Evaluation of Oxidative Stress In Patients With Giardia Lamblia In Duhok City, Iraq

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KEYWORDS
Giardia lamblia, Oxidative stress, Malondialdehyde, Glutathione, Albumin

ABSTRACT
Giardia lamblia (Giardia intestinalis) is a widespread enteric parasite known as giardiasis, which infects the small intestine of human at different ages, particularly children. This parasite has direct and indirect effects on the host's body. This study aimed to evaluate the role of oxidative stress is expressed by estimating the malondialdehyde concentration (As oxidative indicator), and estimating the non-enzymatic antioxidants concentration in the serum of 39 patients (males and females), whose ages ranged between (3-38) years patients infected with the G.lamblia or suspected of being infected with this parasite who were referred to Public Health Prevention Center, Center, Heevi Pediatric Hospital, Shariya and Kaberto Camp inside or outside Dohuk city. The diagnosis of parasitic infection was confirmed on the clinical symptoms of patients, the macroscopic examination of stool infected patients, which cotained trophozoites and / or cysts of G. lamblia, by confirming the infection microscopically in the laboratory by two method the direct wet smear examination and the antigen detection test. in addition to 30 serum samples healthy controls who did not have this disease, of both genders and the same age as a healthy control.The results of current study showed a significant increase in the malondialdehyde (MDA) concentration by 158% in the serum of patients with giardiasis compared to healthy controls of both genders, and by 183% in males, 134% in females. compared to healthy controls according to gender. And the results also showed a significant decrease in the concentration of both glutathione (GSH) and albumin in the serum of infected patients by 70% and 15%, respectively, compared to healthy controls of both genders. The significant percentage of decrease was in males by 71%, 16% respectively, and in females by 70% and 14% respectively compared with healthy controls according to gender. We conclude that infection with G. lamblia caused oxidative stress and dysfunction in physiological and metabolic processes.

1. Introduction

Giardiasis is an enteric disease arising from the protozoan parasite *Giardia duodenalis* informally known as *Giardia lamblia* or *Giardia intestinalis* and is a flagellated enteric protozoon that infects humans different ages and other mammals globally. It is a worldwide cause of gastrointestinal infection known as giardiasis. This parasite is commonly infected by contaminated drinking water, eating contaminated foods or direct fecal-oral contact which cause health problems in the digestive system such as diarrhea, poor absorption and weight loss (Solaymani-Mohammadi, 2022; Alharbi, 2020). 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. Which infects the small intestine of human at different ages, articularly children leading to chronic malnutrition and perhaps stunted growth in developing countries (Solaymani-Mohammadi, 2022; Riba, 2020).

G.lamblia exists in trophozoite and cyst forms, and the infective form is the cyst. Trophozoites of G. intestinalis are found in the upper part of the small intestine, where they live closely attached to the mucosa. They are found at times in the gallbladder and in biliary drainage. *G.lamblia* infection causes severe intestinal disorder, most commonly, diarrhea and related symptoms due to malabsorption of disaccharides, fat and fat-soluble vitamins (Mageed,2019).

Oxidative stress is defined as an imbalance between oxidants and antioxidants, which leads to a decrease in the defense capacity of antioxidants (Blagojevic et al., 2022). The imbalance between free radicals and antioxidants leads to oxidative damage to biomolecules such as proteins, lipids, and nucleic acids (Adwas *et al.*, 2019; Kadhim *et al.*, 2020).

Free radicals are known as self-oxidants that include molecules or atoms that have one or more electrons in their outer orbit (Meda *et al.,* 2019). It has the ability to oxidize other molecules within the body or reduce them by giving them the single electron they have in their outer orbit. There are many oxidizing compounds of different types, and these can cause various diseases and are called Reactive species that contain or do not contain free radicals. Among these reactive types that contain oxygen are called Reactive Oxygen Species (ROS), Reactive Nitrogen Species (RNS) and other types containing sulfur, carbon, or halides (Ighodaroab & Akinloyeb, 2018).

