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The Effect of *Giardia Lamblia* Infection on the Host Immunity and Inflammatory Cytokines

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Annotation: The Giardia lamblia is an intestinal parasites in humans, it's a flagellate protozoan that lives in the host's upper small intestine. The tissue damage results from the parasite G. lamblia adhering toward the gut, this activate the host's immune system, which releases the chemicals and slow down the adsorption of nutrient. A persistent infection may have an impact on the immune cell population, such as increasing the number of T lymphocytes and encouraging an alternative immunological response. When compared to healthy controls, the increase in MN cells cultivated with raised levels of TNF-a, IL-2, IL-4, and IL-10 exhibited the highest activity, suggesting that these interleukins play a significant role in Giardia lamblia infection.

Keywords: Giardia lamblia, giardiosis, Mononuclear cells.

1. Introduction

Giardia is a parasite infection distributed all over the world. Its causes is the parasite *Giardia lamblia*, which also causes giardiasis, a disease that also affects animals and damages the human small intestine [1] . The virus, which is common around the world and the rate of occurrence varies by the location and is impacted by a number of factors, such as age, social and economic situations,

and health habits [2]. Clinical indicators that follow an injury might range from the onset of numerous pathological symptoms to the absence of any symptoms at all, depending on the parasite strain and the host's immunological response. These symptoms include greasy diarrhea and cramping in the colon, stomach cramps, nausea, decrease in weight, and exhaustion are some of these symptoms [3].

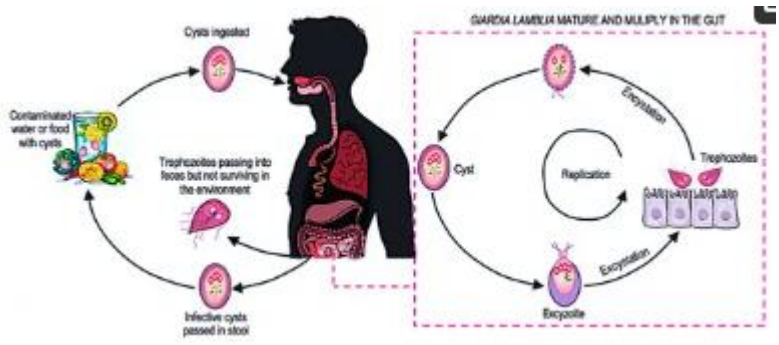


Figure 1. The parasite life after entering inside the body [2] .

The immunological response to *G. lamblia* involves a number of routes, including general responses like phagocytic cell activity. The innate immunity is body defending against *G. lamblia* [4]. Several elements of the innate immune system, including the macrophage, natural killer (NK) cells, dendritic cells (DCs), and neutrophils, rapidly identify *G. lamblia* once they enter the small intestine. To identify the parasite's distinctive molecular patterns (PAMPs), required a sets off a series of defensive responses, triggering signaling pathways within the cells [5] . Along with chemokine's and other inflammatory mediators, these pathways result in the processing of some cytokines such as IL-2, IL-4, TNF-α, IL-10, and interferon's [6]. Additionally, monocytes and macrophages which participate in phagocytosis are the primary immune cells that eliminate pathogens. Macrophages are essential for parasite management, as evidenced by their recruitment in *G. lamblia* infections. Furthermore the activation of the adaptive immune response involves both T and B cells and cytokines which directly contribute to the immunity profile and depending on the stimuli which could identify the various immune responses against the parasite [7] .

