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Assessment Digestive Enzyme Activities, Level of Electrolytes and Alpha-1-Antitrypsin Infected Patients with *Giardia lamblia* in Dohuk City

Ismail H. Slevaney **Muna H. Jankeer** **Senaa A. Al-Jarjari**

Department of Biology/ College of Science/ University of Mosul/ Mosul/ Iraq

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Corresponding author:
Ismail H. Slevaney
ismail.23scp15@student.uomosul.edu.iq
Muna H. Jankeer
munsbio12@uomosul.edu.iq
Senaa A. Al-Jarjari
Sensbio@uomosul.edu.iq

ABSTRACT

Giardia lamblia (*Giardia intestinalis*) is one of the most important flagellated intestinal protozoa. It exists in two stages: The trophozoite stage, which parasitizes in the upper part of the small intestine (duodenum and jejunum) and is responsible for the symptoms of the disease, and the cyst stage, which is the infective stage and is excreted in the stool. The current study aimed to evaluate the effect of infection with *Giardia lamblia* on the digestive enzyme activity (α-amylase and lipase) and malabsorption (electrolytes) and infections (Alpha-1-antitrypsin) 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, of the total 508 patients 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 contained trophozoites and/or cysts of *G. lamblia*, by confirming the infection microscopically in the laboratory by two methods: The direct wet smear examination and the antigen detection test. In addition, 30 serum samples from healthy individuals not infected with this parasite, of both sexes and the same ages, were considered a control group. The results of this study showed a significant decrease in the activity of both α-amylase and lipase enzymes by 38% and 54%, respectively in the serum of patients with giardiasis compared to healthy controls of both genders and in male 44%, 54%, and in female 35%, 57%, respectively compared to healthy controls according to gender. The results also showed a slightly significant decrease in sodium (Na+) and potassium (K+) levels in the serum of infected patients by 6% and 4%, respectively, compared to healthy controls of both genders. The significant percentage of decrease was in males by 5% and 7%, respectively, and in females by 5% only for sodium compared to healthy controls according to gender. The results of the current study showed a significant increase in the Alpha-1-antitrypsin level by 54% in the serum of patients with giardiasis compared to healthy controls of both genders, in male 53% and in female 55% compared to healthy controls according to gender. We conclude that infection with *G. lamblia* causes diarrhea, which affects the digestive tract, causing malabsorption and intestinal inflammation, disrupting metabolic and physiological processes.

Keywords: *Giardia lamblia*, amylase, lipase, electrolytes, alpha-1-antitrypsin.

INTRODUCTION

Giardiasis is an enteric disease caused by the protozoan parasite *Giardia lamblia* or *Giardia intestinalis*. This flagellated protozoon infects humans of all ages and other mammals globally, leading to digestive issues like diarrhea, poor absorption, and weight loss (Solaymani-Mohammadi, 2022). Its primary impact is on the absorption of nutrients and minerals in the small intestine, often causing chronic malnutrition and stunted growth in children in developing countries. Severe intestinal disorders, including diarrhea, result from malabsorption of disaccharides, fats, and fat-soluble vitamins (Mageed, 2019).

Amylase [EC 3.2.1.1] catalyzes the hydrolysis of starch and glycogen into simpler sugars (Hans *et al.,* 2003). It is secreted from the salivary glands and pancreas as isoenzymes. It is called Salivary amylase (S-Isoamylase) and acts as a digestive enzyme, its activity is short-lived, as it loses Its efficacy upon swallowing due to stomach acidity (Almståhl *et al.,* 2001). The pancreas then takes over the primary role in carbohydrate digestion in the small intestine, for pancreatic amylase (P- Isoamylase) (Beckett, 2005). Pancreatic amylase breaks carbohydrates into smaller molecules like maltose and glucose, requiring calcium and chloride ions (Date *et al*., 2020). Giardia infection can reduce pancreatic amylase activity by interfering with pancreatic function and disrupting intestinal digestion (Dua and Shaker, 2016).

Lipase [E.C.3.1.1.3] digests lipids by hydrolyzing triglycerides into fatty acids and glycerol. Pancreatic lipase (PLT) is essential for lipid digestion, aided by bile to emulsify fats for absorption (Brownlee *et al*., 2010; Lambré, *et al*., 2023; Uehira, *et al*., 2023). Giardia can affect PLT activity by causing intestinal inflammation, reducing lipid absorption, and disrupting pancreatic secretions (Katelaris *et al*., 1992).