There are two types of reactive species sources, which are: Exogenous Sources External sources of reactive species include water and air pollution, radiation, alcohol consumption, smoking, heavy metals, certain medications, solvents like benzene and pathogens include parasites, bacteria, viruses and fungi (Rahal et al., 2014). Endogenous Sources These sources are produced through inflammatory mechanisms resulting from the stimulation of immune cells in patients with asthma, cancer, aging and strenuous exercise (Rahal et al., 2014). The formation of these reactive radicals can initiate many diseases, such as diabetes, liver diseases, heart diseases, atherosclerosis, and kidney failure (Halliwell, 2007; Al-Ali,2020; Al-Jawadi & Jankeer, 2021).

Some types of intestinal parasite infestation, such as *Giardia lambilla*, lead to the production of free radicals (ROS), which cause cell damage by interacting with cellular components such as proteins, lipids, and nucleic acids. The infection causes intestinal inflammation, which triggers an immune response that includes the production of free radicals in an attempt to eliminate the parasite. This excessive production of free radicals can lead to oxidative stress, causing damage to cells and cell membranes in the intestine. Free radicals may play a role in the clinical symptoms associated with Giardia infection, such as diarrhea, abdominal pain, and bloating. *Giardia* infection is one of the causes of oxidative stress through the host's immune response, leading to the production of free radicals. This stress contributes to the development of the symptoms experienced by the patient (Ismail *et al.*, 2022; Kadhim & Al-Naemy, 2020). Malondialdehyde (MDA) is an indicator of oxidative stress resulting from lipid peroxidation of polyunsaturated fatty acids, and the level of MDA increases under conditions of increased ROS production. oxidative stress can be studied in patients infected with Giardia or in the host by monitoring MDA levels and biomarkers of antioxidants such as glutathione (GSH) and albumin (Ismail *et al.,* 2022; Kazim and Al-Naimi, 2020).

The current study aimed to show the role of this intestinal parasite (*G. lamblia*) cause oxidative stress by determined malondialdehyde (final product of lipid peroxide process) concentration as an indicator of oxidative stress and concentration of non - enzymatic antioxidants in serum of patients with acute giardiasis.

2. Materials and Methods

Chemical Materials Used

We used some of ready-made analysis kits from BT-Laboratory from China international companie, to estimate the concentration of both MDA and GSH, by using the Enzyme-Linked ImmunoSorbent Assay (ELISA) technique. And the albumin concentration in serum was determined by the colorimetric method (bromocresol green (BCG) method), by using a Cobas c 501 device at a wavelength of 628 nm.

Location and Duration of Study

The study targeted patients infected with the *G. lamblia* parasite or suspected of being infected with this parasite who were referred to Public Health Prevention Center, AMR Lab, Doban Health Center, Heevi Pediatric Hospital, Shariya and Kaberto Camp inside or outside Dohuk city. The study included a survey of patients with *G. lamblia* in these areas.

Stool samples were collected from 508 patients (male and female), aged between (3-38) years, who presented symptoms consistent with giardiasis, such as diarrhea, abdominal cramps, bloating, nausea, and weight loss, were included. Patients with chronic gastrointestinal diseases unrelated to infectious causes and those unwilling or unable to provide stool samples were excluded from the study. Ethical approval was obtained from [Duhok Ethics Committee], and informed consent was secured from all participants or their guardians. And who visited from the beginning of the month of November (2023) until the end of April (2024). The diagnosis of parasitic infection was confirmed on the clinical symptoms of patients, the macroscopic examination of stool 39 (males and females) infected patients , which cotained trophozoites and / or cysts of *Giardia lamblia* , by confirming the infection microscopically in the laboratory by two method: the first is direct wet smear examination and the second method is antigen detection test.

