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Biochemical changes of liver that infected with *Entamoeba histolytica* in white rats

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KEYWORDS

Entamoeba histolytica, Biochemical changes, Apoptosis

A B S T R A C T

This study was carried out in the animal house of the Science College, Babylon University for the period of October 2012–February 2013. This study included 20 white Swiss males *Rattus rattus* aged between 3-4 months, and weight between 250–300 gm. Animals were divided into five groups (2, 4, 8, 12 weeks) and the fifth group considered as control group and each group included (four males). Four groups were infected with suspension of *E. histolytica* that bring from stools of patients attending to the Al-Hilla Hospital laboratory, every rat gives about 10^3 cysts per mililiter of *E. histolytica* through oral administration. And the fifth group treated orally with distilled water. The rats liver were studied functionally by measuring the level of enzymes GOT, GPT and ALP. The tests of liver enzymes, showed that the level of the enzyme GOT, GPT and ALP having significant increase ($P < 0.05$) in the male rats treated with *E. histolytica* suspension compared to control group. The infection with *E. histolytica* established histological changes in the organs especially the liver as apoptosis death of cells as well as changes in biochemical parameters (ALP, GOT, GPT).

Introduction

Amoebiasis caused by *Entamoeba histolytica* and is the second cause of global morbidity and mortality due to parasitic diseases in humans. It causes more than 100,000 deaths each year and is responsible for 50 million cases of diarrhea each year (Huston, 2004; WHO, 2009).

Infected persons display a wide range of disease severity, reflecting the contribution of the patient's immune and nutritional status (Al-Kubaissi, Abdul – Wahab Badawi Hussien, 2002). *E. histolytica* inhabits the large intestine; it is acquired when infective cysts are ingested through contaminated

food or water. Excystation releases trophozoites into the terminal ileum and from there parasites migrate to the colon where they colonize (Mortimer and Chadee, 2010). Some parasites undergo encystment in the descending colon, resulting in passage of mature infective cysts in the stool and perpetuation of the life cycle through fecal-oral spread. In 90% of cases amoebic infections are asymptomatic and self-limiting (Haque *et al.,* 2003). But approximately in 1% of the cases, trophozoites penetrate the intestinal mucosa and spread to other organs, producing extra-intestinal amoebiasis, among which amoebic liver abscess (ALA) is the most common (Bernal – Redondo, 2001).

E. histolytica induces apoptosis, both, in human cells and during the development of ALA in hamsters and mice (Boettner *et al.,* 2008). It has also been found that the death of hepatocytes and immune cells during amoebic invasion is not only due to the cytolysis activity of the trophozoites, but also because of an apoptotic process (Pelosof *et al.,* 2006). Abscesses located just below the diaphragm can lead to pleural pain or referred right shoulder pain. Liver alkaline phosphatase levels (ALP) and alanine aminotransferase levels (GOT, GPT) are elevated in acute liver abscess, which may, however, reverse over time. Males are ten times more likely to present with liver abscess than females and middle-age and young adults more than children (Eichinger, 2009)

Aminotransferase levels are sensitive indicators of liver-cell injury and are helpful in recognizing hepatocellular diseases such as liver abscess, levels of this enzyme are accordingly more specific indicators of liver injury. Both enzymes are released into the blood in increasing amounts when the liver cell membrane is damaged. Necrosis of liver cells is required for the release of the aminotransferases (Dufour, 2001). The aim of this study is to determine the level of liver enzymes (alkaline phosphatase levels (ALP) and alanine aminotransferase levels (AST, ALT) and its relationship with *E. histolytica* infection in rats.

Materials and Methods

Isolates of *E. histolytica* were obtained from some patients in Babylon Hospital in Hilla city. Samples were collected in the period lies between October 2012 and February 2013, stool patients selected for this study were usually suffering from diarrhea, vomiting, dysentery, abdominal pain. The stools were contained blood and mucus. Then kept in sterile plastic container and this stool samples were examined in advanced parasitology lab of Science College for women by direct smear methods.

A drop of Lugol□s iodine was added and mixed with small piece of feces and examined under compound microscope to diagnose the cysts and trophozoite of *E. histolytica.* Normal saline was added to the stool in the proportion of 1:1 and one milliliter of suspension was used that contains about 10^3 cysts and used to infect 20 male rats (divided into five groups 2 weeks, 4 weeks, 8 weeks, 12 weeks and the fifth group consider as control group. Each group included four males) by oral administration, and their weight range was 250–300 gm.