Giardia infection also depletes electrolytes like sodium (Na+) and potassium (K+) due to diarrhea-induced fluid loss, necessitating rehydration with oral solutions (Mageed, 2019).

Alpha-1-Antitrypsin (AAT), a glycoprotein and protease inhibitor, protects tissues by regulating harmful enzymes like neutrophil elastase. An imbalance in AAT levels can lead to tissue damage, liver, and lung diseases (Molmenti *et al*., 1993; Song, 2018). Intestinal parasitic infections, including Giardia, can trigger chronic inflammation, where AAT helps reduce damage by regulating the immune response (Ghandour *et al*., 2008).

MATERIALS AND METHODS

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 [the 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 contained trophozoites and/or cysts of *G. lamblia*, by confirming the infection microscopically in the laboratory by two methods: 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 *G. lamblia* infection. In addition, 30 healthy individuals of both sexes and the same age group, who

were not infected with this parasite were considered a control group. Venous blood for patients and healthy controls were drowned with volume (4-5) ml by using a 5 ml syringe. Blood samples were then placed in jell tubes free of any anticoagulant and left 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 placed in plastic Eppendorf tubes, and stored in the freezer at a temperature of (-20°) C until biochemical tests were conducted.

Determination of some of the biochemical parameters in patients' serum

The α-amylase and lipase activity were determined using an enzymatic colorimetric method by a Cobas c 501 device at a wavelength of 405 nm and 546 nm respectively. Sodium (Na^+) and potassium (K+) were determined using an ion-selective electrode (ISE) method by a Cobas c 501 device. Alpha-1-antitrypsin level was determined using an immunoturbidimetric method by a Cobas e 601 device**.**

RESULTS AND DISCUSSION

The results in (Table 1) showed a significant decrease of 38% in the amylase (Pancreatic amylase) activity in the serum infected patients with *G. lamblia* compared to healthy control of genders. Additionally, the results in (Table 2) also showed a significant decrease in amylase activity in the serum of male and female patients with this infection parasite by 44% and 35% respectively, compared to the amylase activity in the healthy control male and female.

The results of this study are consistent with what was found by (Buret *et al.,* 2007), as it indicated a significant decrease in amylase activity in serum of patients infected with *G. lamblia* compared to healthy controls. They suggest that parasitic infections can impact enzyme activity because of occurrence of epithelial dysfunction, which leads to disorders that may indirectly affect the efficiency of the digestive system in general, and as a result, malabsorption occurs, which weakens the activity of amylase (Buret *et al.,* 2007). Chronic giardiasis can lead to secondary impairment of pancreatic function, resulting in reduced enzyme activity, including amylase and lipase. The decrease in the amylase and lipase activity was attributed to the damage caused to the cell's pancreas producing these, especially in the Giardia infections which damaged its target (Carroccio *et al.,* 1997). Pancreatic amylase deficiency (decreased activity) can lead to the malabsorption of carbohydrates, causing symptoms such as failure to thrive, poor weight gain, diarrhea, and abdominal bloating (Hopson *et al.,* 2019). Infection also induces an immune response that includes T cell activation and cytokine release leading to mucosal inflammation that further impairs digestive enzyme activity by damaging the mucosal surface and changing the optimal kinetic properties of enzyme (Farthing *et al.,*1986).

The results in (Table 1) showed a significant decrease of 54% in the lipase activity (Pancreatic Triacylglycerol Lipase PLT) in the serum infected patients with *G. lamblia* compared to healthy control of genders. Additionally, the results in (Table 2) also showed a significant decrease in PLT activity in the serum of male and female patients with this infection parasite by 54% and 57% respectively, compared to the PLT activity in the healthy control male and female.

The results of this study showed a decrease in the lipase activity in the serum infected patients with *Giardia lamblia* compared to control. This is attributed to the fact that the parasite causes slight mucosal changes in the small intestine, which leads to malabsorption of fats, lipids, accompanied by a weakening of the secretory function of the pancreas in those infected with Giardia. The reason may also be due to the parasite's significant effect on the decomposition of triglycerides by pancreatic lipase, which leads to inhibition of the activity of lipase in the serum of patients, so the enzyme activity decreased compared to healthy controls.