Collection and Preservation of Blood Samples

Patients diagnosed with Giardia lamblia infection were selected for the study, along with age- and sex-matched healthy controls. Inclusion criteria required symptomatic presentation confirmed by diagnostic tests, while exclusion criteria included other underlying health conditions unrelated to *Giardia lamblia* infection. In addition, 30 healthy individuals of both sexes and the same age group, who were not infected with this parasite and who were considered a control group. Venous Blood for patients and healthy controls were drawned with volum (4-5) ml by using a 5 ml syringe. Blood samples were then placed in jell tubes free of any anticoagulant, and leave at room temperature for 30 minutes to clot. The blood was separated using a centrifuge for 15 minutes at a speed of 3000 revolutions per minute to obtain serum. The serum was then drawn using a micropipette and placed in plastic Eppendorff tubes, and stored in the freezer at a temperature of (-20)°C until biochemical tests were conducted.

Statistical Analysis

The statistical analysis of the results was performed using the Complete Randomized Design (C.R.D) and the differences between the patients group and the control group were determined using the T-Test for the studied variables at a probability level of ($p \leq 0.01$). The results values were expressed as the mean ± standar deviation (M±SD). While the difference between the patients and control groups according to gender and age groups was determined using Duncan's multiple range test for the studied parameters at the probability level ($p \geq 0.05$) and was considered a difference.The statistical software SAS was used to find the mean and standard error (Hinton, 2004).

Results and Discussion

The results in table (1) showed a significant increase at a probability level of ($P<0.01$) in malonaldhyde (MDA) concentration (As oxidative indicator) in the serum of infected patients with *Giardia lamblia*, by 158% compared to the healthy control group of both genders. Additionally, the results in table (2) also showed a significant increase at a probability level of ($p \leq 0.05$) in MDA

concentration in the serum of male patients by 183% and in female patients by 134% compared to the healthy control group according to genders.

The results of the current study are consistent with what was reached by (Kadhim and Al-Naemy 2020), as it indicated a significant increase in the MDA concentration in serum of patients infected with the *Giardia lamblia* parasite compared to healthy controls. And They found (Onchuma Mueangson *et al.*, 2023; Abdel Aziz *et al.*, 2015; Eze *et al.*, 2008; Oliveira & Cecchini, 2000) that the MDA concentration in the serum of patients infected with Plasmodium, *Schistosoma mansoni*, *Trypanosoma brucei*, and *Leishmania (L.)* was higher than in healthy control subjects.

The reason for the increase in the MDA concentration is attributed to the increase in the process of lipid peroxidation due to infection with this parasite. Since infection with the *Giardia lamblia* weakens the defense mechanism of antioxidants, so the production of free radicals

Table (1) The effects of *Giardia lamblia* infection on Malondialdehyde and non- enzymatic antioxidant concentration in the serum of patients compared to healthy control of both genders

Studied Groups / **Parameters Studied**	**Control**			**Patients**		
	Concentration Mean ± S.E.	**% Concentration**	**% Change**	**Concentration Mean ± S.E.**	**% Concentration**	**% Change**
Malondialdehyde (MDA) (nmol/ml)	13.853±0.664	100	–	35.795±3.377 **	258	+ 158
Glutathione (GSH) (ng/ml)	19.726±1.720	100	–	5.833± 0.291 **	30	- 70
Albumin (g/dl)	4.740±0.032	100	–	4.070± 0.076 **	85	- 15

- The numbers followed by the sign (**) indicate significant differences at ($p \leq 0.01$) according to (T-Test).

- The sign (-) means a decrease.
- The sign (+) means an increase.

exceeds the concentrations of antioxidants in the infected body, phospholipid compounds become more susceptible to free radicals and ROS generated, as lipid peroxidation occurs, the most important components resulting from this process is MDA, which is considered a biomarker of oxidative stress. Lipid peroxidation plays a critical role in the increase of MDA concentration during parasitic infections. Lipid peroxidation is a well-established mechanism of cellular injury and is used as an indicator of oxidative stress in cells and tissues. It involves the oxidative degradation of lipids, leading to the formation of reactive carbonyl compounds, with MDA being the most abundant (Saraymen 2004). Both (Ismail 2022; Demirci 2003) have reported elevated MDA concentrations in giardiasis, attributing this to increased lipid peroxidation due to oxidative stress. which led to increased MDA concentrations and decreased antioxidant activity in infected individuals.