Chloroform was used for anesthesia and the abdominal cavity was opened until the sternum using medical scissors and blood directly from the heart in a way of heart puncture was drawn using a sterile 5 ml syringe. The blood was collected in the jell tube containing gelatinous material, helps to

increase serum formation after centrifugation. Left the samples for 30 minutes at room temperature and then centrifuged at 3000 rpm /10 minutes for the purpose of separation of serum. Serum was taken to measure the level of liver enzymes GOT, GPT and ALP (Bruchhaus *et al.*, 2003). The numbers of apoptosis cells in liver tissue infected with amoebic dysentery was estimated according to the following equation following Carranza-Rosales *et al.* (2012).

$$\text{Percentage} = \frac{\text{Number of dead cells}}{\text{Number of living cells}} \times 100$$

Result and Discussion

Result reveals a significant differences ($p< 0.05$) in liver enzymes (GOT, GPT, ALP) compared with control group. The GOT enzyme tested was high in all infected animals (all groups) when compared with control group. In spite of the first group (2 weeks), non significant differences ($p> 0.05$) found when compared with second group (4 weeks) and third group (8 weeks) as well as fourth group (12 weeks). But the first and second groups when compared with third and fourth groups reveals significant differences ($p<0.05$) as shown in Table 1. The result of GPT enzyme increased significantly in the second, third and fourth groups when compared with first group as well as compared with fourth groups with others. The ALP enzyme reveals high significant differences in the second, third and fourth when compared with control and first groups, in spite of the differences not reach to significance among all groups as well as the first group when compared with control group.

The histogram (Fig.1) revealed apoptotic index in rats cells infected with *E. histolytica*. The number of apoptotic cells increased directly proportional to the time of infection. Apoptotic cells were recognized to exhibit morphological characteristics, such as nuclear pyknosis and chromatin fragmentation, with cytoplasmic extrusion.

The histogram (Fig. 2) exist positive relationship between number of apoptotic cells and mean levels of enzyme GOT (I.U/L). The greater levels of enzyme GOT increases the numbers of damaged cells by the process of apoptosis.

The histogram of Figure 3 exist positive relationship between number of apoptotic cells and mean levels of enzyme GPT (I.U/L). The greater level of enzyme GPT increased the number of damaged cells by Apoptosis.

The histogram of Figure 4 exist positive relationship between number of apoptotic cells and mean levels of enzyme ALP (I.U/L). The greater level of enzyme ALP increased the number of damaged cells by Apoptosis.

Amebiasis is a cosmopolitan disease of high prevalence in Africa, Middle east Asia, India, South America and Mexico. Although these countries have improved their living conditions and level of sanitation, the disease is still a major public health problem (Cenavece, 2010). Following exposure, *E. histolytica* inhabits the large intestine, where it causes intestinal amebiasis. However, in approximately 1% of cases, trophozoites disrupt the intestinal mucosa and spread to other organs, causing various forms of extra intestinal amebiasis, of these, amebic liver abscess (ALA) is the most common (Stanley, 2003).

The pathogenesis of ALA is very complex and involves host and parasite factors, as

well as micro-environmental conditions (Carranza-Rosales *et al.,* 2012). The ability of amoebae to destroy host tissue and survive in the liver is accompanied by a strong adaptive response and regulation of proteins, such as amebic virulence factors (Bruchhaus *et al.,* 2002). The most studied virulence factors of *E. histolytica* are the adhesion molecule Gal/Gal NAC lectin (Gilchrist and Petri, 1999), cysteine proteinases (Bruchhaus *et al.,* 2002), amoebapore protein (Leippe *et al.,* 2005), and lipophosphoglycan molecules (Moody-Haupt *et al.,* 2000). Because of its important role in the pathogenesis of ALA in humans and in animals, cysteine proteinases from *E. histolytica* have the property to degrade collagen, fibrinogen, elastin and laminin, extracellular matrix elements that trophozoites have to break through in order to cause invasive disease (Carranza-Rosales *et al.,* 2012). These proteins are involved in the disruption of cellular monolayers (Lauwaet *et al.,* 2004). Its inhibition with antisense codons decreases amebic phagocytosis, inflammation of the intestine, and the formation of ALA. It has also been proposed that cysteine proteinases (CP) contribute to create the anaerobic environment that trophozoites require to grow *in vivo* during ALA development (Pérez –Tamayo *et al.,* 2006)

Amoebapore proteins from *E. histolytica* are also involved in the formation of ALA (Zhang *et al.,* 2004). They cause lysis of bacteria and eukaryotic cells (Leippe *et al.,* 2005). Its cytolytic capacity and participation in apoptosis and necrosis induction has been demonstrated *in vivo* (Andra *et al.,* 2003). According to Tsutsumi *et al.* (1984), amoebic liver abscess formation after intraportal inoculation of virulent trophozoites of *E. histolytica* in hamster involves three consecutive phases. Stanley (2003) showed that diffusion of amoebic molecules occurs to the endothelium and hepatocytes located further away die by necrosis.

