Amoebic dysentery patients and two important signs (Malondialdehyde and Glutathione) of oxidative stress

Raad Abbas Kadhim[1]Rana A. Jawad[2] Nada Naji Shaalan[3]

[1] College of sciences for women/ University of Babylon, Babylon/Iraq

Author for correspondence: albaytee2188@gmail.com

[2] College of Veterinary Medicine/ University of Kerbala, Kerbala, Iraq

[3] College of Education for pure sciences/University of Kerbala, Kerbala, Iraq

Abstract

A total of 50 human blood samples with Amoebic dysentery were obtained from people between the ages of 7-66 years for a period of three months from November 2017 to January 2018 from three hospitals (Murjan Medical City hospital, AL-HillaTeachinghospital, and AL-Mahaweel hospital) in Babylon province, Iraq. While the other 30 samples of healthy people (7- 63 years) were seen as a control group, were collected from donors from Babylon Governorate to assess the state of oxidative stress in people with acute *Entamoebahistolytica* infections.

The state of oxidative stress was determined by valuing the levels of serum malondialdehyde (MDA) and serum glutathione (GSH) as an indicator of lipid peroxidation and antioxidant status respectively. Serum MDA and serum GSH levels for Group of sick people were 18.125± 4.56µmol/L and 194.434±23µmol/L respectively, which MDA level was significantly higher than that in the control group 13.285 ± 2.87µmol/L whereas the GSH level was lower than that in the control group 559.25±55µmol/L ($P<0.001$). Serum MDA levels were increased significantly with increasing age ($P<0.001$). The acute infection of the amoeba parasite under study was directly associated with a sharp increase in free radicals by increasing MDA levels and lower GSH levels in people with parasite compared to uninfected persons, confirming the important role of parasites in inducing oxidative stress.

KEY WORDS: Amoebic dysentery, oxidative stress

How to cite this article: Kadhim RA, Nada AJ, Shaalan NN (2019): Amoebic dysentery patients and two important signs (Malondialdehyde and Glutathione) of oxidative stress, Ann Trop Med & Public Health; 22(VI): S188. DOI http://doi.org/10.36295/ASRO.2019.22068

Introduction

Entamoebahistolytica is a protozoan pathogen responsible for amoebiasis, which is important for diarrhea in developing countries (1). To succeed parasite infection must bind to the enormous intestinal epithelial cell and destroy the tissue (2). Oxidative stress is produced from a non-balancing between mechanical defense against oxidants and levels of oxidants (3).

Free radicals are produced through many natural processes in the body of the organism, in addition to the fact that oxidative stress has an essential role in increasing these radicals when exposed to many diseases (4, 5). Free radicals are a double edged weapon within the organism, on the one hand, which have defensive roles against pathogens and contribute to some important life processes in vivo, on the other, have destructive effects on cells and tissues(6). The increase above the normal limit of

active oxygen species leads to oxidative changes in the large molecules inside cells such as DNA, proteins and fats, resulting in irreparable oxidative damage (7), Free radicals of oxygen have been documented as interfering with the development of human cancer(8).

Numerous studies have confirmed the role of some free radicals in the killing of parasites such as H_2O_2, which inhibits the movement of the parasite *Leishmania donovani* and thus its death, and also recorded the increase in the radicals of superoxide when the parasite *Trypanosoma brucei* infection happened (9). Wen et al. (10) demonstrate that there was a rise in oxidative stress markers in the fatty tissue of *Trypanosoma cruzi* -infected mice including lipid peroxidation and protein carbonylation .

Malondialdehyde is an effective carbon compound, which is the most copious in human body and produced from lipid peroxidation when unsaturated fatty acids are exposed to free radicals (11). An increase in lipid oxidation outputs occurred when it was combined with various parasitic infections in humans (12, 13) and animals (14, 15). The chiefaims were to appraise the oxidative stress grade in persons with acute *Entamoebahistolytica* infections by determining the serum concentration ofmalondialdehyde and glutathione as an indicator of lipid peroxidation and antioxidant status respectively.