Reactive oxygen species (ROS) generated during parasitic infections lead to an increase in MDA levels in the host by initiating lipid peroxidation. ROS are highly reactive molecules that can damage cellular components, including lipids, proteins, and DNA. During parasitic infections, the imbalance between ROS production and the antioxidant defense system results in

Table (2) The effects of *Giardia lamblia* infection on Malondialdehyde and non- enzymatic antioxidant concentration in the serum of patients compared to healthy control according to gender

Studied Groups / **Parameters Studied**	**Gender**	**Control**			**Patients**		
		# Concentration Mean ± S.E.	**% Concentration**	**% Change**	**Concentration Mean ± S.E.**	**% Concentration**	**% Change**
Malondialdehyde (MDA) (nmol/ml)	**Male**	13.32± 1.019 b	100	-	37.83± 4.955 a	283	+ 183
	Female	14.38± 0.818 b	100	-	33.75± 4.480 a	234	+ 134
Glutathione (GSH) (ng/ml)	**Male**	19.047± 2.317 a	100	-	5.502± 0.411 b	29	- 71
	Female	20.405± 2.524 a	100	-	6.164± 0.383 b	30	- 70
Albumin (g/dl)	**Male**	4.74± 0.046 a	100	-	4.02± 0.089 b	84	- 16
	Female	4.74± 0.0469 a	100	-	4.12±0.120 b	86	- 14

The numbers followed by different letters horizontally indicate a significant difference at ($p \leq 0.05$) according to Duncan is Test.

- The sign (-) means a decrease. The sign (+) means an increase.

oxidative stress (Demirci 2003). This oxidative stress triggers lipid peroxidation, a process where

ROS attack polyunsaturated fatty acids in cell membranes. The oxidative degradation of these lipids leads to the formation of lipid peroxides, which subsequently decompose to form reactive carbonyl compounds, with MDA being the most abundant (Saraymen 2004). In giardiasis increased MDA levels have been observed, reflecting the increased lipid peroxidation due to ROS generated during the infection (Ismail 2022; Demirci 2003). This process suggests a role of ROS in mediating cellular damage through lipid peroxidation, leading to increased MDA levels in the host.

MDA levels are increased as a consequence of ROS production during parasitic infection, which is a part of the host immune response. The immune cells such as macrophages and neutrophils produce ROS to eradicate the parasites, but high levels of ROS can lead to toxic effects on the host tissues and lipids (Demirci 2003). The connection between antioxidant system imbalance and the increase in MDA levels during parasitic infections such as giardiasis lies in the disruption of the oxidant-antioxidant balance. During parasitic infections, the production of ROS increases as part of the host's immune response. When the antioxidant defense system is insufficient to neutralize these ROS, oxidative stress occurs (Demirci 2003). The tissue damage caused by the parasite further exacerbates this process by continuously stimulating the immune response and ROS production, creating a vicious cycle of oxidative stress and lipid peroxidation (Ismail 2022; Demirci 2003). And both (Ismail 2022; Demirci 2003) pointed out that tissue damage caused by the parasite exacerbates this process by stimulating the immune response and continuously producing reactive oxygen species, creating a vicious cycle of oxidative stress and lipid peroxidation.

The results in table (1) showed a significant decrease in glutathione (GSH) concentration (As an indicator of oxidative stress) in the serum of infected patients with *Giardia lamblia*, by 70% compared to the healthy control group of genders. Additionally, the results in table (2) showed a significant decrease in GSH concentration in the serum of male patients by 71% and in female patients by 70% compared to the healthy control group according to genders.