The study by (Katelaris *et al.,* 1992) indicated that Giardia lamblia infection causes minor mucosal changes in the small intestine, but fat malabsorption may occur. Some evidence suggests that the secretory function of the pancreas is impaired in people infected with Giardia, although the mechanism and significance of this are unclear.

Table 1: The effects of *Giardia lamblia* infection on digestive enzymes activities, level of electrolytes and alpha-1-antitrypsin in the serum of patients compared to healthy control of both genders.

Study groups / Variables studied	Control			Patients		
	Mean ± SE	% Activity or level	% Change	Mean ± SE	% Activity or level	% Change
Amylase activity (U/L)	78.00 ± 2.947	100	_	48.00 ± 1.726 **	62	- 38
Lipase activity (U/L)	37.00±2.222	100	_	17.00±0.450 **	46	- 54
Sodium (Na^+) level (mmol /L)	141.00±0.566	100	_	133.00±0.746 **	94	- 6
Potassium(K^+) level (mmol /L)	4.53±0.055	100	_	4.38±0.053 **	96	- 4
Alpha-1-antitrypsin level (AAT) (mg/dl)	133.5± 2.408	100	_	205.4± 5.464 **	154	+ 54

- 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.

This *in vitro* study was conducted to determine the effect of *Giardia lamblia* on triglyceride hydrolysis by pancreatic lipase extracted from pigs. The study found that live *Giardia lamblia* hydrolyze fats significantly, so the degree of inhibition increases with increasing duration of exposure of the lipase to the parasite. Enzyme activity decreased by 89.7% compared to controls after 4 h incubation with the parasites. As for *Trichomonas vaginalis*, a flagellate protozoan, this parasite failed to inhibit lipase in this assay system. The effect of this effect *in vivo* is still unknown but may contribute to fat malabsorption in giardiasis.

Table 2: The effects of *Giardia lamblia* infection on digestive enzymes activities, level of electrolytes and alpha-1-antitrypsin in the serum of patients compared to healthy control according to gender.

Study groups / Variables studied	Gender	Control			Patients		
		# Mean±SE	% Activity or level	% Change	Mean ± SE	% Activity or Level	% Change
Amylase activity (U/L)	**Male**	78.0± 2.232 a	100	-	44.0± 1.719 b	56	- 44
	Female	79.0± 5.452 a	100	-	51.0± 2.580 b	65	- 35
Lipase activity (U/L)	**Male**	37.0± 2.464 a	100	-	17.0± 0.709 b	46	- 54
	Female	37.0± 3.844 a	100	-	16.0± 0.551 b	43	- 57
Sodium (Na^+) level (mmol /L)	**Male**	141.0±0.872a	100	-	134.0± 0.607 b	95	- 5
	Female	140.0± 0.670 a	100	-	133.0± 1.328 b	95	- 5
Potassium (K^+) level (mmol /L)	**Male**	4.64± 0.054 a	100	-	4.33± 0.064 b	93	- 7
	Female	4.41± 0.084 b	100	-	4.43±0.082 ab	100	0
Alpha-1-antitrypsin level (AAT) (mg/dl)	**Male**	130.6± 2.136 b	100	-	200.0±7.151 A	153	+ 53
	Female	136.3± 4.125 b	100	-	210.7±7.909 A	155	+ 55

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.

(Farthing *et al.,*1986) attributed Giardia infection to an immune response characterized by T-cell activation and cytokine release, which contributes to mucosal inflammation. This inflammatory response can impair the activity of pointed out that infection with *G. lamblia* is a known causative agent of common infectious diarrhea. In rare cases, this parasite has been found to be involved in the development of diseases of the pancreas and biliary tract, whether inflammatory or neoplastic (Pisani *et al.,* 2023).

The results in (Table 1) showed a slight significant decrease in sodium level (Na^+) in the serum of infected patients with *G. lamblia*, by 6% compared to the healthy control of genders. Additionally, the results in (Table 2) also showed a slight significant decrease in Na level in the serum of male patients this infection parasite by 5% and in female patients by 7% compared to the Na^+ level in healthy control according to genders.

