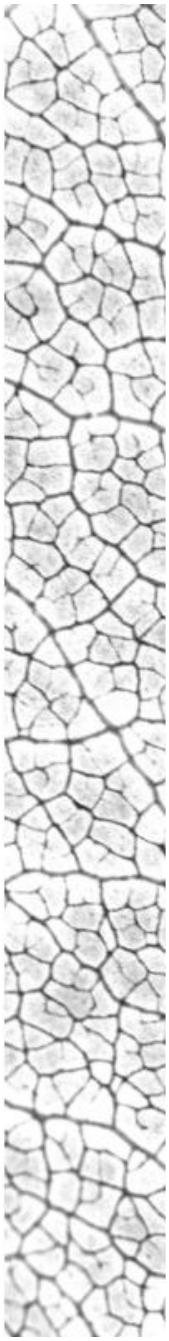

EurAsian Journal of BioSciences
Eurasia J Biosci 14, 5679-5683 (2020)

Giardiasis and oxidative stress: a case-control study

Raad A. Kadhim [1*], Nadia Zeidan Al -Naemy [2]

[1] Biology Department, College of Science for Women, University of Babylon, IRAQ
[2] General Directorate of Education in Babylon, Ministry of Education, IRAQ
*Corresponding author: wsci.raad.a@uobabylon.edu.iq

Abstract
Giardiasis is spread internationally and is prevalent in both children and adults. In developing nations, the incidence of *Giardia* infection is higher. About 200 million cases of giardiasis are diagnosed worldwide annually. This parasite has direct and indirect effects on the host's body, and its most prominent feature is its effect on the absorption of beneficial substances and minerals in the small intestine. In the current study 38 blood samples (20 males and 18 females) were collected, including 18 blood samples (11 males and 7 females) for people with acute giardiasis, and 20 samples (9 males and 11 females) were collected from healthy, uninfected persons from Imam Ali Hospital in Kotha district (Jableh) North of Babylon governorate, to determine the state of oxidative stress by determining the serum malondialdehyde (MDA) levels in infected and healthy people, this compound is considered as an indicator of the lipid peroxidation resulting from oxidative stress, and the determination of the serum levels of reduced glutathione (GSH), which in turn is the master of antioxidants in the body, which works to maintain a balance between oxidizing substances and antioxidants, The work was carried out in the Advanced Parasitology Laboratory in the department of biology / college of sciences for women at the university of Babylon for the period from October 2019 to February 2020. the statistical results using the t-test indicated that there was a significant increase ($P < 0.05$) in the MDA concentration (8.961 ± 3.262 µmol/L) in people with acute giardiasis compared with the control group (6.298 ± 2.975 µmol/L), the statistical analysis showed a significant decrease ($P < 0.05$) in the levels of serum GSH (447.440 ± 336.374 µmol/L) in patients with acute giardiasis compared to the control group (703.492 ± 458.337 µmol/L). When comparing the difference in MDA concentration between males and females, no significant differences were observed. MDA levels were distributed among age groups, it was found that their levels increased with increasing age group. We conclude from the current study that the oxidative stress represented by the formation of free radicals that led to the process of lipids peroxidation through the formation MDA and the reduction of antioxidants by decreasing the GSH concentration, that parasite had an active role in its emergence.

Keywords: giardiasis, oxidative stress, malondialdehyde, glutathione

Kadhim RA, Al -Naemy NZ (2020) Giardiasis and oxidative stress: a case-control study. Eurasia J Biosci 14: 5679-5683.

INTRODUCTION

The Giardia lamblia is a widespread enteric parasite that affects large population groups, leads to morbidity and possibly deaths (Adam, 2001). It is one of the most important causes of diarrhea in children in developing countries (Nkrumah and Nguah, 2011). Parasites play an important role in stimulating and modulating the host's immune system (Hewitson et al. 2009). The immune response behaves in many ways, including the cellular and humoral response (Vesely et al. 2012). Some aspects of this immune response may provoke a state of oxidative stress in the host's body (Chen et al. 2018).

