

# Evaluation of interleukin -1 alpha, lipase and vitamin D3 in patients with giardiasis

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**Abstract.** Background: Giardiasis is one of most gastrointestinal parasite infection around world, affecting all people especially children under age five. Methods: The present research performed from the start of September 2021 to the end of March 2022, (50) blood samples were taken from suspected infected patients with *G. lamblia* and proved to be infected with the Giardia and (40) blood samples collected from healthy people as a control group. human IL-1A was measured by ELISA technique. The concentration of serum Lipase was measured by dry chemistry in the Fujifilm method, while the level of vitamin D3 was evaluated by Cobas E411 device Results: This study found that the level of interleukin -1 alpha has significantly increased in ( $p < 0.05$ ) in the infected patients with parasite further were the study of biochemical parameters of patients infected with parasite showed increase in concentration of lipase, and decrease in concentration of vitamin D3 in patients when compared to the healthy group. The results also showed that activity of lipase enzyme have been shown to be reduced when *G. lamblia* incubated with some intestinal parasites in vitro. Conclusions: it is concluded that giardiasis is a significant inflammatory process that results in changes in and vitamin D3lipid metabolism.

**Keywords.** Giardiasis, interleukin-1 alpha, vitamin D3, lipase.

## 1. Introduction

Giardiasis is a common parasitic infection that can cause diarrhea. It can be spread through contaminated water or food, and it is often seen in daycare centers and among travelers. Giardiasis is caused by *Giardia lamblia* which is a flagellate protozoan parasite. Which is spread through the fecal-oral route, which is commonly through the intake of contaminated water or food, or through contact with an infected person's stool [1]. *Giardia lamblia* has two stages during life cycle: trophozoite and cyst. Trophozoite being the pathogenic stage and cyst being the infective stage, both of which were observed in the feces of giardiasis patients [2-3] Giardiasis can cause a range of clinical symptoms, including watery diarrhea, epigastric pain (pain in the upper abdomen), nausea, vomiting, and weight loss. It is also characterized by small intestine malabsorption and fatty diarrhea. Malabsorption is a condition in which the body is not able to absorb nutrients from food properly. This can lead to a variety of symptoms, including diarrhea, weight loss, and fatigue [4]. The prevalence rate of giardiasis in developing states, can be ranging between 20% to 30%, with some studies reporting a 100% frequency, while in developed countries, the prevalence can be ranging from 3% to 10% [5]. In both humans and animals, giardia causes a strong adaptive immune response. For many years, it has been known that following infection, high quantities of parasite specific IgA are formed, and that CD4+ T cell responses contribute to this IgA formation and infection control. Over the last decade, we have gained a better comprehension of the non-antibody effector mechanisms used by the host to combat giardiasis [6]. Many innate defense models, including nitric oxide (NO) formation, lactoferrins, hypermotility, phagocytes, defensins, dendritic cells and mast cells, play an early role in the defense against giardia [7-8]. However, the traditional method of diagnosis is to look for *G. intestinalis* trophozoites or cysts in the stool of infected patients by examining fecal eggs and parasites. However, in recent years, with more objective techniques (e.g., immunoassay, Nucleic Acid Amplification Techniques (NAAT) [9].

## 2. Materials and Methods

### 2.1. Blood Samples Collection

(50) blood samples were taken from suspected infected subjects with *G. lamblia* when they returned to the laboratory to receive the result of microscopic analysis and proved to be infected with the parasite and (40) blood samples collected from healthy people as a control group. The samples were taken from both sexes' male and female and different ages group (adult 20-45 years male and female, children 4 months to 10 years male and female) [10].

### 2.2. Examination Stool Samples

The stool samples were examined with the naked eyes before being microscopically examined for color, consistency, and blood mucous, then the first step in the present study was to identify positive samples from whole samples collected from suspected patients with giardiasis. This was done by microscopically examining the samples to determine the presence of the parasite. A light microscope was used to detect trophozoite and cyst stages of *Giardia lamblia* after mixing a double-blind check mixed with saline (normal saline solution) which was formed by dissolving 8.5 gm of pure sodium chloride (NaCl) in one liter of distilled water [10].

