Original Article

The Relationship Between MDA and TAOS in Liver Infected with Hydatid Cysts

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Abstract

The aim of the study is to investigate oxidative stress in sheep infected with hydatid cyst by determining the activities of Total antioxidants (TAOS) and Malonaldehyde (MDA) and their relationship in liver tissues. Fifteen infected livers and 15 healthy livers were included in the present study. The levels of TAOS and MDA in homogenized liver tissue and in parasite tissue were measured using ELISA. The findings of the study revealed that high levels of MDA were found in infected livers compared to healthy livers (P value <0.05) and low levels of TAOS were found compared to healthy livers. Pearson correlation coefficient between MDA and TAOS was inversely negative. The study comes to a conclusion that there is a relationship between TAOS antioxidants and MDA oxidants in the parasite and their role in oxidative stress in sheep livers.

Keywords: Hydatid Cysts, Total antioxidants (TAOS) and Malonaldehyde (MDA).

1. INTRODUCTION

Hydatidosis is one of the most dangerous and common diseases due to its wide spread between humans and animals alike over the world (Mohammed, 2021). Hyditosis is a global endemic disease caused by infection with the larval stage of Echinococcus granulosus. The disease spreads to humans by ingesting egg of parasite that is discharged through dog feces (the final), and infection can result in single or multiple hydatid cysts (Jafari et al., 2019; Tamarozzi et al., 2019).

Oxidation is defined as a chemical reaction that occurs through the transfer of electrons or hydrogen from a substance to an oxidizing agent, and this process results in free radicals that in turn produce another series of free radicals, which lead to the death and damage of cells (Mustafa, 2012). The role of anti-oxidants lies in destroying free radicals and enhancing the body's immunity. They also have a role in distinguishing between normal cells and cancer cells and works to reduce infections emerging in the body (Rahil, 2017). Oxidative stress occurs, in the body, when the production of oxidants exceeds the antioxidant capacity in the body and plays a role in the pathogenesis of many chronic diseases such as diabetes, cardiovascular disease, stroke and kidney failure (Maleki et al., 2015). This known as oxidative stress (OS), which leads to a group A variety of physiological and biochemical changes that cause damage to biological molecules such as nucleic acids, proteins, lipids, etc., which consequently lead to deterioration and death of cells, when free radicals are highly reactive within cells (Rosa, 2010).

Malondehyde (MDA), on the other hand, is the final result of the process of lipid peroxidation, as it occurs spontaneously inside the body and is a by-product of this process. The activities of a number of enzymes, including NADPH and Xanthine Oxidase, as MDA is characterized by its ability to interact with phosphorylated lipids and proteins, resulting changes in its work and characteristics (Rahil, 2017). Studies have shown that measuring the concentration of MDA in blood gives an indication of body fat oxidation (Singh and Rupinder, 2014).Many studies refer that most parasitic infectin induce oxidative stress that forced with antioxidants which expose the host body to oxidative stress(Sagkan-Ozturk et al.,2015, Martínez-González et al.,2022).The aim of the study is to investigate oxidative stress in sheep infected with hydatid cyst by determining the activities of Total antioxidants (TAOS) and Malonaldehyde (MDA) and their relationship in liver tissues.

2. Materials and Methods

During the study period, 15 livers of sheep infected with hydatid cyst in addition to 15 non infected livres of sheep (aged ,1-3 years) collected from a slaughterhouse in the city of Baquba , northeast of Baghdad, Iraq. In order to make sure of the

health status and gender of animals, each slaughtered animal was examined separately by the naked eye, completely and accurately, and the carcass was examined in terms of color and texture for any abnormal changes. Then an examination for the internal organs including livers, lungs, and other internal organs was carried out by naked eye and touch to diagnose hydatid cysts. Another examination is done by the use a knife to cut when necessary for the purpose of diagnosis (Nissafi et al., 2010). The procedures recommended by the Food and Agriculture Organization- United Nations Program -The United Nations Environment- World Health Organization are followed (Al-Maliki and Ahmed, 2022). The naturally infected livers were moved to the animal laboratory, Department of Biology, College of Education for Pure Sciences - University of Diyala by plastic bags in a container containing crushed ice, where the infected organs were thoroughly washed with water for the purpose of getting rid of blood, stains and suspended materials resulting from the slaughter process. Then, the liver was placed in a steriled dish where the outer surface of the bag was sterilized with 70% ethyl alcohol (Nisafi et al., 2010).

3. Cysts Isolation

Cysts were isolated from the infected livers and then each cyst was sterilized with alcohol, their number was calculated, and the size of each cyst was determined for each isolated hydatid cysts taken by measuring the diameter of the cyst in centimeters using a tape measure. Then, they were classified in terms of: Small (1) cm, Medium (2-5) cm and Large (more than 5 cm).

4. Biopsy of the Infected Liver and of Disinfected Areas

Three biopsies (5 grams each) were taken. A biopsy was taken from different regions of the livers according to cysts positions , a biopsy close to the cyst and biopsy from a far from the cyst. and all were placed in a sample container.

Then, phosphate buffer saline (PBS) was added. The containers were locked well and placed in a cool place in the refrigerator (-20 °C) until the homogenization of the samples process is carried out. Information about each biopsy were recorded in terms of biopsy location, sample number, sample gender, and date of biopsy (Nisafi et al., 2010).

5. Homogenization of the samples

To obtain the supernatant material of biopsies taken from infected livers, The samples were crushed with PBS and homogenized with homogenizer. After that, 3 ml of the homogenized materials were added to the samples and all are put in tubes. Then, centrifugation by cold centrifuge at 3000 cycles for 30 minutes at a temperature of 4 °C. Finally, the supernatant material is taken and kept in a cool place (Noeman et al., 2011).

