

## Study of Some Epidemiological and Immunological Aspects on Patients Infected with Giardiasis in Thi-Qar Province

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### ABSTRACT

The current study was aimed to find the correlation between giardiasis and some Epidemiological aspects such as (Sex, Age) among infected patients and explore the effect of *G. Lamblia* infection on some immunological biomarkers such as IL-4, TNF- $\alpha$ . Among 225 fecal samples were collected from patients with diarrhea from Thi-Qar hospitals after microscopic examination in order to diagnose the parasite. During the period of October 2019 to January 2021. The results of present study were not significant between female and male, high infection was noted in males 52.8% more than females (47.2%). However, this result was not significant., while The present study showed The highest frequency of infection of *Giardia lamblia* (36.1 %) was found at age group (1 - 10 years old), while the lowest (11.1%) was recorded at ages (21-30) years old. the present study showed significant ( $P \leq 0.05$ ) increasing in Interlukine-4 The result of IL-4 and Tummor necrosis factor-alfa (TNF- $\alpha$ ) concentration were rising in serum levels of infected group compared with control group and TNF- $\alpha$ .

### KEYWORDS

Giardia Lamblia, IL-4, TNF- $\alpha$ , ELISA.

### Introduction

*Giardia lamblia* is one of intestinal flagellated parasite return to protozoan phylum, Giardiasis disease can infects small intestine in human, wild and domestic animals. (Serradell *et al.*, 2018). *Giardia* can infects individuals in any age stage Adult and childhood. The healthy individuals may carry the parasite with no symptoms whereas the children more considerably apparent signs and symptoms of this parasitic disease that consider a prevalent infection of childhood age in the developing countries (Zemene and Shiferaw., 2018). The infections are more recurrent and acute in the young children, especially in child health day-care centers, and travelers, passengers, and martial personnel in the domain. Transmission of *Giardia lamblia* to humans by ingestion the cysts of parasite out of contaminated food and drinking water (Shahnazi *et al.*, 2019) The signs and symptoms pass after a 1–2 weeks after ingestion (incubation period). (Thomas Iv *et al.*, 2014). An predestined 280 million patients of indicative giardiasis every year in worldwide. (Esch and Petersen 2013) Giardiasis is recognized by a broad range of clinical manifestations, like vomiting severe diarrhea, intestinal pain, malabsorption syndrome and weight loss, in human is especially recognize by weak or no inflammation in mucosa. (Einarsson *et al.*, 2016). The chronic Giardiasis disease is related with stunting. (less height according to age), wasting (less weight for height) and cognitive failure on children in the developing countries. and can elicit protracted post-infectious syndromes, including irascible bowel syndrome and chronic fatigue (Hanevik *et al.*, 2014). villus and brush border microvillus leanness are current, leading to deficiencies in digestive enzymes and chronic lamblasis can lead to obvious mucosa inflammation and with damage on villus. (Miyamoto and Eckmann,2015). The disease riskiness can depend on multiplied parasite and host linked factors.so, Some cases, specially the variant specific surface proteins (VSPs) have been suggest to be engaged in pathogenicity, but antigenic variance is obviously response for the chronic Giardiasis infection. So, the stimulus causing VSP switching still evasive. (Serradell *et al.*, 2018). Symptoms of giardiasis disease are less severe in the endemic region than non-endemic, that suggest progressive buildup of immune response (Miyamoto and Eckmann,2015). The parasite progressing sophisticated, just now uncompletely disclosed. The mechanisms to escape immune response and development in the intestine. The developing of defensive immunity to *G. lamblia* depend not only on (B cell - mediated antibody produce and (T cell – mediate immune response). (Faria *et al.*, 2020). The cytokines participate in innate and adaptive immune response via receptors and cytokines can modulate balance between cellular and humoral immune response, also facilitate correlation among the cells and enhance the production of antigen-specific effector cells, cytokine also mediate systemic and local inflammation. (Popko *et al.*, 2010). But else on induction of the