### 2.3. Statistical Analysis

Descriptive statistics: (Frequency and percentage tables; mean and standard deviation). Inferential Statistics: (Independent sample t-test, to test the difference between two independent groups study and control groups). ANOVA test was used to test differenced among age categories. Post-hoc analysis was done by least significant difference [11].

### 3. Human Interleukin 1 alpha ELISA kit

#### 3.1. Assay Principle

This kit is an ELISA, which stands for Enzyme-Linked Immunosorbent Assay. In this assay, the plate is coated with a specific antibody that binds to the target molecule, in this case, human IL-1A. When the sample is added to the plate, the IL-1A in the sample binds to the antibodies on the plate. A biotinylated antibody is then added, which binds to the IL-1A in the sample. Finally, streptavidin-HRP is added, which binds to the biotinylated antibody. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm.

#### 3.2. Determination Of Serum Lipase and Vitamin D3

The concentration of serum Lipase was measured by dry chemistry in the Fujifilm method [12], while the level of vitamin D3 was evaluated by Cobas E411 device [13].

### 4. Results and Discussion

The blood samples for patients and adult infected with *Giardia lamblia* parasite which numbered (50) samples, and (40) samples of healthy people without parasite, were subjected to the study of cytokines (interleukin 1-alpha) and biochemical markers study, the results of current study revealed increase in the level of interleukin 1-alpha as shown in the (table 1), *Giardia lamblia*, a parasite, can cause the release of a variety of inflammatory cytokines, including IL-8, GROa, GM-CSF, IL-6, and IL-1a, from human epithelial and stromal cell lines. IL-1a is particularly important for initiating the secretion of inflammatory cytokines in response to *Giardia lamblia* infection. This is because IL-1a differs from most other proinflammatory cytokines in several ways. For example, IL-1a can induce physiological responses at very low concentrations (1-10 pg/ml), while other proinflammatory cytokines, such as chemokines, require much higher concentrations (>10 ng/ml) to mediate their biological activity [10]. Interleukin-1 alpha (IL-1a) is a cytokine that lacks an amino terminal signal peptide, which is required for efficient secretion. This suggests that IL-1a may be able to exert some of its activities even when it is not secreted. Despite the absence of a signal peptide, IL-1a can still be secreted by cells in the mature form in response to various stimuli. For example, murine macrophages secrete IL-1a after being stimulated with LPS, or after being infected with *Shigella flexneri*. In contrast, keratinocytes do not secrete IL-1a, although they have high levels of intracellular IL-1a whose biological functions are unknown [7]. The physiological role of intracellular IL-1a in the host response to lytic pathogens is that it can be released from cells when their membranes are damaged. This release of IL-1a can activate a cascade of proinflammatory events in the immediate vicinity of the lysed cells. Membrane damage and subsequent cell lysis can be caused by contact with pathogens such as *Giardia lamblia*. Additionally, IL-1a released by damaged cells can increase IL-1a production by neighboring cells. This mechanism could serve to amplify the inflammatory response by increasing the capacity of neighboring cells to release additional IL-1a when they are infected. IL-1a also upregulates adhesion molecules for inflammatory cells on the endothelium, and induces prostaglandin production. These actions may contribute to the diarrhea that is often associated with *Giardia lamblia* infection [12].