Materials and methods

This study was carried out during the period between November 2017 and January 2018 from three hospitals (Murjan Medical City, AL-HillaTeaching, and AL-Mahaweel hospitals) in Babylon province, Iraq. A total of 80 subjects aged (7-66) years were comprised in the study. There were 50 patients with acute amoebiasis, while the rest 30 subjects (collected from different areas of the province outside of hospitals) were healthy individuals and considered as a control group.The infection was diagnosed by the pathological laboratories in the above mentioned hospitals and followed up by specialist physicians.An information form was used for people under trial and excluded all people with chronic diseases.Three ml of venous blood was withdrawn for all subjects in the study. it was permitted to clot in plain tube and then centrifuged at 3000 round / minute for 10- 15 min and sera were separated to be used for quantifying MDA and GSH.

The level of malondialdehyde determined by an improved procedure described by Guidet & Shah (16) by using a spectrophotometer at 532 nm. Serum reduced glutathione was determined by colorimetric method at 412 nm according to Burtis and Ashwood (17), The serum GSH concentration was obtained from the following curve in µM Fig. (1).

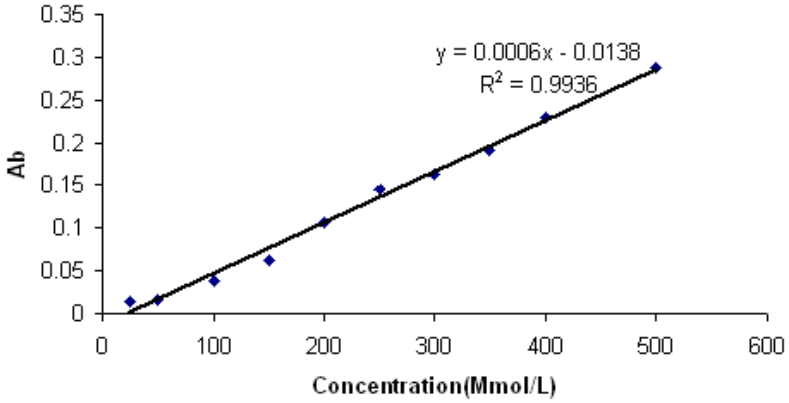

Fig. 1: The stander curve of reduced glutathione under this study.

Statistic

The data were analyzed statistically by using t-test (student). The value of P was considered significant if it is less than 0.05 and in case of less than 0.01 it is more significant. The results were expressed as mean ± SD (stander division).

RESULTS

Eighty human serum samples(44 male and 36 female) were studied for the purpose of estimating the level of MDA concentrations, the data were evaluated by using a student`s t-test. There were 50 patients (28 male and 22 female) with amoebiasis, while the rest 30 subjects (16 male and 14 female) were apparently healthy individuals and considered as control group, The demographic characteristics of the people in the experiment are shown in the table1.

Table 1. The most important specifications (demographic characteristics) in the people under experiment

Specifications	**Patients** (Amoebic dysentery)	**Healthy** (Without infection)
Age/year	7- 66	7- 63
Gender **Male (number)** **Female (number)**	28 22	16 14
Location of samples	Al Mahaweel Hospital 16 Hilla Teaching Hospital 12 Murjan Medical City Hospital 22	Collected from different areas of the province outside of hospitals 30
Smoking	No smoking	No smoking
Pathological history	They do not have chronic diseases such as blood pressure and diabetes as indicated in the information form through questions addressed to them	Same history in the patient group except for amoebic dysentery parasite
Drug use	No	No

The result show higher serum levels of MDA in patients with amoebiasis compared with controls ($p<0.01$) and lower levels of GSH in patients with amoebiasis compared to the control group ($p<0.01$) table 2.

Table 2. MDA and GSH Concentration in patients and control groups.

Variables	patients Mean ± SD N=50	control Mean ± SD N=30	P value
MDA (µmol/L)	18.125 ± 4.56	13.285 ± 2.87	p<0.01
GSH(µmol/L)	194.434±23	559.25±55	p<0.01

There was no statistical significance as compared between males and females in amoebiasis patients. In general, there was a higher level of serum MDA and lower level of GSH in males patients in contrast with a females, table 3.

Table 3: MDA Concentration in patients according to gender factor.