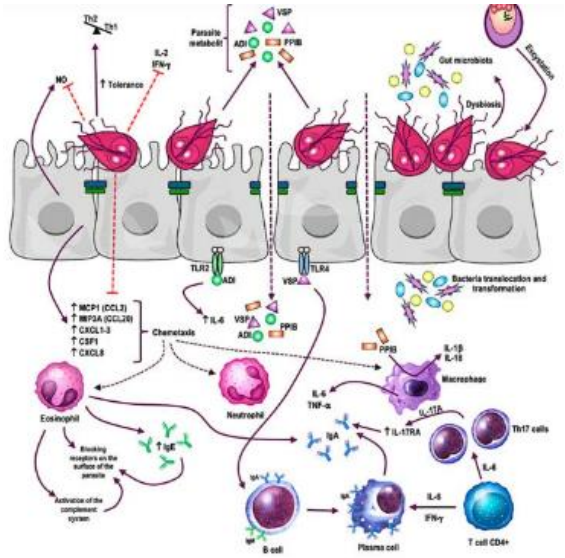


Figure 2. The immunity response against the *Giardia lamblia* [8]

However, it is still unclear how these signaling pathways function in these cells during interactions with *G. lamblia* and how these cytokines' effector mechanisms contribute to the cellular activation. The impact of cytokines on phagocytes in protozoan infections is little understood, despite the fact that phagocytic cells are essential in giardiasis [9]. The age of the patient and any underlying medical conditions may affect the immune system abnormalities brought on by giardiasis. This may be the

cause of some people's delayed giardiasis diagnosis. The levels of cytokines and the activity of MN cells with the cytokine production were investigated in this work to determine the effect of this parasite on host immunity [10].

2. Materials and Methods

2.1 The serological analysis of *G. lamblia*

Five milliliters of blood were drawn from each participant who attending the Baghdad Medical City at Laboratory department between 48 and 72 hours. To extract the serum, the samples were put in anticoagulant-free tubes and centrifuged for 15 minutes. For the purpose of determining the *G. lamblia* serology, the serum samples were kept at -80°C [11]. The ELISA test (enzyme-linked immunosorbent assay) was used to measure the activity of IgM and IgG antibodies to *G. lamblia* in the serum. Additionally, 5 ml of blood samples were collected from both healthy people and the control group and patients who were confirmed to have giardiosis. The serum was prepared by centrifuging them at 3000 rpm for five minutes. The serum then separated into four sections, each of which was stored to determine the levels of cytokines [12] .

2.2 ELISA test evaluation of TNF-a, IL-2, IL-4, and IL-10

The (ELISA) test was used to measure the levels of serum interleukins (IL-2, IL-4, TNF-a, and IL-10) in both patients with confirmed giardiosis and seemingly healthy controls in accordance with the manufacturer's [13]

2.2.1 Detection of IL-2, IL-4

The IL-2 and IL-4 kits' directions were followed while bringing frozen blood samples to room temperature and properly mixing them. Each well received 100 µl of the assay diluent, 100 µl of the control, and 100 µl of the sample and shaken at 3000 rpm for two hours at room temperature (25°C) [14]. About 0.4 ml of the washing solution was poured to wash the plate twice, and the contents of each well were then aspirated. Each well received 200 µl of interleukin conjugate. Fifty µl of solution with substrate was mixed, then incubated for 1 hour at room temperature 25°C after being cleaned up and incubated for 2 hours. Finally 50 µl of stop solution was added, the absorbance was estimated at 490 nm using an ELISA microplate reader [15] .

2.2.2 Identification of TNF-a and IL-10

Following the guidelines provided by the US Biological IL-10 and TNF-a kit procedure, practical work was completed. The antibody-coated microtiter plate was filled with 100 microliters of each standard and sample and it was then allowed to sit at room temperature for an hour [16]. About 50 µl of biotin was applied to each well without discarding the standards or samples. The plate was then washed to get rid of any unconjugated antibodies after it had been incubated for an hour. After adding 100 µl of the Avidin attached with HRP enzyme to each well. About, 100 µl of the substrate was added about a 15-minute stand period at in the dark , and 100 µl of stop solution was mixed . Each TNF-a and IL-10 concentration was determined [17].

2.3 MN cell separation and blood samples

Eight milliliters of blood were extracted from patients and put in tubes with EDTA for fifteen minutes, the samples were centrifuged to prepare the plasma. The cells were prepared from a (Ficoll-Paque gradient) and then examined under a light microscope. 200 µL of acridine orange was used to color the sediment for one minute after the supernatant was disposed in a fluorescence microscope was used to estimate the MN cells [18] .

