Original Article

The effect of infection with the Entamoeba histolytica on oxidative stress status in Kirkuk hospital patients

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Abstract

Entamoeba histolytica is one of the principal intestinal pathogenic parasitic protozoans that cause health issues globally. The purpose of this study is to evaluate how the oxidative stress indicators glutathione (GSH), malondialdehyde (MDA), catalase(CAT), and superoxide dismutase (SOD) are affected by E. histolytica infection. This study, which involved 476 patients of all ages and genders, was conducted between September 2020 and the end of August 2021 to look in to the prevalence of E. histolytica among those who had diarrhea, and tow diagnostic methods were used, including the direct smear method and ELISA technology to detect the parasite in stool samples. 20 blood samples were collected from those infected with the parasite of both sexes, 20 samples from patients suffering from diarrhea symptoms, in addition to 20 samples from healthy individuals as a control group. The results showed that the total percentage of infection with E. histolytica reached to 29.6%. and showed significantly increased the concentration of malondialdehyde ($P<0.01$) compared to the diarrhea-infected group and the healthy group and the decreased in the activity of glutathione concentration and the activity of catalase enzymes and superoxide dismutase in the infected with the parasite significantly ($p<0.01$) compared to the diarrhea-infected group and the healthy group.

It is concluded from the results of the current study that infection by E. histolytica induced oxidative stress status of the infected people, which may have different health consequences.

Keywords: E. histolytica, Diarrhea, ELISA, Malondialdehyde, Antooxidants.

INTRODUCTION

Amoebic dysentery is caused by the parasite protozoan Entamoeba histolytica and affects over 90 million individuals annually worldwide (Wesel et al.,2021(. This parasite causes amoebiasis which can also be asymptomatic or can cause two serious infections (Shirley and Moonah.,2016), The life cycle of this parasite goes through two stages. It has two stages the vegetative phase or the so-called trophozoite stage and infected cyst stage . By ingesting these infected cysts, humans gets infected , which travel to the small intestine (terminal ileum) through the lumen of the gut, where each excysts to generate eight daughter trophozoites. The intestinal epithelial cells that line the gastrointestinal tract are adhered to and invaded by the motile trophozoites. Trophozoites move by pulling themselves along with creeping cytoplasmic extensions known as pseudopodia. (John and Petri .,2006).

One of the most important feature that is distinctive for its type is their ability to infect and Lysis tissues and hence the name was derived. In most of the cases the infection with remain in the mucosal layer of the gut will minute damage to the mucus membrane with mild to asymptomatic symptoms (Pritt and Clark.,2008). However, in some cases the patient may remain carrier with no symptoms in such cases the infection is considered noninvasive and the patient could shed cystic form in their stool leading to increase the rate of environmental contamination and assume to the elevation of the spread on the disease (John and petri ., 2006).

Various studies have shown that parasite infections in both humans and animals can cause oxidative stress. (Chandramathi et al.2010), All biomolecules (polynucleotides, proteins, lipids, and carbohydrates) can be damaged by oxidative stress, which can seriously impair biological processes and cause cell death (Gulec peker et al.,2018 ; Marian et al.,2004), However, persistent

oxidative stress may result in diseases including diabetes, cardiovascular disease, and cancer. Overproduction of Reactive oxygen and Nitrogen species (ROS,NOS) can cause oxidative stress. Environmental factors and cellular metabolic activities both produce ROS (Patlevic et al.,2016), It has a crucial role in the beginning and development of several gastrointestinal disorders, as well as in the removal of intracellular pathogens in many infections (Carneiro et al.,2018). The purpose of this research is to determine the level of oxidative stress in the patients infected with the E.histolytica.

Materials and Methods

A total of 476 stool samples from individuals with diarrhea and other symptoms, ranging in age from less than a year to more than 51, were studied in total. 20 blood samples were collected from those infected with the parasite of both sexes, 20 samples from patients suffering from diarrhea symptoms, in addition to 20 samples from healthy individuals as a control group. Three ml of venous blood was withdrawn for all subjects in the study. After allowing to coagulate in a plain tube, the serum was separated and centrifuged at 3000 rounds per minute for 5–10 minutes to measure MDA, GSH, CAT, and SOD.

Laboratory test

1. Direct smear

A stool sample was taken using a wooden stick and placed on a glass slide with a drop of iodine stain prepared previously by Luna (1968) method, and a Coverslip was placed and examined using a microscope under x40 and x100 power magnification.

2. Enzyme–Linked Immunosorbent assay test of stool samples

Before being tested by ELISA for E. histolytica/E. dispar stool antigen, the portion of stool specimens (0.5-3 mg) was kept in sterile screw cap containers containing potassium dichromate (K2Cr2O7) at -20°C using deep freezing (Diagnostic Automation, INC. Suit , Netherlands).

Oxidative Stress Markers

The levels of MDA and GSH, as well as the CAT and SOD enzyme activity, were assessed spectrophotometrically in the blood serum utilizing the techniques of (Moron et al.,1979; Goth,1991; Rao at al, 1998 ; and.Fridovich, 1998). Respectively.

Results and Discussion

As shown in Table (1), the infection rate using the ELISA method was 29.6% while the infection rate using the direct swab method was 27.7%.