Variables	Male N=28	Female N=22
MDA(µmol/L)	21.15 ± 2.22	19.194± 2.87
GSH(µmol/L)	185.75 ± 16.14	188.607 ± 24.64

Table (4) shows the relationships between MDA concentrations and ages of different groups (patients). A positive correlation was determined between MDA and age(P<0.01).

Table 4: MDA Concentration according to the ages in patients and control groups.

Ages groups (year)	No. of samples	MDA (µmol/L) Mean ± SD
<20	19	13.628 ± 1.52
20-30	17	17.822 ± 1.22
31-40	15	15.208 ± 2.3
41-50	16	25.136 ± 4.22

≥50	13	15.61 ± 3.6

Figure 1 shows a negative correlation between the MDA levels and the GSH level in patients group. When the MDA values rise, the GSH values decrease and correlation value was -0.7611.

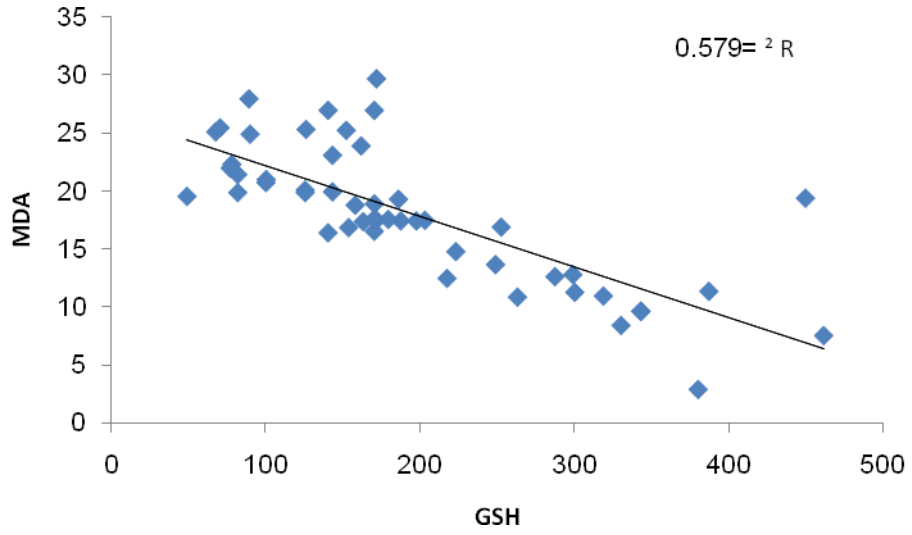

Figure 1: illustrates the relationship between MDA and GSH levels in patients

Discussion

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, many studies have indicated the occurrence of oxidative stress in parasitic infection, they recorded a high concentration of Malonaldahyde in patients with acute and chronic fascioliasis (18) *Toxoplasma gondii* (13)*Leishmania*,(12, 19,and 20) *Giardia lamblia*(21) and *Entamebahistolytica* (22). Macrophages, neutrophils and other phagocytic cells are mainconstituents of the anti-parasitic, anti-microbial and tumoricidal immune responses. These cells are capable of producinghuge amounts of ROS and reactive nitrogen species (RNS). ROS and RNS have a probable role in the pathogenesis of parasitic diseases (23). As an immune response to parasitic infection with *Trypanosomacruzi* , free radicals are produced that keep the parasite chronic and at the same time harmful to the heart muscle. These radicals may be beneficial for acute infection and need to be developed by the parasite(24).Many different chronic diseases affecting humans are associated with increased production of lipid peroxidation(25). The present study powerfully suggests that one of the main causes for high MDA levels in patients due to parasitic infection. ROS are highly reactive, they attach the important molecule in the cell and thus leading to damage the cell constituents. To survive under such dangers, humans or animals have established defense mechanisms for extermination of free radicals. However, the antioxidant mechanisms are not enough to remove all ROS produced during pathological or physiological processes. Then oxidative damage occurs, if the damage cannot be repaired, its effects will accumulate with age (26). In the present study serum MDA level was increased with increasing age for Age group(41-50 years). This result can support other previous studyhad suggested that oxidative damage is accrued during elderly (27).