2.4 Statistical analysis

The SPSS statistical (version 20) was used to the statistical analysis, the data was subjected to analysis of variance (ANOVA). The mean standard error is used to display the data. The least significance difference (LSD) was used to evaluate group mean differences, and $P \leq 0.05$ considered statistically significant [19] .

3. Results and Discussion

An infectious condition called giardiosis is found worldwide, but it is more common in third-world nations like Iraq. According to our study's findings, there are gender-specific variations in infection rates, as shown in Table 1, as the males significantly higher than females. These result was consistent with multiple result for many research that noted that there are more males than females infected with *G. lamblia*. Males are more contact with their surroundings than females, which is the reason for the rise in the rate of infection in males [20].

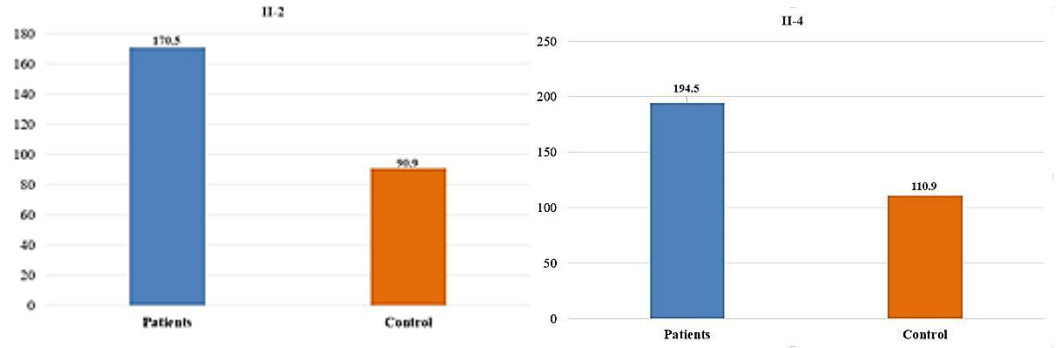
Table 1: The infection duration according to gender

Gender	The participate	The patients with positive sample	The rate of infection
Male	200	79	39.5%
female	150	57	28.5%
total	350	136	38.85%

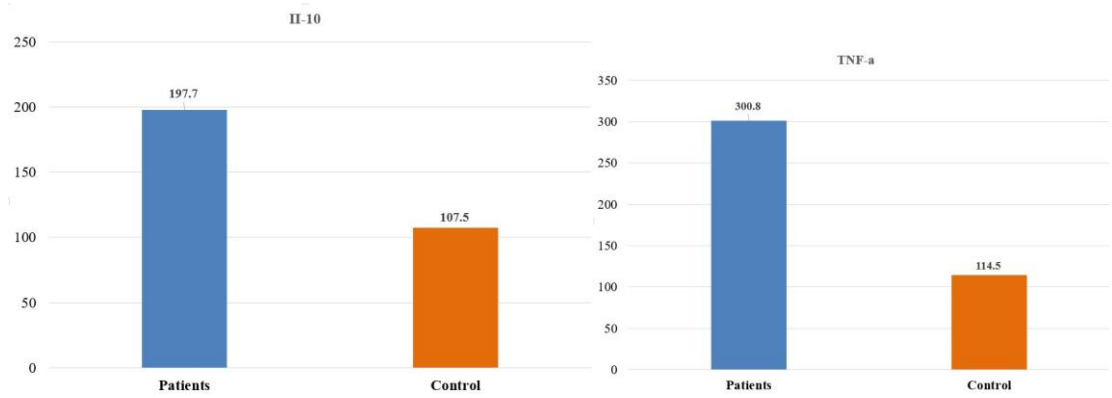
According to these findings the patients the levels of interleukins (IL-2, IL-4, TNF-a, and IL-10) were higher than those of the healthy control group (Table 2) P≤0.05 indicates that this rise was statistically significant (Table 2).