interleukin 17 A (IL-17A) in intestinal response. It is clear now that up regulation of IL-17A is need to releasing of IgA to the lumen of intestine in order to the production of anti-microbial peptides, The regulate of complement activation become utmost relevance during the acute symptomatic Giardiasis infection in human. (Paerewijck *et al.*, 2017). The secretory IgA intestinal hypermobility (Miyamoto and Eckmann,2015), anti-microbial peptides and nitric oxide compound (Tako *et al.*, 2013) all have been shown, the direct effector activity against parasite, although their related contributions to clear of *Giardia* may be difference. (Miyamoto and Eckmann,2015). Giardiasis -Specific immunity response also have been inspected in humans. In on study, the human CD<sup>4+</sup> cells in both intestinal and blood were stimulated by trophozoites of *Giardia*. The IFN- $\gamma$  secretion was increased and cells proliferation in order to response against trophozoites, That suggest *Giardia* - specific proliferation of CD<sup>4+</sup> T cells also exists in humans. The CD<sup>4+</sup>T cell which is the memory immune response in peripheral blood mononuclear cells for human were investigated 5 years after wide prevalence of *Giardia* occurred. *Giardia*-specific CD<sup>4+</sup> T cell response presents by up regulation of activation of markers surface (CD25 /CD26 and CD45RO /HLA-DR) and higher proliferation of (T-cells) in *Giardia* exposed person compared with control. (Saghaug *et al.*, 2016). Currently, different genotype (A-H) identify for *G. intestinalis*. Within these genotypes, A and B assemblages have the potential to infect human and mammalian animals, but other genetic groups (C-H) often show specificity for animals. The human isolates are located in two large genotype groups pedantic as A and B in which subgroups (AI, All, BIII, and BIV) are essentially relate with human isolates. (Shahnazi *et al.*, 2019).

## Material and Methods

### 1-Collection of Data

The present study was performed on 225 patients sever from sever diarriah in the hospitals and special laboratories in Thi-Qar province. Information from 225 infected patient with Giardiasis were collected about sex and age for the period from october 2019 to January 2021.

### 2- Diagnosis Giardia Lamblia

By direct smear method and sedimentation technique

### 3-Using (ELISA)

The Enzyme-linked Immuno-Sorbent Assay technique to find the concentration of Cytokines (IL-4 and TNF- $\alpha$ ).

### Assay Procedure

We used 100  $\mu$ L of the standard or sample to each well. Then Incubate for 90 minute at (37°C), after that remove liquid, then add (100  $\mu$ L) of HRP Conjugate. Incubate for 30 min at 37°C. Also aspirate and wash 5 times, then add 90  $\mu$ L of Substrate Reagent solution. Incubate for 15 min at (37°C), finally add 50  $\mu$ L Stop Solution and Read at (450 nm) directly. Calculation of results.

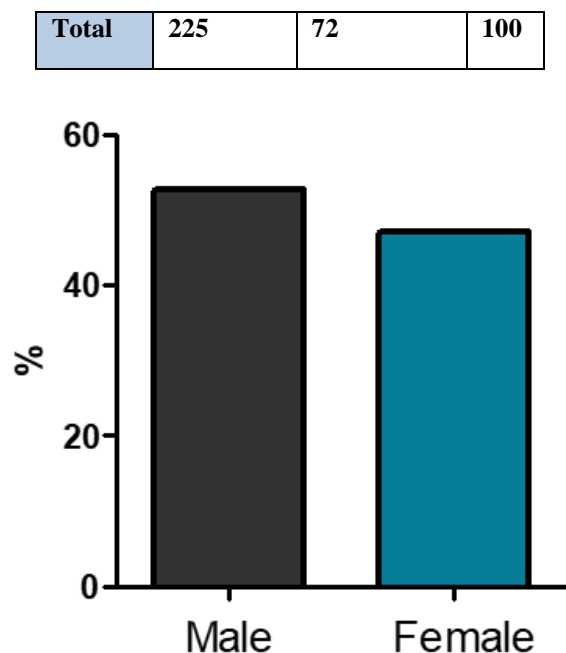
## Result

1- The result of 225 examined diarrheal stool specimens, 72 (32 %) were found to be infected with *Giardia Lamblia*. by microscopic examination. Of these 72 positive samples, 38 (52.8%) were male and 34 (47.2%) were female. The sex showed insignificant association with giardial diarrhea ( $P > 0.05$ ) as in table (1) and figure (1).