The results in (Table 1) showed a slight significant decrease in sodium level (K^+) in the serum of infected patients with *G. lamblia*, by 4% compared to the healthy control of genders. Additionally, the results in (Table 2) also showed a slight significant decrease in K^+ level in the serum of male patients this infection parasite only by 7% but in female patients it was not affected compared to the healthy control according to genders.

The results of the current study are consistent with what was reached by (Khalaf and Shaker, 2022; AL-Haboobi, 2014), as they indicated a significant decrease in sodium and potassium levels in the serum of patients infected with the *G. lamblia* parasite compared to healthy controls. Giardiasis often causes profuse diarrhea, which can lead to significant loss of fluids and electrolytes, including sodium and potassium, and this loss through the gastrointestinal tract leads to decreased levels of these electrolytes in the serum of infected patients (Khalaf and Shaker, 2022). Powell *et al.,* (2022) noted that giardiasis causes diarrhea, which leads to dehydration and electrolyte imbalance, resulting in the loss of sodium and potassium, which are excreted from the body with the stool. In some cases, giardiasis can lead to increased secretion of potassium into the intestinal lumen, further exacerbating potassium loss (Gomes *et al.,* 2012). Habbari *et al.* (2000) confirmed that diarrhea causes decreased absorption and leads to a reduction in ions entering the body, which leads to a decrease in the level of ions in the blood.

Several studies have indicated that infection leads to changes in the levels of hormones that play a vital role in the digestive processes in the gastrointestinal tract and the regulation of other hormones such as a significant increase in the levels of both cholecystokinin and somatostatin in patients infected with giardiasis, which affects the absorption and secretion processes in the intestine, leading to decreased levels of sodium and potassium. Infection can also affect the absorption of nutrients and electrolytes in the small intestine, so malabsorption contributes to decreased levels of these electrolytes (Troeger *et al.,* 2007; Buret, 2007).

The results in (Table 1) showed a significant increase at a probability level of ($P<0.01$) in Alpha-1-antitrypsin (AAT) level (specific protein) in the serum of infected patients with *G. lamblia*, by 54% compared to the healthy control of genders. Additionally, the results in (Table 2) also showed a significant increase at a probability level of ($p \leq 0.05$) in AAT level in the serum of male patients this infection parasite by 53% and in female patients by 55% compared to the AAT level in the healthy control male and female.

The results of this study are consistent with what was reached by (Gandour *et al.,* 2008), as they indicated a significant increase in alpha-1-antitrypsin level in serum of patients infected with the *G. lamblia* parasite compared to healthy controls. AAT has great potential for use as a biomarker and therapeutic agent for various infectious diseases (Indalao *et al.,* 2019). AAT level increase in response to inflammation, this suggests that AAT acts as an acute phase protein, increasing in level during inflammatory responses (Ahsan *et al.,* 2014).

Pérez-Holand *et al.,* (2014) found significant increase in AAT levels in the serum of patients with colorectal cancer (CRC) compared to healthy controls, suggesting an association between elevated AAT levels and intestinal conditions. AAT plays multifaceted roles in tissue protection through its anti-inflammatory, immunomodulatory, and anti-apoptotic properties. AAT inhibits several enzymes, including neutrophil elastase, cathepsin G, proteinase III, thrombin, trypsin, and chymotrypsin, which are involved in tissue damage and inflammation (Wang *et al.,* 2017). AAT also plays a role in

promoting tissue repair mechanisms in the intestine. It has been shown to increase intestinal recovery after intestinal injury induced colitis, helping to repair damaged intestinal tissue (Collins *et al.,* 2013).

Alpha-1-antitrypsin (AAT) acts as an acute phase protein whose level increases in response to inflammation. It is a member of the serine proteinase inhibitor (SERPIN) superfamily and functions primarily by inhibiting proteases such as neutrophil elastase, which can cause tissue damage during inflammatory responses (Ahsan *et al.,* 2014). Kulakov *et al.,* (2022) were indicated that an AAT plays an important role in the context of intestinal health and diseases, as AAT is a protein that inhibits the neutrophil elastase enzyme, and is released during inflammatory processes to reduce the activity of proteolytic enzymes in areas of inflammation.