Table 2: The compared levels of (IL-2, IL-4,TNF-a and IL-10) between patients and control .

Parameter	Patients	Controls	P value
Il-2 (pg/ml)	170.5±11.31	90.9±2.14	≤0.05
Il-4 (pg/ml)	194.5±13.44	110.9±4.64	≤0.05
Il-10 (pg/ml)	197.7±3.15	107.5±12.2	≤0.05
TNF-a (pg/ml)	300.8±15.69	114.1± 5.561	≤0.05



(Figure 3.1) Shows the different levels of (IL-2 and IL-4) between patients and controls



(Figure 3.2) Shows the different levels of (IL-10 and TNF-a) between patients and controls

The eosinophils, basophils, and mast cells are all activated by IL-4 which represent a lower concentration in patients as compared with healthy controls (Table 3). It has been demonstrated to suppress the IFN-γ which inhibit the ability of macrophages to kill the intracellular and extracellular parasites, and the immune-mediated host tissue damage were stopped in response to this cytokine [21] .

Table 3: The monocytes number in patients compared to healthy

parameter	The number of participate	MN cells / mm3	P value
Infected	136	5.231 ±8.0654	≤0.05
Control	136	9.298 ± 1.3256	≤0.05

Although the defense mechanisms in charge of containing giardia are not fully known, multiple studies have shown the development of acquired immunity mechanisms [22]. The response to this infections, including giardia, is dependent on both the natural and acquired immune response [23]. This study explains the relationship between the concentration of MN phagocytes and the levels of the cytokines IL-2, IL-4, TNF-a, and IL-10, as well as the impact of these cytokines on parasite infection. Numerous studies have documented the function of MN cells and the effector pathways in the response to *G. lamblia* [24]. Since many cells activated by the cytokines show higher microbicidal activity, a link between cytokines and the cytotoxic activity of MN cells against *G. lamblia* has been proposed [25]. These cytokines exhibited the greatest levels and increased phagocytic activity in the current investigation because they enhance their microbicidal capability by upregulating the surface receptor expression and the phagocytosis rates [26] . The early control of *G. lamblia* infection by the parasite elimination may be facilitated by the high production of cytokines, which trigger the synthesis of mediators that can activate the phagocytic cells [27] . The current study indicates that the cytokine is a crucial immune mediator that controls the proliferation of macrophages and benefits the host by stimulating MN cells. But this cytokine's role in MN phagocyte-parasite interactions might be linked to its mucosal immunity activity and direct impacts on phagocytes and IgA production [28]. These MN cells may enhance the intestinal mucosa's secretory production of IgA and directly contribute to the removal of parasites once the cytokine is activated .Thus, cytokines had an impact on the phagocytes' microbicidal activity [29] .

4. Conclusion

This work demonstrated how cytokines modulate the phagocyte functional activity and highlights the significance of cytokines' interaction with blood cells and soluble components in the removal of *G. lamblia*.