Oxidative stress known as a state of imbalance between oxidants and antioxidants. It may result from several processes in the body of the organism. It is either caused by a decrease in antioxidants or from an increase in the production of oxidants (Srivastava et al. 2015). Oxidizers are naturally generated from metabolic processes within cells such as cellular respiration and energy production in mitochondria (Valko et al. 2007), but in normal conditions they are controlled by the defense systems of antioxidants. In abnormal circumstances, such as long hours of work, heavy work, lack of sleep hours, fatigue, and psychological problems lead to increased oxidative stress (Masahiro et al. 2001). Also infection, physiological diseases, or exposure to contaminated chemical agents, oxidizing substances are produced, including active oxygen and nitrogen species, which makes the system that suppresses them

Received: September 2019
Accepted: March 2020
Printed: November 2020

unable to confront and control their levels (Valko et al. 2007). This leads to the interaction of those roots with important molecules in living body cells such as lipids, proteins and nitrogen bases of the DNA molecule (Davies, 1991; Masahiro et al. 2001). The outcome of the interaction of free radicals with molecules leads to the formation of a toxic compound such as malondialdehyde and the limit may end up creating genetic mutations that are responsible for causing various cancers and other disorders in human body (Agarwal and Said, 2005; Nita and Grzybowsk, 2016).

Numerous studies have documented the role of parasitic infections in inducing oxidative stress in people with it (Abd Al-Wahab et al. 2009; Jasim and Al-Azzauy, 2009; Kadhim, 2013. Kadhim et al. 2019). The parasite G. lamblia did not have wide significance in this regard. Allain et al. (2017) study indicated that this parasite produces arginine diemnase enzyme that contributes to the regulation of some factors leading to oxidative stress in the intestine, including nitric oxide. The current study came to show the role of this intestinal parasite in the process of oxidative stress by identifying two pivotal factors: malondialdehyde and glutathione in patients with acute giardiasis.

MATERIALS AND METHODS

Sample collecting

The current study included collecting 3 ml of 38 blood samples (20 males and 18 females) to estimate the levels of MDA and GSH from the Imam Ali Hospital in Kotha district in Babylon province, of which 18 samples (11 males and 7 females , aged between 3 and 60 years/ Mean ± SD 30.38±17.44 years) were for people with acute giardiasis, the diagnosis of parasitic infection was approved on the clinical symptoms of patients, the macroscopic examination of stool samples, and then confirming the infection microscopically in the hospital laboratory) and 20 samples (9 male and 11 female, aged between 2 and 58 years/ Mean ±SD 21.9±17.26 years) of healthy uninfected persons as a control group. People with other acute and chronic diseases, smokers, and those receiving any treatments and drugs were excluded from this study. Samples were collected in flat tubes free of anticoagulants. Serum was separated from blood samples by central centrifugation at 3000 rpm for a 15 min, then the serum was withdrawn using a micro-pipette and placed in new tubes in the refrigerator until use. The study was carried out for the period from 27-October /2019 to 20- February /2020 in the laboratory of advanced parasitology in biology department / college of sciences for women at the university of Babylon.

Measurement of the serum Malondialdehyde level

The working principle is based on the interaction that takes place between Malondialdehyde with Thiobarbituric acid (TBA) and according to the method of Guidet and Shah (1989).

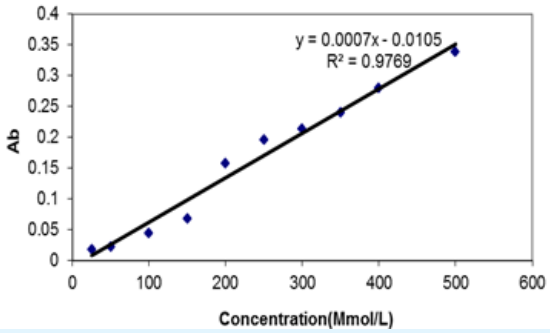

Fig. 1. Standard curve of glutathione under study

Measurement of the serum reduced glutathione level

The reagent 5.5-di-thiobase (2-nitro-benzoc acid) is reduced by the sulfhydryl group present in the reduced glutathione into a yellow-colored compound, and the absorbance of this compound is measured at a wavelength of 412 nm and the intensity of the yellow color is proportional to the concentration of glutathione in the sample (Burtis and Ashwood, 1999).