**Table 1.** Distribution of giardiasis infection depending on gender

Gender	Exam No.	Infected No.	%
Male	117	38	52.8
Female	108	34	47.2

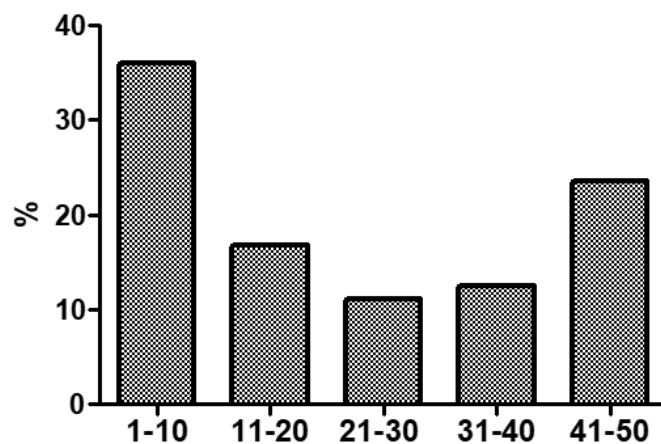
P=0.6

**Figure 1.** Relationship between *G.lamblia* infection and gender

2- The results of infection regarding to the age, of current study showed the highest infection ratio was detected at age ( 1-10 years old) with almost 36.1% while the lowest recorded at age (21-30 years old) 11.1%, The results appears that there was a significant correlation between Age groups and infection of *G. lamblia* at ( $P<0.05$ ) as in table (2) and figure (2).

**Table 2.** Distribution of giardiasis according to age groups

Age	Exam No.	Infection No.	%
1-10	79	26	36.1
11-20	29	12	16.7
21-30	37	8	11.1
31-40	34	9	12.5
41-50	46	17	23.6
<b>Total</b>	<b>225</b>	<b>72</b>	<b>100</b>
t=4.372 df=4 P=0.0119			

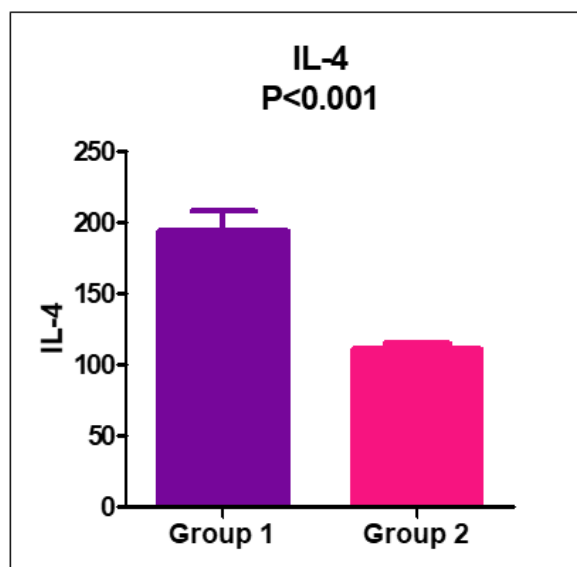
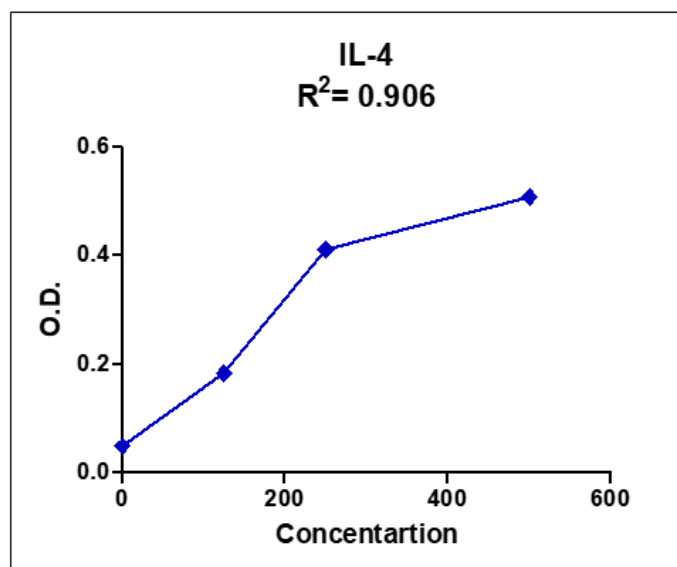


**Figure 2.** Showed related between the rate *Giamblia* infection and the age groups

3- The present results showed the interleukin levels (IL-4) of infected group was higher than those of controls group (Table 3 and figure 3). infected group which recorded  $194.5 \pm 13.44$  pg/ml concentration compared to the control which was  $110.9 \pm 4.648$  pg/ml. The increase ratio of IL-4 was appears statistically significant ( $P \leq 0.05$ ).