Reference

1. hang, Q.-Y.; Yan, Z.-B.; Meng, Y.-M.; Hong, X.-Y.; Shao, G.; Ma, J.-J.; Cheng, X.-R.; Liu, J.; Kang, J.; Fu, C.-Y. Antimicrobial peptides: Mechanism of action, activity and clinical potential. *Mil. Med. Res.* 2021, *8*, 48.
2. Zhao, P.; Li, J.; Li, X.; Dong, J.; Wang, X.; Zhang, N.; Li, S.; Sun, M.; Zhang, X.; Wang, Z.; et al. The NLRP3 inflammasome recognizes alpha-2 and alpha-7.3 giardins and decreases the pathogenicity of *Giardia duodenalis* in mice. *Parasites Vectors* 2023, *16*, 85
3. Fernández-Lainez, C.; de la Mora-de la Mora, I.; Enríquez-Flores, S.; García-Torres, I.; Flores-López, L.A.; Gutiérrez-Castrellón, P.; de Vos, P.; López-Velázquez, G. The Giardial Arginine Deiminase Participates in Giardia-Host Immunomodulation in a Structure-Dependent Fashion via Toll-like Receptors. *Int. J. Mol. Sci.* 2022, *23*, 11552.
4. Unterberger, S.; Mullen, L.; Flint, M.S.; Sacre, S. Multiple TLRs elicit alternative NLRP3 inflammasome activation in primary human monocytes independent of RIPK1 kinase activity. *Front. Immunol.* 2023, *14*, 1092799.
5. Evans-Osses, I.; Ansa-Addo, E.A.; Inal, J.M.; Ramirez, M.I. Involvement of lectin pathway activation in the complement killing of Giardia intestinalis. *Biochem. Biophys. Res. Commun.* 2010, *395*, 382–386
6. Amezcua Vesely, M.C.; Bermejo, D.A.; Montes, C.L.; Acosta-Rodríguez, E.V.; Gruppi, A. B-Cell Response during Protozoan Parasite Infections. *J. Parasitol. Res.* 2012, *2012*, 362131
7. Khalaf, M.M.; Hussein, M.H.; Hafedh, A.A. Evaluation of IL-2, IL-4 and IL-10 levels in patients with giardiosis. *Ann. Parasitol.* 2021, *67*, 697–702
8. Exum, N.G.; Pisanic, N.; Granger, D.A.; Schwab, K.J.; Detrick, B.; Kosek, M.; Egorov, A.I.; Griffin, S.M.; Heaney, C.D. Use of Pathogen-Specific Antibody Biomarkers to Estimate Waterborne Infections in Population-Based Settings. *Curr. Environ. Health Rep.* 2016,
9. Li, J.; Casanova, J.-L.; Puel, A. Mucocutaneous IL-17 immunity in mice and humans: Host defense vs. excessive inflammation. *Mucosal Immunol.* 2018, *11*, 581–589.
10. Inger, S.M.; Fink, M.Y.; Angelova, V.V. Recent insights into innate and adaptive immune responses to Giardia. *Adv. Parasitol.* 2019, *106*, 171–208.
11. Shi, C.; Cheng, M.; Yang, X.; Lu, Y.; Yin, H.; Zeng, Y.; Wang, R.; Jiang, Y.; Yang, W.; Wang, J.; et al. Probiotic *Lactobacillus rhamnosus* GG Promotes Mouse Gut Microbiota Diversity and T Cell Differentiation. *Front. Microbiol.* 2020, *11*, 607735.
12. Khattak, I.; Yen, W.-L.; Usman, T.; Nasreen, N.; Khan, A.; Ahmad, S.; Rehman, G.; Khan, K.; Said, M.B.; Chen, C.-C. Individual and Community-Level Risk Factors for Giardiasis in Children under Five Years of Age in Pakistan: A Prospective Multi-Regional Study. *Children* 2023, *10*, 1087.
13. Bhargava, A., Cotton, J. A., Dixon, B. R., Gedamu, L., Yates, R. M., and Buret, A. G. (2015). Giardia duodenalis surface cysteine proteases induce cleavage of the intestinal epithelial cytoskeletal protein villin via myosin light chain kinase. PLoS One 10:e0136102. doi: 10.1371/journal.pone.0136102
14. Haidar, Z.; Fatema, K.; Shoily, S.S.; Sajib, A.A. Disease-associated metabolic pathways affected by heavy metals and metalloid. Toxicol. Rep. 2023, 10, 554–570
15. Al-Aboody B.A., Al-Rumaidh Sh.Z., Abdul-Alhasan A.S. 2017. Investigation of infection of intestinal parasites Entamoeba histolytica and Giardia lamblia among patients which attending of the Health Centers of Gharraf City/Thi-Qar province.Journal of Thi-Qar Science 6: 25–29.