**Table 1.** Comparison between infected patients and healthy control interleukins (IL-1 $\alpha$ ) concentration

Groups	Mean $\pm$ Standard Error	
	No.	IL-1 $\alpha$ (Pg/ml)
Infected Patients	50	2.828 $\pm$ 0.07 <sup>a</sup>
Healthy Control	40	0.721 $\pm$ 0.02 <sup>b</sup>
L.S. D		0.52

The current study also indicate that concentration of lipase is increase ( $p > 0.01$ ) as (table 2) below, this agree with [14] which indicate that mucosal enzymes (alkaline phosphatase and lipase) levels were changed in infected rats after (1-2) weeks post infection compared to control group both enzymes were increase during infection and this phenomenon was taken as mucosal marker for decreased absorption, while disagree with [15]. The results showed that activity of lipase enzyme have been shown to be reduced when *G. lamblia* incubated with some intestinal parasites in vitro. That difference between the current result and the results of the few previous researches may be activity of parasite itself, strain of parasite, experimental conditions and activity of lipase enzyme, intestinal parasite effect in the level of activity of pancreatic enzymes by reducing the absorption in the intestine [16].

As well as the current study showed significant decrease ( $p > 0.05$ ) in the level of vitamin D3 in patients infected with *G. lamblia* than control group (table 3). *G. lamblia* represented the highest incidence (22.9%) among studied cases of diarrhea in parasitic infection, the parasite lives in upper intestine and cause damage by action of its suction discs and paving the surface of intestinal mucosa Giardia develops into cysts which are intermittently passed in the feces [15]. Malabsorption i.e., decrease absorption of vitamins by the inflamed mucosa. Furthermore, intestinal losses may play a role. So, it can affect vitamin absorption from small intestine, as detected in our study, as well as the parasite effect the liver there for effect on vitamin D3 maturation to active form [15].

**Table 2.** Comparison between infected patients and healthy control Lipase concentration

Groups	Categories	N	Mean $\pm$ S.E	Sig.
Patients	< 1-14	17	45 $\pm$ 0.67 <sup>a</sup>	0.002
	15-29	9	33 $\pm$ 1.22 <sup>c</sup>	
	30-44	10	41 $\pm$ 2.1 <sup>ab</sup>	
	> 45	14	39 $\pm$ 0.99 <sup>b</sup>	
Total	50			
Control		40	17 $\pm$ 0.893 <sup>d</sup>	
L.S.D			5.22	

**Table 3.** Comparison between infected patients and healthy control vitamin D3 concentration

Groups	Categories	N	Mean $\pm$ S.E	Sig
Patients	< 1-14	17	4.11 $\pm$ 0.96 <sup>d</sup>	0.042
	15-29	9	9.08 $\pm$ 2.22 <sup>c</sup>	
	30-44	10	12.23 $\pm$ 4.32 <sup>b</sup>	
	> 45	14	8.71 $\pm$ 2.33 <sup>cd</sup>	
Total	50			
Control		40	44.27 $\pm$ 4.92 <sup>a</sup>	
L.S.D			2.17	

## 5. Conclusions

it is concluded that giardiasis is a significant inflammatory process that results in changes in and vitamin D3 lipid metabolism.

**Conflict of interest:** Nil

**Ethical approval**

This case-control study was approved by the medical ethics committee in the Faculty of Science/ Thi-Qar University (Reference#: MEC-17 on March 3, 2022).

**Author Contributions**

Fatima Assad Baker Al-musawy, data collection, manuscript concept, writing, results analysis. Murtada Hafedh Hussein: Corresponding author, manuscript submission, revision and gallery proof, manuscript concept and writing.