The concentration of glutathione in the samples is calculated through the standard curve (**Fig. 1**) obtained through the values of absorbance against each given concentration of reduced glutathione during the study and by using the computer excel system, and by dropping the absorbance values in the samples for sick and healthy people into the standard curve by substituting in the equation.

The Study ethics

The study was approved by the Ethical Committee at the University of Babylon and Prior consent was taken from patients and healthy people.

The statistical analysis

The results values were expressed as the mean ± standar deviation (M±SD), T-test for students. The one-way of variance (ANOVA) and identifying the least significant difference (LSD) for the significant cases was used for comparison between more than 2 groups. All these statistical operations were performed using by Microsoft Excel, values were significant when P was less than 0.05.

RESULTS

The study included the use of 38 blood samples (20 males and 18 females) to estimate levels of malondialdehyde and glutathione, of which 18 samples (11 males and 7 females) were for people with acute giardiasis and 20 samples (9 males and 11 females) were for healthy (uninfected people). Statistical analysis using the t-test that there is a significant increase in the concentration of malondialdehyde in people with acute giardiasis compared to the control group, while the statistical analysis showed a significant decrease in the levels of glutathione in patients with acute Giardiasis compared to a group the control as in **Table 1**.

Table 1. MDA concentration in people infected with the parasite and the control group

Variables	patients Mean ± SD N=18	control Mean ± SD N=20	P Value
MDA(µmol/L)	8.961 ± 3.262	6.298 ± 2.975	0.0129
GSH(µmol/L)	447.440±336.374	703.492±458.337	0.0273

Table 2. The concentration of MDA in the sick persons and the control group according to the sex factor

Variables	Male N=20	Female N=18	P Value
Patients group	8.534 ± 3.369 (11)	9.633 ± 3.219 (7)	0.499
Control group	5.366± 2.448 (9)	7.061± 3.256 (11)	0.201

Table 3. Concentrations of MDA in sick and healthy people according to age groups

Ages groups	No. of samples	MDA (µmol/L) Mean ± SD
≤10	10	4.574 ± 2.400
11-20	7	6.146 ± 1.830
21-30	7	8.131±1.647
31-40	6	8.719±3.149
41-50	5	10.556±2.886
≥50	3	12.164±3.405

F calculated= 7.308909 *F crit*= 2.512255
P-value= 0.000115 *LSD*= 5.230879

Table 2 shows the relationship between the levels of MDA with the sex factor, as the statistical analyzes showed that there were no significant differences between the concentrations of MDA between males and females, although there was a slight non-significant increase in the concentration of MDA in females in both the patient and control groups.

Table 3 shows the relationship between the levels of MDA and the different age groups in the affected and healthy people together, as the table shows a significant increase in MDA levels with the advancement of age groups below the probability level (P <0.01).

DISCUSSION

The primary mechanism of immune attack against parasites is the development of reactive oxygen species by phagocytes. Commensurately, elevated amounts of antioxidants including superoxide dismutases (SOD), catalases, peroxidases of glutathione and thioredoxin, and peroxiredoxins are generated by most parasites **(**Hewitson et al. 2009).