16. Ouattara M., N'guessan N.A., Yapi A., N'goran E.K.2010. Prevalence and spatial distribution of Entamoeba histolytica and Giardia lamblia among school children in Agboville Area. PLOS Neglected Tropical Diseases. doi:10.1371/journal.pntd.0000574

17. Al-Kahfaji M.S.A., Al-Masoudi H.K., Almosawy A.M. 2019. Serum interleukins (IL-4, IL-10) and immunoglobulin a as biomarkers in patients with giardiasis. Plant Archives 19: 1932–1934.

18. Sanchez A.L., Mahoney D.L., Gabrie J.A. 2015.Interleukin-10 and soil-transmitted helminth infections in Honduran children. BMC Reseach Notes Evaluation of IL-2 7018: article number 55. doi:10.1186/s13104-015-1019-x

19. Coffey, C. M., Collier, S. A., Gleason, M. E., Yoder, J. S., Kirk, M. D., Richardson, A. M., et al. (2021). Evolving Epidemiology of Reported Giardiasis Cases in the United States, 1995–2016. Clin. Infect. Dis. 72, 764–770. doi: 10.1093/cid/ciaa128

20. Cheng, L., Zhang, Z., Li, G., Li, F., Wang, L., Zhang, L., et al. (2017). Human innate responses and adjuvant activity of TLR ligands in vivo in mice reconstituted with a human immune system. Vaccine 35, 6143–6153. doi: 10.1016/j.vaccine.2017.09.052

21. Cromarty, R., Sigal, A., Liebenberg, L. J. P., McKinnon, L. R., Abdool Karim, S. S., Passmore, J. A. S., et al. (2019). Diminished HIV Infection of Target CD4+ T Cells in a Toll-Like Receptor 4 Stimulated in vitro Model. Front. Immunol. 10:1705. doi: 10.3389/fimmu.2019.01705

22. Akram, R.S. (2018). Molecular detection and prevalence of Giardialamblia,Entamoeba histolyticaand Cryptosporidium parvumamong patients with diarrhea at Al Rifea city. (Thesis),University of Thi-Qar College of Science.

23. Al-Ammash, M.S.J. (2015). Study on prevalences of Entameoba histolytica and Giardia lamblia in Samarra city. Kufa Journal for Veterinary Medical Sciences, 6(2), 194-204.

24. Al-Difaie, R.S.(2016). Molecular Study to Detect Genotyping of Giardia lambliafrom Human and Cattle Feces in Al -Qadisiya Governorate, Iraq. Ibn Al-Haitham Journal for Pure and Applied Sciences, 29(3), 1-13.

25. Bazzaz, A.A.,Shakir, O.M., & Alabbasy, R.H. (2017). Prevalence of two gastrointestinal parasites Enta-moeba histolytica and Giardia lamblia within Samarra city, Iraq.Advances in Bioscience and Biotechnolo-gy,8(11), 399-410.

26. Ejaz, M., Murtaza, G., Ahmad, M., Khan, S.A., Najam-us-Saqib, Q., Asad, M.H.H.B., & Hussain, I. (2010). Determination of the prevalence of Entamoeba histolytica in human at a private fertilizer company hospital in Pakistan using microscopic technique.African Journal of Microbiology Research,5(2), 149-152.

27. El-Tantawy, N.L., & Taman, A.I. (2014). The epidemiology of Giardia intestinalis assemblages A and B among Egyptian children with diarrhea: A PCR-RFLP-based approach. Parasitologists United Jour-nal,7(2),104–109.

28. Einarsson, E., Ma'ayeh, S., & Svärd, S.G. (2016). An up-date on Giardia and giardiasis.Current opinion in microbiology,34, 47-52.

29. Faria, C.P., Neves, B.M., Lourenço, Á., Cruz, M.T., Martins, J.D., Silva, A., & Do Céu Sousa, M. (2020). Giardia lamblia Decreases NF-κB p65 RelA Protein Levels and Modulates LPS-Induced Pro-Inflammatory Response in Macrophages.Scientific reports,10(1), 1-17.