GSH is an important component for protective mechanisms inside the cells against numerousharmful stimuli as oxidative stress. It prevents conversion of hemoglobin into methemoglobin as the result of oxidation, as well as it conserves the -SH groups in proteins against oxidation (28). 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.The

result of GSH observed in this study agreed with two previous studies carried out on parasitic infection in humans such as *T. gondii* (29) and *E. coli* (30).Also Kaya et al.(18) found that people with fascioliasis had a lower activity of glutathione peroxidase (enzymes convert H_2O_2 to water and alcohols by using reduced glutathione) in serum and erythrocytes compared to non-infected people.

The results of the present study showed that there is an inverse association between the level of MDA and the level of GSH in the serum of people with parasite, and this is consistent with other studies.A number of studies have shown that there is an inverse relationship between GSH and MDA, In their study, Ahmadvand et al. (31) 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. (32) reported a decrease in the value of MDA and a change in oxidative stress when GSH was supplied orally to obstructive jaundice rats.

In conclusion, this study demonstrates that, The infection by *E. histolytica* in women and men induced oxidative stress through free radicals formation. The infection by *E. histolytica* leads to increase lipid peroxidation in human host through MDA formation. In the present study serum GSH level was decreased in patients with amoebasis.

References

1.Ben Ayed L and Sabbahi S. Entamoebahistolytica. In: J.B. Rose and B. Jiménez-Cisneros, (eds) Global Water Pathogens Project. http://www.waterpathogens.org (R. Fayer and W. Jakubowski, (eds) Part 3 Protists) www.waterpathogens.org/book/entamoeba-histolytica Michigan State University, E. Lansing, MI, UNESCO. 2017.

2. Stanley S L and Reed S L. Microbes and microbial toxins: paradigms for microbial-mucosal interactions VI. *Entamoebahistolytica:* parasite-host interactions. Am. J. Physiol. Gastrointest. Liver Physiol.2001;280:G1049.

3. Pineda E. and Perdomo D.*Entamoebahistolytica* under Oxidative Stress: What Countermeasure Mechanisms Are in Place?. Cells. 2017;6(44):1-14.

4. Anderson KM, Ells G, Bonomi P, and Harris J E. Free radical spin traps as adjuncts for the prevention and treatment of disease. Medical Hypotheses.1999; 52:53-57.

5. Jameel Z I,Tolaifeh Z A,Radi N K, Amid T H and Murad A F.Investigation of Oxidative Stress in Patient with Varicella Zoster Virus (VZV) Infection. Journal of Global Pharma Technology. 2017; 10(9):94-97.

6.Kunwar A and Priyadarsini K I.Review Free radicals, oxidative stress and importance of antioxidants in human health. J Med Allied Sci.2011 ; 1 (2) : 53-60.

7.Trachootham D, Weiqin L, Ogasawara MA, Valle NRD, Huang P. (2008) Redox Regulation of Cell Survival. Antioxid Redox Signal.2008; 10:1343-1374

8.Carmia B. Free Radicals: The Pros and Cons of Antioxidants,Antioxidants and Radiation Therapy**.** American Society for Nutritional Sciences. J. Nutr.2004; 134: 3207S–3209S.

9. Mehta A and Shaha C. Apoptotic death in *Leishmaniadonovani*promastigoites in response to respiratorychain inhibition: complex II inhibition results inincreased pentamidine cytotoxicity; J Biol Chem.2004;279: 11798-11813.

10. Wen J-J, Nagajyothi F, Machado FS, Weiss LM, Scherer PE, Tanowitz HB and Garg NJ. Markers of oxidative stress in adipose tissue during Trypanosomacruzi infection. Parasitol Res. 2014; DOI 10.1007/s00436-014-3977-7.

11.Kilic E,Yazar S,Saraymen R and Ozbilge H. Serum malondialdehyde level in patients infected with *Ascarislumbricoides*. World J. Gastroenterol.2003; 9(10):2332-2334.

12.Kocyigit A, Gurel M, Ulukanligil M. Erythrocyte antioxidative enzyme activities and lipid peroxidation levels in patients with cutaneous leishmaniasis; Parasite. 2003; 10: 277–281.

13.Yazar, S. ; Kilic, E. ; Saraymen, R. and Sahin, I. Serum malodialdehyde levels in *Toxoplasma* Seropositive patients.Annals of Saudi Medicine. 2003; 23 :413-415.