In the current study, malondialdehyde and glutathione were used as an indicator of the prevalence of oxidative stress in people with giardia, our findings during the current study revealed an increase in the level of MDA and a decrease in the levels of glutathione in the serum of people with the parasite compared to healthy people, this result is consistent with other previous studies that have linked oxidative stress to the incidence of different parasitic species, As a study of Abd Al-Wahab et al. (2009), which was conducted on 100 people infected with various parasites, including 9 people infected with the giardia parasite, through which it was found that infection with the latter parasite caused an increase in the serum levels of MDA and a decrease in the levels of both serum zinc and erythrocytes SOD when compared to a group. Control numbering 130 people. Jasim and Al-Azzauy (2009) study, which were conducted in people with Kala-azar in Iraq, they found an increase in the level of malondialdehyde associated with Erythrocytes in people with Kala-azar disease compared to healthy people. Kadhim study (2013) which found a moral increase in the concentration of MDA and significant decrease in GSH in man and women infected with chronic toxoplasmosis in the province of Babylon. Kadhim et al. (2019), also found a high level of serum MDA and low level of serum GSH in patients with amoebic dysentery, compared to the non-infected control group. The reason for this can be explained that the invasion of parasites stimulates immune cells in the human body to produce reactive oxygen species (ROS) and reactive nitrogen species (RNS) as a tool to defend against the invasion of the parasite and that the excessive production of these free radicals instead of being beneficial to the body by eliminating the parasite and killing it leads to damage to the body more and more as it works to oxidization of many compounds and chemical components in the human body, including lipid in the membranes of cells and finally made up of toxic and harmful substances (Agarwal and Said, 2005). The study of Demirci et al. (2003) concluded that chronic giardiasis in children led to a decrease in both serum iron and zinc elements, an increase in MDA levels and a decrease in the activity of the superoxide dismutase enzyme bound on the walls of red blood cells compared to uninfected children.

Many other studies have shown that chronic Giardia infection during childhood leads to malnutrition of protein-energy, vitamin A deficiency, anemia of iron deficiency, and low cognitive and educational output (Gendrel et al. 2003; Al-Mekhlafi et al. 2010). A study by Quihui et al. (2010) demonstrated that infection with the Giardia parasite is a risk factor for zinc deficiency. Also, in an Egyptian study on hamsters, it was found that when infected with G. lamblia experimentally, they lead to a significant decrease in serum manganese values compared to uninfected animals (Aly et al. 2014). The decrease in zinc and manganese in the aforementioned studies as a result of infection with G. lamblia may give a great impetus to the increase of free radicals, which in turn generate MDA compound and depletion or decrease of GSH because of these minerals have role in the oxidative stress process as they act as a cofactor for some antioxidant enzymes (Marreiro et al. 2017; Olechnowicz et al. 2018). The results of the current study indicate that MDA levels rise with age, which supports the theory of the relationship between aging and oxidative stress (Schöttker et al. 2015).

CONCLUSION

In conclusion, *G. lamblia* infection, as shown by considerably higher serum MDA and lower serum GSH levels among such patients, are associated with a substantial degree of free radical development.

REFERENCES

Abd Al-Wahab SA, Mahdi JK, Mahdi N K (2009) Oxidative stress among patients with some different parasitic infections. The Medical Journal of Basrah University, 27(2):66-70.

Adam RD (2001) Biology of *Giardia lamblia*. Clin. Microbiol. Rev., 14: 447–475.

Agarwal A, Said TM (2005) Oxidative stress, DNA damage and apoptosis in male infertility: a clinical approach. B.J.U. International, 95: 502–507.

Allain T, Amat CB, Motta J-P, Manko A, Buret AG (2017) Interactions of *Giardia* sp. with the intestinal barrier: Epithelium, mucus, and microbiota, 5:1, e1274354, DOI: 10.1080/21688370.2016.1274354.

Al-Mekhlafi HM, Surin J, Sallam AA, Abdullah AW, Mahdy MAK (2010) Giardiasis and poor vitamin A status among aboriginal school children in rural Malaysia. Am. J. Trop. Med. Hyg., 83:523–527.

Aly EM, Sabry HY, Fahmy ZH, Zalat RS (2014) Efficacy of combination therapy (metronidazole and/or artemether) in experimental giardiasis and its impact on non-enzymatic oxidative stress biomarkers Parasitologists United Journal, 7:68–74.

Burtis CA, Ashwood ERT (1999) Text Book of Clinical Chemistry. 3rd ed. Philadelphia WB SAUNDERS, 45.

Chen Y, Zhou Z, Min W (2018) Mitochondria, Oxidative Stress and Innate Immunity. Front. Physiol., 9:1487. doi: 10.3389/fphys.2018.01487.