Table (1): The percentage of patients infection with E.histolytica

The total number of examined samples	positive samples				negative samples			
	Direct		Elisa		Direct		Elisa	
476	No	%	No	%	No	%	No	%
	132	27.7	141	29.6	344	72.2	335	70.3

The result agreed with Hadi (2014) in Dhi Qar who recorded 29.9% Infection rate for E. histolytica and (Kadir and Ali, 2011) which recorded a rate of 31.6% in Kalar town, Sulaimani province 31.6%. It also agrees with Delialloglu et al . (2004) in Turkey who recorded 29.5 % Infection rate for E. histolytica and with Braga et al . (2001) in Fortaleza , Northeastern Brazil , who reported 25.4 % infection rate using Entamoeba TechLab ELISA.

The results of the present study was higher than the rate recorded by Shameeran (2011) in Duhok province in which he recorded a rate of infection with pathogenic strains of E. histolytica among children in Duhok province was 15% and Ahmed (2010) n Duhok province in which he

recorded a rate of 10.68%. Hama (2007) in Erbil which he recorded a rate of 2.33%.

In other research Al-Najar (2000) estimated a higher infection incidence of 49.5% and that E. histolytica was present in 60% of cases of bloody diarrhea. AL-Harthi and Jamjoom (2007) in Saudi Arabia reported a 59.6% infection rate for E. histolytica.

The size of the study's population, the length of the study's coverage period, and the quantity of stool samples evaluated per patient are among the factors that contribute to the variation in E. histolytica infection rates.

The results of the study indicated that the concentration of malondialdehyde was significantly increased ($p<0.01$) in the parasite infected group compared to the non infected diarrheal group and the control group , and the glutathione concentration and the activity of catalase and superoxide dismutase were significantly ($p<0.01$) in the parasite-infected group compared to the non infected diarrheal group and the control group as shown in Table (2).

Table (2): Shows the effects of E. histolytica on GSH, CAT, SOD and MDA

Group	No	Mean ± SE			
		MDA	GSH	CAT	SOD
Infection Entamobia	20	2.365±0.391	0.178±0.064	0.989±0.101	0.572±0.086
Diarrhea	20	1.886±0.037	0.267±0.132	1.025±0.137	0.685±0.166
Control	20	1.386±0.093	0.467±0.135	1.669±0.081	0.841±0.075
P-value		< 0.01	< 0.01	< 0.01	< 0.01

One of the potential metabolic pathways linked to the genesis of numerous diseases is oxidative stress. The importance of oxidative stress comes from the damage to the body, and the stress belong to many causes may be hereditary or physiological or the result of microbial infections, Numerous research have shown that oxidative stress occurs in parasitic infection. They found that patients with acute and chronic fascioliasis had high levels of malonaldahyde (Kaya et al., 2007), Toxoplasma gondii (Yazar et al., 2003), and other parasites , Leishmania (Oliveira and Cechini.2002 : Kocyigit et al.,2000), Giardia lamblia and Entameba histolytica (Demirci et al.,2003). All ROS produced by physiological or pathological activities are not entirely eliminated by the antioxidant mechanisms. Then oxidative damage occurs and lipid peroxidation increased and thus led to increase of MDA levels in patients. The increased period of infection with these parasites and the accumulation of free radicals generated by It can play a major role in emptying enzymatic and non-enzymatic antioxidants inside the body, Thus, causing an imbalance in the oxidation balance and reaching oxidative damage for patients, which consequently leads to many chronic diseases such as cancer, cardiovascular disease and diabetes (Reuter et al.,2010 ; Forrester .,2018).

Glutathione is an important component of the antioxidant system inside the body and the mechanisms of intracellular protective against many internal and external indicators that lead to a state of oxidative stress, It is one of the endogenous antioxidants that works against oxidants such as some drugs, carcinogens and other pathological injuries, and the low concentration of glutathione makes the tissue more receptive to oxidation (Halliwell et al.,1994(.

In the current study, A significant reduction in the levels of serum GSH was detected in patients with E. histolytica infection. This proposes that the incidence of this parasite may be related with remarkable oxidative stress causing in the depletion of GSH due to an upturn in the quantity of free radicals (Halliwell and Gutheridge,2015). The result of GSH observed in this study agreed with two previous studies carried out on parasitic infection in humans such as T. gondii (Karaman et al,.2008) and (Kaya

et al.,2008) found that people with fascioliasis had a lower activity of glutathione peroxidase (enzymes convert H2O2 to water and alcohols by using reduced glutathione) in serum and erythrocytes compared to non-infected people.

The present study's findings indicate there is an inverse relationship between the level of MDA and the level of GSH in the serum of people with parasite, and this is consistent with other research. A number of studies have shown that there is an inverse relationship between GSH and MDA, In their study, (Ahmadvand et al., 2019) indicated that the injection of GSH into peritoneal cavity in rats with Kidney ischemia leads to a lower serum MDA level compared to non-injected rats. (Chen et al.,2015) reported a decrease in the value of MDA and a change in oxidative stress when GSH was supplied orally to obstructive jaundice rats.

The findings of the present investigation demonstrated that when evaluating the activity of superoxide dismutase and catalase enzymes in patient blood serum, there were significant differences between the study groups.

Hemorrhagic colitis can result from E.histolytica invading and destroying human colon tissue. While remaining in the human intestine, the parasite lives in an environment with low oxygen pressure. An important component of the host's innate immunological defense against microbial diseases, such as amoebiasis, is the production of reactive oxygen species (ROS) (Paiva and Bozza, 2014).

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