.* 8: 658.

14. Schlebusch, H. (1974). Alkaline Phosphatase Liquicolor. Dtsch.med. Wschr. 99, 765.
15. SAS. ,(2012). Statistical Analysis System, User's Guide. Statistical. Ver. 9.1thed. SAS. Inst. Inc. Cary. N.C.USA.
16. Frances, F. and Marshall B. D. (2015). A Manual of Laboratory and Diagnostic Tests. Lippincott William & Wilkins.9th ed. 405-420.
17. Helmy M.; Rashed L. and Abdel-Fattah H.(2007). Detection and differentiation of *Entamoebahistolytica* and *Entamoebadispar* isolates in clinical samples by PCR. J. Egypt Soc. Parasitol. 2007. 37(1):257-74.
18. Ferri, F. F.; Updated by: Todd Gersten, M. D. (2014). Lactate dehydrogenase. Hematology/Oncology, Florida Cancer Specialists & Research Institute, Wellington, FL. Review provided by VeriMed Healthcare Network. Also reviewed by David Zieve, MD, MHA, Isla Ogilvie, PhD, and the A.D.A.M. Editorial team.U.S. National Library of Medicin_
19. Tenner, S.; Baillie, J.; Dewitt, J. and Vege, S. S.(2013). American College of Gastroenterology. American College of Gastroenterology guideline: management of acute pancreatitis. Am. J. Gastro.; 108:1400-15.