Davies KJA (1991) ed. Oxidative damage and repair: introduction and overview. In: Oxidative damage and repair. Chemical, biological and medical aspects. Pergamon Press, New York; pp 341–354.

Demirci M, Delibas N, Altuntas I, Oktem F, Yönden Z (2003) Serum iron, Zinc and Copper levels and lipid peroxidation in children with chronic Giardiasis. J Health popul Nutr., 21(1): 72-75.

Gendrel D, Treluyer JM, Richard-Lenoble D (2003) Parasitic diarrhea in normal and malnourished children. Fundam. Clin. Pharmacol., 17, 189–197.

Guidet B, Shah SV (1989) Enhanced in vivo H_2O_2 generation by rat kidney in glycerol- induced renal failure. American journal of physiology, 1257: 440-444.

Hewitson JP, Grainger JR, Maizels RM (2009) Helminth immunoregulation: the role of parasite secreted proteins in modulating host immunity. *Mol Biochem Parasitol*., 167(1):1-11.

Jasim BT, Al-Azzauy AAM (2009) Oxidative Stress and Antioxidant Status in Human Infected With Kala-azar. AJPS, 6(1):116-122.

Kadhim RA (2013) The Relationship between some physiological and biochemical aspects in persons infected with Toxoplasmosis. Ph.D. Thesis, Babylon University.

Kadhim RA, Jawad RA, Shaalan NN (2019) Amoebic dysentery patients and two important signs (Malondialdehyde and Glutathione) of oxidative stress. Annals of Tropical Medicine and Public Health, 22 (6):72-78.

Marreiro Ddo N, Cruz KJC, Morais JBS, Beserra JB, Severo JS, de Oliveira ARS (2017) Zinc and Oxidative Stress: Current Mechanisms. Antioxidants, 6, 24.

Masahiro I, Shinya A, Nagata S, Miyata M, Hiroshi K (2001) Relationships between perceived workload, stress and oxidative DNA damage. Int Arch Occup Environ Health, 74:153–157.

Nita M, Grzybowski A (2016) The role of the reactive oxygen species and oxidative stress in the pathomechanism of the age-related ocular diseases and other pathologies of the anterior and posterior eye segments in adults. Oxidative Medicine and Cellular Longevity, Article ID 3164734 | 23 pages | https://doi.org/10.1155/2016/3164734.

Nkrumah B, Nguah SB (2011) *Giardia lamblia*: a major parasitic cause of childhood diarrhoea in patients attending a district hospital in Ghana. Parasites & Vectors, 4:163.

Olechnowicz J, Tinkov A, Skalny A, Suliburska J (2018) Zinc status is associated with inflammation, oxidative stress, lipid, and glucose metabolism. J Physiol Sci., 68:19–31.

Quihui L, Morales GG, Méndez RO, Leyva JG, Esparza J, Valencia ME (2010) Could giardiasis be a risk factor for low zinc status in schoolchildren from northwestern Mexico? A cross-sectional study with longitudinal follow-up. BMC Public Health, 10:85.

Schöttker B, Brenner H, Jansen EHJM, Gardiner J, Peasey A, Kubínová R, Pająk A, Topor-Madry R, Tamosiunas A, Saum K-U, Holleczek B, Pikhart H, Bobak M (2015) Evidence for the free radical/oxidative stress theory of ageing from the chances consortium: a meta-analysis of individual participant data. BMC Medicine, 13:300.

Srivastava KK, Kumar R (2015) Stress, oxidative injury and disease. Indian journal of clinical biochemistry, 30(1): 3-10. Doi: 10.1007/s12291-014-0441-5.

Valko M, Leibfritz D, Moncol J, Cronin MTD, Mazur M, Telsar J (2007) Free radicals and antioxidants in normal physiological functions and human disease. The International Journal of Biochemistry and Cell Biology, 39: 44–84.

Vesely MCA, Bermejo DA, Montes CL, Acosta-Rodrıguez EV, Gruppi A (2012) B-Cell Response during Protozoan Parasite Infections. Journal of Parasitology Research Volume, (7):362131.