**Table 3.** Comparison between patients and control groups interleukin concentration

IL-4 (pg/ ml)		
Groups	No.	Mean $\pm$ S.E.M
Giardiasis infected groups	30	$194.5 \pm 13.44$
Control	30	$110.9 \pm 4.648$
P.value	< 0.0001	
Sig	* * *	
t = 5.883	df= 58 $p \leq 0.05$	

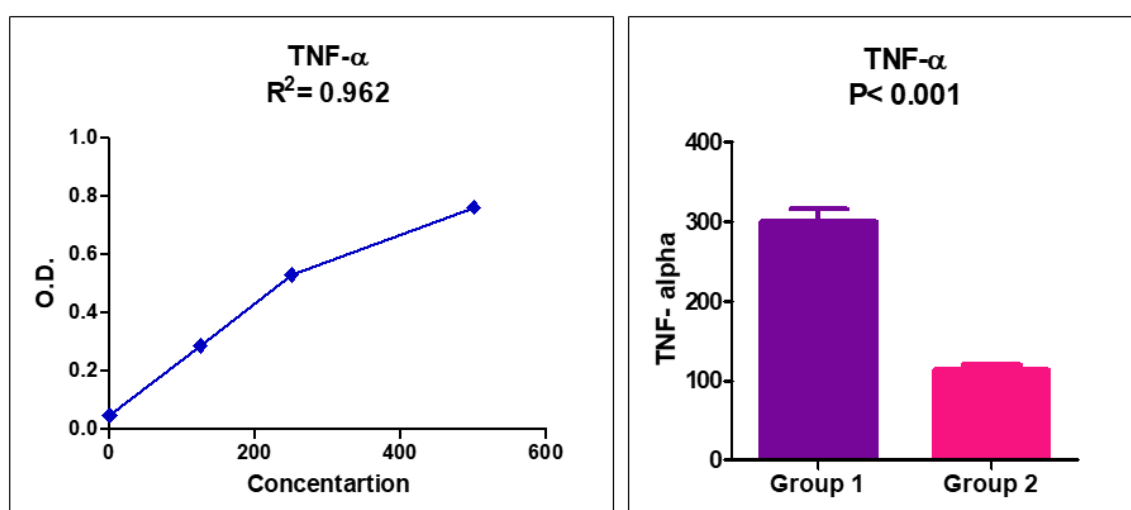


**Figure 3.** Effect of *Giardia* on the levels of interleukin (IL-4)

4-Our results of TNF- $\alpha$  showed significant association ( $P \leq 0.05$ ) between infected group which recorded  $300.8 \pm 15.69$  pg/ml concentration and control group which recorded  $114.1 \pm 5.561$  pg/ml. As in table (4) and figure (4).

**Table 4.** TNF- $\alpha$  concentration in study groups

TNF – alpha ( pg/ ml )		
Groups	No.	Mean $\pm$ S.E.M
Giardiasis infected groups	30	300.8 $\pm$ 15.69
Control	30	114.1 $\pm$ 5.561
P.value	< 0.0001	
Sig	* * *	
t = 11.22                      df= 58 p $\leq$ 0.05		



**Figure 4.** Effect of *Giardia* on the levels of TNF- $\alpha$

## Discussion

Giardiasis is one of parasitic disease that spread around the world, particular in the third world countries like Iraq, that severe from poor in health system and living conditions (Al-Difaie, 2016).

In the current study, the prevalence rate of giardiasis in human was (32 %). This result is agree with 33.12% reported by (El-Tantawy and Taman, 2014) in Egypt and (Al-Kahfaji *et al.*, 2019) in Babylon province, Iraq when they recorded rates of infection 34%.The rate of giardiasis in the present study was highest than results which was reported by (Yasir, 2020 ) in Thi-Qar province, and (Sánchez *et al.*, 2017) in Colombian Amazon Basin, when they recorded infection rate 18.75%, 23.7% respectively. The variation in the prevalence ratio of human giardiasis could be current according to several factors such as socio economic education, sanitation system and size of samples, the different between studies designs and using techniques, in addition to environment and water supply sources, kinds of the food and healthy hygiene, and presence of the domesticated and wild animals. As well as economic level all classified as having a role in the probability of parasite infection. (Thapar and Sanderson, 2004).