**References**

- [1] Leung, A. K., Leung, A. A., Wong, A. H., Sergi, C. M., & Kam, J. K. Giardiasis: an overview. *Recent patents on inflammation & allergy drug discovery*, 13(2), 2019, 134-143.
- [2] Dann, S. M., Le, C. H., Hanson, E. M., Ross, M. C., & Eckmann, L. Giardia infection of the small intestine induces chronic colitis in genetically susceptible hosts. *The Journal of Immunology*, 201(2), 2018, 548-559. DOI: 10.4049/jimmunol.1700824
- [3] Buret, A. G., Motta, J. P., Allain, T., Ferraz, J., & Wallace, J. L. Pathobiont release from dysbiotic gut microbiota biofilms in intestinal inflammatory diseases: a role for iron?. *Journal of biomedical science*, 26(1), 2019, 1-14.
- [4] AL-kahfaji, M. S. A., & Alsaadi, Z. H. *Giardia Lamblia* and Giardiasis. *Journal of University of Babylon for Pure and Applied Sciences*, 27(5), 2019, 66-74.
- [5] Ankarklev, J., Jerlström-Hultqvist, J., Ringqvist, E., Troell, K., & Svärd, S. G. Behind the smile: cell biology and disease mechanisms of Giardia species. *Nature Reviews Microbiology*, 8(6), 2010, 413-422. DOI: 10.1038/nrmicro2317
- [6] Singer, S. M., Fink, M. Y., & Angelova, V. V. Recent insights into innate and adaptive immune responses to Giardia. *Advances in parasitology*, 106, 2019, 171-208. DOI: 10.1016/bs.apar.2019.07.004
- [7] Frontera, L. S., Moyano, S., Quassollo, G., Lanfredi-Rangel, A., Rópolo, A. S., & Touz, M. C. Lactoferrin and lactoferricin endocytosis halt Giardia cell growth and prevent infective cyst production. *Scientific reports*, 8(1), 2018, 1-15. DOI: 10.1038/s41598-018-36563-1
- [8] Hadi WS, Salman RS, Al-Fahham AA, Faryad Khan MU, Kadir S, Laft MH, Saeed BQ, Kadhum WR, Jalil AT, Kadhim MM. Evaluation of IL-17 and IL-35 in patients with giardiasis in Thi-Qar province, Iraq. *J Med Life*. 2022;15(9):1096-1099. doi: 10.25122/jml-2021-0328.

- [9] Heyworth, M. F. Diagnostic testing for Giardia infections. *Transactions of The Royal Society of Tropical Medicine and Hygiene*, 108(3), 2014, 123-125. DOI: 10.1093/trstmh/tru005
- [10] Al-Abodi, H. R. J. Effect and spread of Giardia parasite on children in primary development stages in southern Iraq. *Biochem. Cell. Arch*, 18(2), 2018, 1537-1541.
- [11] Al-Fahham, A.A. Development of New LSD Formula when Unequal Observations Numbers of Observations Are Unequal. *Open Journal of Statistics*, 8, 2018, 258-263. <https://doi.org/10.4236/ojs.2018.82016>
- [12] Kainyu, R. M., Njagi, E. N., George, O., & Kiruki, S. Establishment of Reference Ranges for Liver Biochemistry Tests in Children in Meru County, Kenya. *Biochem Anal Biochem*, 7(349), 2018, 2161-1009. DOI: 10.4172/2161-1009.1000349
- [13] Abdel-Wareth, L., Haq, A., Turner, A., Khan, S., Salem, A., Mustafa, F., ... & Rajah, J. Total vitamin D assay comparison of the Roche Diagnostics “Vitamin D total” electrochemiluminescence protein binding assay with the Chromsystems HPLC method in a population with both D2 and D3 forms of vitamin D. *Nutrients*, 5(3), 2013, 971-980. DOI: 10.3390/nu5030971
- [14] Al-Malaak, M., Sucker, D. K., & Shnawa, B. H. Histochemical changes on small intestine mucosa infected with Giardiasis in wister Albino rats. *Basrah. J. of veterinary research*, 9(1), 2010, 90-100. DOI: 10.33762/bvetr.2010.55082
- [15] Abed, N. T., Mohamed, N. S., Abdel-Gawad, E. R., & Ibrahim, S. G. Vitamin D status in children with recurrent acute diarrhea. *Int J Curr Microbiol Appl Sci*, 3(11), 2014, 858-868.
- [16] Al-Quraishi, M. A., & Al Hrbey, S. R. Biochemical Study for Patient with Mainprotozoa Diarrheal Agents in Babylon Province, Iraq. *Annals of Tropical Medicine and Public Health*, 23, 2020, 231-208. DOI: 10.36295/asro.2020.231208