CONCLUSIONS

We conclude from is study that *Giardia lamblia* infection leads to significant disruptions in metabolic and physiological processes, including reduced activities of digestive enzymes (amylase and lipase) and imbalances in key electrolytes (sodium and potassium). These findings indicate impaired pancreatic and intestinal functions, contributing to malabsorption and gastrointestinal symptoms. Additionally, the marked increase in Alpha-1-antitrypsin (AAT) levels suggest its potential role as a biomarker for inflammation and intestinal damage associated with giardiasis. The results emphasize the need for timely diagnosis and effective treatment of giardiasis to prevent its adverse effects on digestive and systemic health.

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تقييم فعالية الأنزيمات الهضمية ومستوى الإلكتروليتات وألفا–1–أنتيتريبسين لدى مرضى الجيارديا لامبليا في مدينة دهوك

إسماعيل حسن سليفاني منى حسين جانكير سيناء عبد الله الجرجري

قسم علوم الحياة/ كلية العلوم/ جامعة الموصل/ الموصل/ العراق

الملخص

يعد طفيلي الجيارديا لامبليا *Giardia lamblia* من اهم الاوالى المعوية السوطية، اذ يوجد بطورين هما، الطور الناشط، الذي يتطفل في الجزء العلوي للامعاء الدقيقة (الاثني عشر والصائم) وهو المسؤول عن الاعراض المرضية، اما الطور المتكيس وهو الطور المعدي ويطرح مع البراز. هدفت الدراسة الحالية إلى تقييم تأثير الإصابة بطفيلي جيارديا لامبليا *G. lamblia* في فعالية الأنزيمات الهضمية (α–أميليز والليباز) وسوء الامتصاص (الإلكتروليتات) والالتهابات (ألفا–1–أنتيتريبسين) في مصل 39 مريضا (ذكور و اناث) تراوحت أعمارهم بين (3-38) سنة من المرضى المصابين بجيارديا لامبليا أو المشتبه بإصابتهم بهذا الطفيلي، من اجمالي 508 مريض تم احالتهم الى مركز الوقاية من الجيارديا ومركز هيفي للأطفال وشاريا ومخيم كبرتو داخل مدينة دهوك وخارجها، وتم تأكيد تشخيص الاصابة بالطفيليات من خلال الاعراض السريرية للمرضى والفحص العياني لبراز المرضى المصابين والذي يحتوي على الطور الناشط و/او الطور المتكيس لطفيليات الجيارديا لامبليا وذلك من خلال تأكيد الاصابة مجهرياً في المختبر بطريقتين فحص المسحة الرطبة المباشرة وفحص الكشف عن المستضد. بالإضافة إلى 30 عينة مصلية من الاصحاء غير مصابين بهذا الطفيلي، من كلا الجنسين وبنفس الاعمار وعدت كمجموعة سيطرة. أظهرت نتائج هذه الدراسة انخفاضا معنويا في فعالية كل من انزيم α–الأميليز والليباز بنسبة 38% و54% على التوالي في مصل مرضى الجيارديا مقارنة مع السيطرة الاصحاء لكلا الجنسين، وفي الذكور 44% و54% وفي الإناث 35% و57% على التوالي مقارنة مع السيطرة الاصحاء حسب الجنس. وأظهرت النتائج أيضا انخفاضا طفيفا معنويا في مستوى كل من الصوديوم (Na^+) والبوتاسيوم (K^+) في مصل المرضى المصابين بنسبة 6% و4% على التوالي مقارنة بالسيطرة الاصحاء لكلا الجنسين، وكانت النسبة المعنوية للانخفاض في الذكور بنسبة 5% و7% على التوالي وفي الإناث بنسبة 5% فقط للصوديوم مقارنة مع السيطرة الاصحاء حسب الجنس. أظهرت نتائج الدراسة الحالية ارتفاعا معنويا في مستوى ألفا–1–أنتيتريبسين بنسبة 54% في مصل مرضى الجيارديا مقارنة مع السيطرة الاصحاء لكلا الجنسين، وبنسبة 53% لدى الذكور و55% لدى الإناث مقارنة مع السيطرة الاصحاء حسب الجنس. نستنتج أن الإصابة بـ *G. lamblia* تسبب الإسهال، الذي يؤثر على الجهاز الهضمي، ويسبب سوء الامتصاص والتهاب الأمعاء، مما يؤدي إلى اختلال العمليات الأيضية والفسلجية.

الكلمات الدالة: جيارديا لامبليا، الأميليز، الليباز، الإلكتروليتات، ألفا–1–أنتيتريبسين.