14.Şimsek S, Yüce A, and Ütük A E. Determination of serum malonaialdehyde levels in sheep naturally infected with *Dicrocoeliumdendriticum*. F.Ü. SağlıkBil. Dergisi. 2006; 20(3), 217-220.

15. El-Taweel HA, El-Zawawy LA, Said DE and Sharara GM. Influence of the antioxidant drug (Antox) on experimental giardiasis and microsporidiosis; J Egypt Soc Parasitol.2007; 37: 189-204.

16.Guidet B and Shah S V. Enhanced in vivo H_2O_2 generation by rat kidney in glycerol- induced renal failure. American journal of physiology.1989; 1257: 440-444.

17.BurtisC A and Ashwood E R T. Text Book of Clinical Chemistry. 3rd ed. Philadelphia WB SAUNDERS.1999; 45.

18. Kaya S, Sutcu R, Cetin E S, Arıdogan B C,Delibas N andDemirci M. Lipid peroxidation level and antioxidant enzymeactivities in the blood of patients with acute andchronic fascioliasis. International Journal of Infectious Diseases.2007; 11, 251—255.
19. Biswas T, Ghosh DK, Mukherjee N andGhosal J. Lipid peroxidation of erythrocytes in visceral leishmaniasis; J Parasitol.1997; 83(1): 151–152.
20. Oliveira FJA andCechini R. Oxidative stress of liver in hamsters infected with *Leishmania (L.) chagasi*; J Parasitol. 2002;86: 1067–1072.
21. Demirci M, Delibas N, Altuntas I, Oktem F and Yönden Z. Serum iron, zinc and copper levels and lipid peroxidation in children with chronic giardiasis; J health popular Nutr.2003; 21(1): 72-75.
22. Sim S, Yong TS, Park SJ, Im KI, Kong Y, Ryu JS, Min DY and Shin MH. NADPH Oxidase derived reactive oxygen species-mediated activation of ERK1/2 is required for apoptosis of human neutrophils induced by *Entamoebahistolytica*; J Immunol. 2005; 174(7): 4279-4288.
23. El-Badry AA-M. Serum malondialdehyde levels as a biomarker of cellular injury in human fascioliasis; J T U Med Sc.2006; 1(1): 57-64.
24.Paiva CN, Medei E andBozza MT. ROS and *Trypanosomacruzi*: Fuel to infection, poison to the heart. PLoSPathog. 2018; 14(4): e1006928.
25. Kim G H, Kim J E, Rhie S J and Yoon S. The Role of Oxidative Stress in Neurodegenerative Diseases.ExpNeurobiol. 2015; Dec;24(4):325-340.
26.Halliwell B. Antioxidants and human disease: a general introduction; Nutr Rev.1997; 55: 544–549.
27.Muralidharan N, Bhat T andKumari S N. A study on effect of ageing on the levels of total antioxidant and lipid peroxidation. International Journal of Contemporary Medical Research. 2017;4(12):8-10
28. Akkus, I. Effects of free radicals and pathophysiological. Mimoza. Pub.1995; 32:1-76.
29. Karaman U,CelikT,Kiran TR,Colak C and Daldal N U.Malondialdehyde, Glutathione, and Nitric Oxide Levels in *Toxoplasma gondii* Seropositive Patients. Korean J. Parasitol.2008; 46(4): 293-295.
30. Karaman U,Kiran TR,Colak C, Iraz M,Celik T and Karabulut A B. Serum Malondialdehyde, glutathione and nitric oxide levels in patients infected with *Entamoeba coli*. International Journal of Medicine and Medical Sciences.2009;1(5): 235-237.
31. Ahmadvand H, Babaeenezhad E, Nasri M, Jafaripour L andKhorramabadi R M. Glutathione ameliorates liver markers, oxidative stress and inflammatory indices in rats with renal ischemia reperfusion injury. J Renal Inj Prev. 2019;8(2):91-97.
32. Chen J, Wu F, Long Y, Yu W. Glutathione supplementation attenuates oxidative stress and improves vascular hyporesponsiveness in experimental obstructive jaundice. Oxid Med Cell Longev. 2015; 486148. doi:10.1155/2015/486148.