These authors suggest that cytotoxicity can occur due to the secretion of amoebic molecules that can cause toxic effects at a distance, even when there is not close contact between the trophozoites and hepatocytes were observed. In this study apoptotic cells, whose number was increasing with the time of incubation, were observed with the presence of pyknotic nuclei, and/or nuclear fragmentation, which are the important characteristics of apoptotic cell death. A gradual increase of apoptotic cells in the infected slices happened while incubation time increased. ALA development causes severe destruction of the liver tissue. This is consistent with what was said by Carranza-Rosales *et al.* (2012), amoeba induces the programmed death of hepatic cells and noted that it was increased in number with the progress of infected time.

Table 1 reveals the levels of liver enzymes increased directly proportional to the time of incubation, it was also noted high levels of these enzymes in the serum increased the number of damaged cells by apoptosis, probably due to these enzymes mostly reside within the cells of the liver. But when the liver is injured for any reason, these enzymes are spilled into the blood stream.

These enzymes are predominantly contained within liver cells and to a lesser degree in the muscle cells. If the liver is injured or damaged, the liver cells spill these enzymes into the blood, raising the aspartate aminotransferase (AST) and alanine aminotransferase (ALT) enzymes in blood levels and signaling liver disease, while ALP is a substance found in the bile ducts of the liver, intestine and the bone.

Table.1 Effect of infected male rats with *E. histolytica* according to different time periods on mean levels liver enzymes GOT,GPT, ALP (I.U/L)

ENZYMES / GROUPS	MEAN (AST) GOT (I.U/L) SE±	MEAN ()GPTALT (I.U/L) SE±	MEAN ALP (I.U/L) SE±
Control	[a]58.48 ± 2.88	[a]60.15 ± 3.13	[a] 440.50 ± 15.87
Group 1 (2 weeks)	[b] 69.33 ± 3.29	[a] 57.45 ± 2.82	[a] 448.25 ± 19.18
Group 2 (4 weeks)	[b]77.00 ± 2.42	[b]74.10 ± 3.73	[b] 538.25 ± 20.45
Grroup 3 (8 weeks)	[c]91.50 ± 4.92	[c]90.05 ± 4.50	[b] 550.0 ± 18.17
Group 4 12 week)(	[c]98.25 ± 3.94	[d]104.00 ± 4.02	[b] 566.50 ± 19.75

Mean ± SE different index on significant character ($p<0.05$),Number animals in all group,n = 4

Fig.1 Relationship between different time periods (weeks) and mean numbers apoptotic cells after infected with *E. histolytica*

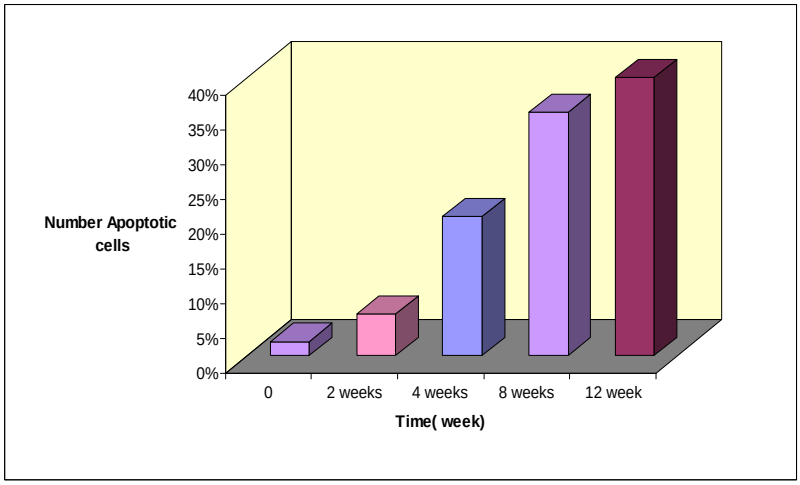


Fig.2 Relationship between number apoptotic cells and mean level of enzyme GOT (I.U/L) after infected with *E. histolytica*