The result of infection rate recorded (32 %), The positive samples involved of (38) male (52.8%) and (34) female (47.2%), the results showed no significant difference between infection rates according to sex. This result was agree with the results of (yasir, 2020) in Thi-Qar, Iraq, (Al-Ammash, 2015) in Samarra, Iraq and (Zylberberg *et al.*, 2017) in united states patients with giardiasis were more likely to be male (57.8% vs 34.1%). Men are more closed to giar-

diarrhea disease due to spend most of their time out of house also drinking water may contaminated of *Giardia* cysts, therefore they would be more exposed to infection.

This results was disagreed with result of (**Bazzaz et al., 2017**) in Samarra, when they recorded high rate of infection in females.

The highest infection ratio was recorded at ages (1-10) year old, while the lowest was recorded at age >40 years old. This result was in accordance with result of (**AKram, 2018**) which reported the highest rate of *G.lambilia* (48.8%) in (1-10 years) age group, while lower rate in (31-40) This result was disagreed with (**Punsawa et al., (2017)** when they recorded high rate of infection in (41-50). This is a clear indication of hygienic correlated cause where children are more sensible to be bared to unhealthy food than the adults or because immune system less developing than adults. (**Stark et al., 2007**) child's lack of committing to many rules of hygiene oral movements of the random and non-washing and no sterilization of the hands required and the periodic and random activity as well as their contaminated with animals Such as cats and birds, also the lacking of full therapeutic doses for giving them to the child and also the impairment of the immune system (**Ejaz et al., 2011**).

Newly, the Center for the Disease Control and Prevention (CDC) in USA government public health agency changed to the prospect of major risk create from travelers to countries where giardiasis disease is widespread, people in child care setting, carriers of parasite, contaminated or non-treated drinking water, more traveling man, campers and people which contact to infected animals. (**Heyworth, 2016**).

In spite of being knowing about *G. lamblia* parasite and its effective on humans and animals, The mechanisms of the host's immune system controls the parasitic infection and different cytokines correlated with this immune response are not fully understand. These cytokines also have main role when designing new vaccines and drugs against this parasitic infection. (**Jiménez et al., 2014**).

The results of this study showed increasing levels of the cytokines (IL-4 and TNF-  $\alpha$ ) in infected group than those of control group. The increase ratio of IL-4 and TNF-  $\alpha$  was statistically significant ( $P \leq 0.05$ ) The results of the present study was in agree with many authors worldwide (**Mahmuod et al., 2018; Al-Kahfaji et al., 2019**) this Results was incompatible with (**Mitra et al., 2012**).

IL-4, this cytokine is essential enhance Th2 cells response and suppress Th1 differentiation major interleukine responsible for the aggregate the eosinophil population in enteric protozoal infection is interleukin-4 and has an essential role. In *G. lamblia* infection, most of the cytokines are produce by CD<sup>4+</sup> cells of Peyer's patches or created from the mucosa of the lymphoid tissue (MALT) owing to long period of antigenic stimulation via the parasite. (**Al-Kahfaji et al., 2019**).

The High level of TNF - $\alpha$  was shown correlation with *Giardia lamblia* diarrhea. TNF- $\alpha$  trigger the cytotoxic activity by enhance neutrophils and macrophages to release NO and ROS to fight the parasite and stimulates the phagocytosis. TNF- $\alpha$  also, is one of pro-inflammatory cytokine that mediate the inflammation and stimulates acute phase proteins. (**Lin et al., 1994**).

Its clear that *Giardia*-derived antigens increasing the production of TNF- $\alpha$  which is the main regulator of pro-inflammatory cytokine production. (**Parameswaran and Patial, 2010**). In addition, the important role of TNF- $\alpha$  in early control of *Giardia* infection was reported by **Matowicka-Karna et al., 2009**.

## Conclusion

The results of this study showed that the Giardiasis disease is common among Thi-Qar population especially in children caused immunological response includes both cellular and humoral immune response in infected patients such as inflammatory mediators IL-4 and TNF- $\alpha$  compared with control.