The results of the current study are consistent with what was reached by (Kadhim and Al-Naemy 2020), as it indicated a significant decrease in the GSH concentration in serum of patients infected with the *Giardia lamblia* parasite compared to healthy controls. The results of this study are consistent with what (Kotepui *et al.,* 2023) found regarding a significant decrease in the GSH concentration in the serum of patients infected with the malaria parasite compared to uninfected controls (Kotepui *et al.,* 2023).

The reason for the decrease concentration of glutathione is that reduced glutathione (GSH) acts as a non-enzymatic antioxidant that helps eliminate ROS in cells that regulate the oxidation and reduction balance in cells. Among the multiple antioxidant enzymes present in the cell, reduced glutathione (GSH) reduces a myriad of ROS and also cooperates with enzymes responsible for detoxification (Cassier-Chauvat *et al.,* 2023). The glutathione system, including GSH-dependent enzymes such as glutathione S-transferases, glutathione peroxidases, and glutathione reductase, acts coordinately to protect against ROS-induced damage and heavy metal toxicity (Hossain *et al.,* 2012; Li, 2009). The GSH/GSSG redox couple is commonly used to determination of oxidative stress status (Presnell *et al.,* 2013). An imbalance in the GSH/ROS ratio can lead to oxidative stress and cell death (Liu *et al.,* 2021). GSH depletion is observed in many diseases and is necessary for apoptosis progression (Franco *et al.,* 2007).

The results in table (1) showed a significant decrease in albumin concentration (As an indicator of

oxidative stress) in the serum infected patients with *Giardia lamblia*, by 15% compared to the healthy control group of genders .Additionally, the results in table (2) also showed a significant decrease in Albumin concentration in the serum of male patients by 16% and in female patients by 14% compared to the healthy control group according to genders.

The results of the current study are consistent with what was reached by (Mageed, 2019; Akkelle *et al.*, 2018), as it indicated a significant decrease in albumin concentration in serum of patients infected with the *Giardia lamblia* parasite compared to healthy controls. While the papers do not directly address albumin levels, they suggest that parasitic infections can impact various metabolic parameters. The complex interactions between parasites and host microbiota may also influence metabolism and immunity (Elsaftawy & Wassef, 2021; Lopes *et al.*, 2012). The decrease in albumin concentration is attributed to increased oxidative stress, the consumption of albumin increases due to its role as a major non- enzymatic antioxidant and is synthesized exclusively by the liver. Albumin acts as a free radical scavenger, particularly through its cysteine and methionine residues, which react with ROS and reactive nitrogen species (RNS) (Roche, 2020; Taverna, 2013). This antioxidant activity leads to the oxidation and subsequent degradation of albumin, thereby increasing its consumption (Anraku, 2015). The oxidative modifications impair albumin's function and promote its removal from circulation, contributing to its increased consumption under conditions of increased oxidative stress.

Albumin can bind to and neutralize ROS. Albumin is non- enzymatic antioxidant which has the properties of closely related to its ability to bind and neutralize ROS through its multiple ligand-binding capacities and free radical-trapping properties. Specifically, the cysteine residue (Amino acid in its composition) in albumin at position 34 (Cys34) plays a crucial role in scavenging ROS such as hydrogen peroxide, peroxynitrite, superoxide, and hypochlorous acid (Roche, 2020; Taverna, 2013). Additionally, methionine residues (Amino acid in its composition) in albumin also contribute to its antioxidant activity by reacting with ROS (Anraku, 2015).

Oxidative stress leads to the modification and degradation of albumin molecules. For example, albumin can be oxidized by ROS such as hydrogen peroxide, resulting in the formation of sulfenic, sulfinic, or sulfonic acids, which can further degrade the protein (Roche, 2020; Taverna, 2013). Additionally, oxidative stress can cause the oxidation of cysteine and methionine residues in albumin, leading to structural changes and reduced antioxidant capacity (Anraku, 2015).

Conclusion

We conclude from current study that infection with parasite of the *Giardia lamblia* cause oxidative stress and dysfunction in physiological and metabolic processes. By studying and monitoring the malondialdehyde concentration as an indicator of oxidative stress and non - enzymatic antioxidants.

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