6. MDA and TAOS assay by ELISA

MDA ELISA Kit and TAOS ELISA Kit (Sunlong Biotech Co.,Ltd , China) were used to determine the levels of MDA and TAOS

7. Results

Regarding the effect of the parasite Echinococcus granulosus on the level of TAOS and MDA, there is significant statistical difference (P value < 0.05) between the infected animals and the control group. The study indicated higher level of TAOS in females than males (except In the liver tissue surrounding the cyst)and in infected samples compared to the control group and in samples that were isolated more than 10 cysts compared to those from which less than 10 cysts were isolated.

Table (1) The concentration of TAOS in the livers of infected animals and the control groups for males and females and its concentration in different locations of the liver

Sex	concentration rate U/ml TAOS			P value
	In the liver tissue surrounding the cyst	In the liver tissue adjacent to the cyst	In the parasite tissue	
male	0.630	0.493	0.487	0.025
Female	0.613	0.508	0.554	0.034
overall average	0.622	0.500	0.521	
Control(male)	0.428			0.021
Control(female).	0.542			0.021
overall average	0.655			
P value	0.025	0.026	0.038	

Table (2) TAOS concentration in livers containing less than and more than ten bags and the control group

NO. of cyst	U/ml TAOS Conce.rate			P value
	In the liver tissue surrounding the cyst	In the liver tissue closed to the cyst	in the parasite tissue	
Less than 10	0.502	0.487	0.438	0.039
More than 10	0.742	0.513	0.604	0.026
Oerall average	0.622	0.500	0.521	0.036
Control	0.655			
P value	0.038	0.033	0.034	

The results also indicated that the level MDA in females were higher than males, and they increased in the samples that were isolated from liver infected with more than 10 cysts compared to those from which infected with less than 10 cysts, while the overall level of MDA increased in the infected samples compared to the control group a shown in tables 3 and 4 .

Table (3) Concentration of MDA in the livers of infected animals and control groups for males and females and its concentration in different locations of the affected liver

Sex	MDA concentration rate ng/mL			P value
	In the liver tissue surrounding the cyst	In the liver tissue closed to the cyst	In the parasite tissue	
Male	23.882	27.362	25.980	0.025
Female	24.774	28.719	28.010	0.032
Overall average	24.328	28.040	26.995	
Control (male)	26.309			0.046
Control (female)	20.005			0.034
Overall average(control)	23.157			
P value	0. 044	0.049	0.044	

Table (4) MDA concentration in livers containing less than and more than ten bags and the control group

No. of cyst	ng/ml MDA Conce. rate			P value
	in the tissue surrounding the cyst	In the tissue adjacent to the cyst	in the parasite tissue	
Less than10	25.001	24.617	20.429	0.035
More than 10	23.654	31.464	33.562	0.022
Overall average	24.328	28.040	26.995	
Control	23.157			0.033
P value	0.024	0.045	0.026	

8. Discussion

The overall rate of TAOS concentration in the parasite tissue is lower when compared to the infected liver tissue and its concentration in the control group, but in a very simple and unremarkable difference, and its level increase with increase of cysts number and this indicates the parasite's adaptation to the effort the oxidative stress that it is exposed to in its host while its concentration rate increases with the increase in the number of cysts and this agrees with the findings of Martínez-González et al. (2022) who refer that parasite size plays an important role in the concentration of antioxidants, which increases with the increase in the size of the parasite, as TAOS is One of the antioxidants and that its concentration is caused by oxidative stress due to the high percentage of free radicals (Abd and Murad, 2019), this statement is consistent with Moti et al. (2015) and as indicated by Skrzychi et al (2012).

The present results does not agree with Samadieh et al. (2017) who did not find a difference in its rate between infected and uninfected animals but agree with Stumbo et al. (2012) who showed an increase in the livers of fish infected with the larval stage of two types of trematode , and agrees with Fahmy et al. (2019) who recorded an increase in MAD in the affected liver tissue with Blastocystosis.

Malonaldehyde represents 20% of the final product derived from the oxidation of super lipids. It is also characterized by stability and a long half-life compared to other types of super-oxidation. Therefore, malonaldehyde is an indication of the occurrence of this oxidation (Schaur et al., 1991). Similarly, it has been shown that the level of MDA has been adopted as a biomarker when the production of free radicals, oxidative stress, tissue damage. It is significantly increased in hosts affected by hydatid cysts (Deghbar et al., 2019). Evidently, lipid peroxidation resulting from ROS leads to blockage and ultimately disruption of cell membranes, which leads to cell death and necrosis where high levels of malonaldehyde peroxidation in serum infected animals host defense against parasitic infections (Yazar et al., 2003). Much research has been done on antioxidants and their effect on health. It has been emphasized that the balance between oxidation and antioxidants is critical to a healthy biological system. Low doses of antioxidants may be beneficial for this system, but higher amounts may violate the balance (Mordvinov et al., 2021).

There are several possible mechanisms for increased lipid peroxidation and protein carbonyl in liver, heart and kidney tissues. ROS and lipid peroxidation products impair the respiratory chain in hepatocytes either directly. Or indirectly, through oxidative damage to the mitochondrial genome, which in turn, leads to the generation of more types of ROS. Mitochondrial dysfunction can lead to apoptosis or necrosis, depending on the energy state of cell damage (Noeman et al., 2011).

9. Conclusions

The result of this study is that malonaldehyde levels are increased in hepatocytes infected with E. granulosus compared to healthy liver cells, and TAOS levels are decreased in liver cells infected with E. granulosus, which is evidence of oxidative stress.

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