## References

- [1] Akram, R.S. (2018). Molecular detection and prevalence of *Giardia lamblia*, *Entamoeba histolytica* and *Cryptosporidium parvum* among patients with diarrhea at Al Rifea city. (Thesis), University of Thi-Qar College of Science.
- [2] Al-Ammash, M.S.J. (2015). Study on prevalences of *Entamoeba histolytica* and *Giardia lamblia* in Samarra city. *Kufa Journal for Veterinary Medical Sciences*, 6(2), 194-204.
- [3] Al-Difaie, R.S. (2016). Molecular Study to Detect Genotyping of *Giardia lamblia* from Human and Cattle Feces in Al -Qadisiya Governorate, Iraq. *Ibn Al-Haitham Journal for Pure and Applied Sciences*, 29(3), 1-13.
- [4] Al-Kahfaji, M.S.A., Al-Masoudi, H.K., & Almosawy, A.M.A. (2019). Serum Interleukins (IL-4, IL-10) and immunoglobulins as biomarkers in patients with giardiasis. *Plant Archives*, 19(2), 1932-1934.
- [5] Bazzaz, A.A., Shakir, O.M., & Alabbasy, R.H. (2017). Prevalence of two gastrointestinal parasites *Entamoeba histolytica* and *Giardia lamblia* within Samarra city, Iraq. *Advances in Bioscience and Biotechnology*, 8(11), 399-410.
- [6] 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.
- [7] 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 Journal*, 7(2), 104-109.
- [8] Einarsson, E., Ma'ayeh, S., & Svärd, S.G. (2016). An up-date on *Giardia* and giardiasis. *Current opinion in microbiology*, 34, 47-52.
- [9] Esch, K.J., & Petersen, C.A. (2013). Transmission and epidemiology of zoonotic protozoal diseases of companion animals. *Clinical microbiology reviews*, 26(1), 58-85.
- [10] 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- $\kappa$ B p65 RelA Protein Levels and Modulates LPS-Induced Pro-Inflammatory Response in Macrophages. *Scientific reports*, 10(1), 1-17.
- [11] Hanevik, K., Wensaas, K.A., Rortveit, G., Eide, G.E., Mørch, K., & Langeland, N. (2014). Irritable bowel syndrome and chronic fatigue 6 years after giardia infection: a controlled prospective cohort study. *Clinical Infectious Diseases*, 59(10), 1394-1400. <https://doi.org/10.1093/cid/ciu629>.
- [12] Heyworth, M.F. (2016). *Giardia duodenalis* genetic assemblages and hosts. *Parasite*, 23.
- [13] Jiménez, J.C., Fontaine, J., Creusy, C., Fleurisse, L., Grzych, J.M., Capron, M., & Dei-Cas, E. (2014). Antibody and cytokine responses to *Giardia* excretory/secretory proteins in *Giardia intestinalis*-infected BALB/c mice. *Parasitology research*, 113(7), 2709-2718.
- [14] Lin, J.Y., Seguin, R., Keller, K., & Chadee, K. (1994). Tumor necrosis factor alpha augments nitric oxide-dependent macrophage cytotoxicity against *Entamoeba histolytica* by enhanced expression of the nitric oxide synthase gene. *Infection and immunity*, 62(5), 1534-1541.
- [15] Mahmoud, A., Bakir, H., Mohamed, Y., Zaid, M., & El-Mokhtar, M. (2018). Assessment of the intestinal immune response in *Giardia duodenalis* experimentally infected rats using quantitative real-time PCR. *Parasitologists United Journal*, 11(2), 75-81.
- [16] Matowicka-Karna, J., Dymicka-Piekarska, V., & Kemon, H. (2009). IFN- $\gamma$ , IL-5, IL-6 and IgE in patients infected with *Giardia intestinalis*. *Folia Histochemica et Cytobiologica*, 47(1), 93-97.
- [17] Mitra, Z., Nasrin, D., Nahid, E., & Arezoo, J. (2012). Evaluation of Cytokines Changes in Patients Infected with *Giardia lamblia* In Comparison with Healthy Subjects. *Danashki Jalna*, 8(4), 293-299.
- [18] Miyamoto, Y., & Eckmann, L. (2015). Drug development against the major diarrhea-causing parasites of the small intestine, *Cryptosporidium* and *Giardia*. *Frontiers in microbiology*, 6, 1208.