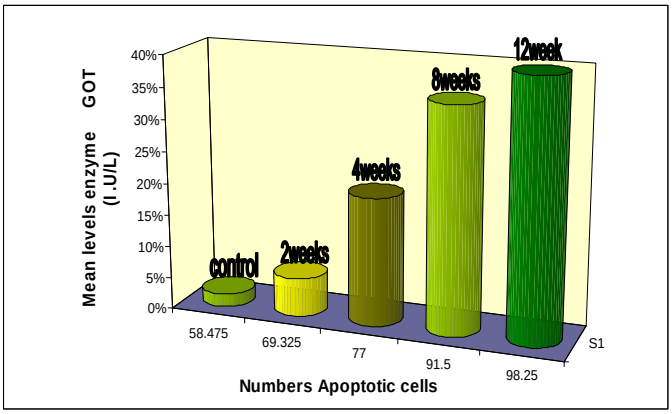


Fig. 3 Relationship between number apoptotic cells and mean level of enzyme GPT (I.U/L) after infected with *E. histolytica*

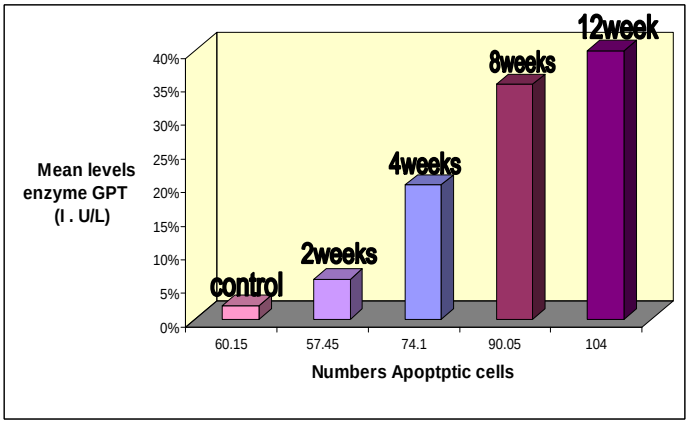
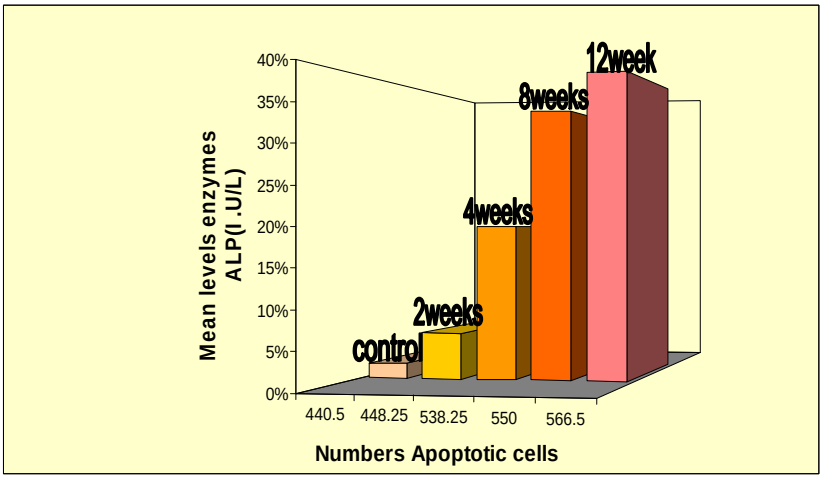


Fig.4 Relationship between number Apoptotic cells and mean level of enzyme ALP (I.U/L) after infected with *E. histolytica*



Damage or obstruction of the bile ducts may result in elevated levels of ALP. These tests can provide a host of information on a range of disease processes (Dufour, 2001).

This is consistent with what was found by Al-Kubaissi, Abdul – Wahab Badawi Hussien (2002). He noted a high level of concentration of the enzyme ALP that reached 90% of the cases with a high level of enzyme GOT, GPT in the serum of patients infected with dysentery. This result matched the findings of the Pluta and Pluta (2002) as the very high levels of liver enzymes in the serum of patients infected with the parasite, as well as demonstrated (Al - Ghanimi, FatimaYusufKtan, 2013) for an increase in the levels of liver enzymes in mice infected with parasite *Giardia lamblia*.

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