<https://doi.org/10.3389/fmicb.2015.01208>

- [19] Paerewijck, O., Maertens, B., Dreesen, L., Van Meulder, F., Peelaers, I., Ratman, D., & Geldhof, P. (2017). Interleukin-17 receptor A (IL-17RA) as a central regulator of the protective immune response against *Giardia*. *Scientific reports*, 7(1), 1-14.
- [20] Parameswaran, N., & Patial, S. (2010). Tumor necrosis factor- $\alpha$  signaling in macrophages. *Critical Reviews<sup>TM</sup> in Eukaryotic Gene Expression*, 20(2), 87-103.
- [21] Popko, K., Gorska, E., Stelmazczyk-Emmel, A., Plywaczewski, R., Stoklosa, A., Gorecka, D., & Demkow, U. (2010). Proinflammatory cytokines IL-6 and TNF- $\alpha$  and the development of inflammation in obese subjects. *European journal of medical research*, 15(2), 1-3.
- [22] Punsawad, C., Phasuk, N., Bunratsami, S., Thongtup, K., Siripakonuaong, N., & Nongnaul, S. (2017). Prevalence of intestinal parasitic infection and associated risk factors among village health volunteers in rural communities of southern Thailand. *BMC public health*, 17(1), 1-9.
- [23] Saghaug, C. S., Sørnes, S., Peirasmaki, D., Svård, S., Langeland, N., & Hanevik, K. (2016). Human memory CD4+ T cell immune responses against *Giardia lamblia*. *Clinical and Vaccine Immunology*, 23(1), 11-18. <https://doi.org/10.1128/CVI.00419-15>
- [24] Sánchez, A., Munoz, M., Gómez, N., Tabares, J., Segura, L., Salazar, Á., & Ramírez, J. D. (2017). Molecular epidemiology of *Giardia*, *Blastocystis* and *Cryptosporidium* among indigenous children from the Colombian Amazon Basin. *Frontiers in microbiology*, 8, 248.
- [25] Serradell, M.C., Gargantini, P.R., Saura, A., Oms, S.R., Rupil, L. L., Berod, L., & Luján, H. D. (2018). Cytokines, antibodies, and histopathological profiles during *Giardia* infection and variant-specific surface protein-based vaccination. *Infection and immunity*, 86(6).
- [26] Shahnazi, M., Naghizadeh, F., Hajialilo, E., Alizadeh, S. A., Saraei, M., & Alipour, M. (2019). Investigation of *Giardia intestinalis* Genotypes among the Food Handlers of Qazvin, Iran. *Iranian journal of parasitology*, 14(4), 534-541.
- [27] Stark, D., Van Hal, S., Marriott, D., Ellis, J., & Harkness, J. (2007). Irritable bowel syndrome: a review on the role of intestinal protozoa and the importance of their detection and diagnosis. *International journal for parasitology*, 37(1), 11-20.
- [28] Tako, E.A., Hassimi, M.F., Li, E., & Singer, S.M. (2013). Transcriptomic analysis of the host response to *Giardia duodenalis* infection reveals redundant mechanisms for parasite control. *MBio*, 4(6). <https://doi.org/10.1128/mBio.00660-13>
- [29] Thapar, N., & Sanderson, I. R. (2004). Diarrhoea in children: an interface between developing and developed countries. *The Lancet*, 363(9409), 641-653.
- [30] Thomas IV, L.J., Zweig, A.P., & Tosh, A.K. (2014). An adolescent with chronic giardiasis mimicking anorexia nervosa. *International journal of adolescent medicine and health*, 26(2), 293-295. <https://doi.org/10.1515/ijamh-2013-0506>
- [31] Yasir, S.K. (2020). *Detection of heat shock protein70 and Diagnosis of Giardia lamblia polymorphism in Thi-Qar province*. College of Education for pure science Biology department, 174.
- [32] Zemene, T., & Shiferaw, M. B. (2018). Prevalence of intestinal parasitic infections in children under the age of 5 years attending the Debre Birhan referral hospital, North Shoa, Ethiopia. *BMC research notes*, 11(1), 1-6.
- [33] Zylberberg, H.M., Green, P.H., Turner, K.O., Genta, R.M., & Lebwohl, B. (2017). Prevalence and predictors of giardia in the united states. *Digestive diseases and sciences*, 62(2), 432-440.