

Original paper

Evaluation of IL-2, IL-4 and IL-10 levels in patients with giardiasis

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ABSTRACT. The aim of this study was to determine IL-2, IL-4 and IL-10 levels, in patients with giardiasis and to compare their interleukins levels with healthy controls. A total of 375 patients (211 males, 164 females) in Thi-Qar Province, southern of Iraq were examined. Twenty-four (16 males, 8 females) patients confirm to have giardiasis and 20 healthy control group were withdrawn (5) ml of venous blood to conduct immunological tests to determine the quantitative for level of IL-2, IL-4 and IL-10 in a manner (ELISA). The result showed, the overall infection rate of *G. lamblia* was 6.40%, according to gender, higher infection rate was recorded in males 7.58% compared to the females rate 4.87% no significant differences were observed between gender. The highest rates of giardiasis observed in age group (30–45), which reached 7.04% and there is no significant difference showed in the infection of different ages under study ($P \leq 0.05$). The results showed a significant increases in the level of interleukins and the amount of IL-2, IL-4 and IL-10 in patients (26.90, 17.43 and 14.71), respectively, was higher than those of healthy control (13.32, 10.25 and 10.55). The conclusion of this study demonstrated that the rate of infection was higher in males than in females and the age group (30–45) have the highest infection rates. The levels of interleukins (IL-2, IL-4 and IL10) were increased in the infected patient when compared to healthy persons, from this, we can deduce that pro-inflammatory and anti-inflammatory interleukins have an important role in the infection of *Giardia lamblia*.

Keywords: *Giardia lamblia*, giardiasis, IL-2, IL-4, IL-10

Introduction

Giardiasis is one of the famous gastroenteritis, that is caused by a microscopic parasite, called *Giardia lamblia* (syn. *G. duodenalis*, *G. intestinalis*) [1,2]. It is a flagellated unicellular eukaryotic intestinal parasite found in the gastrointestinal tract of humans and a range of other vertebrates [3,4]. *G. lamblia* is transmitted by the swallowing of the mature cyst in faecally contaminated water or foods through faecal-oral route [5].

It is characterized by a broad range of clinical manifestations from asymptomatic carriage, chronic diarrhea to severe malabsorption [6]. The symptoms of giardiasis patients mainly suffer from abdominal pain, diarrhea, nausea, chills and fever in addition to weight loss [7].

The variability of clinical illness seems to be due to differences both in the parasite and the host's immune response [8], as well as to previous

exposure to this pathogen [9]. Defense of immune system is vital for destruction of the *G. lamblia* during the period of infection and progress of effective immunity against it [10]. Both, humoral and cell-mediated immune responses play a role in acquired immunity, but the mechanisms involved are unknown [11]. Since an immune response is directly affected by interleukins (ILs), study of ILs changes in patients with giardiasis is of particular importance [12].

This present study was undertaken to estimate the immunological effect of *G. lamblia* infection on IL-2, IL-4 and IL-10 levels in patients with giardiasis compared to control group in Thi-Qar Province, southern of Iraq.

Materials and Methods

Stool and blood samples

From June 2020 till March 2021, a total of 375

stool samples were collected from patients and 20 healthy persons, from Al-Hussein Teaching Hospital and Al-Shatrah General Hospital in Thi-Qar, southern Iraq.

Freshly voided stool samples (included 211 males and 166 females aged from 1–50 year) were processed and examined microscopically using $\times 40$ objective lens for *G. lamblia* detection as described by Paniker [13].

Blood samples (5 ml) also were assembled from patients who confirm to have giardiasis and healthy individuals as a control. The blood specimens centrifuged at 3000 rpm for 5 minutes (Backman/counter, Germany) to separate the serum and collected, each sample of serum was divided into three parts; each of them was store until used for the determination of IL-2, IL-4 and IL-10 levels.

Evaluation of IL-2, IL-4 and IL-10 by ELISA test

Serum interleukins (IL-2, IL-4, IL-10) levels were estimated in 24 patients confirm suffering from giardiasis and 20 apparently healthy controls by Enzyme Linked Immunosorbent Assay (ELISA) test according to the manufacturer instructions.

Detection of IL-2, IL-4

Following the instructions of IL-2 Kit and the IL-4 Kit, frozen serum samples were brought to room temperature and mixed thoroughly. One hundred μ l of assay diluent was added to each well, 100 μ l of control and sample were added to each per well. The covered plate with a fresh sealer was incubated for 2 h at room temperature 25°C and shaker at 500 \pm 50 rpm. The plate was washed (repeated twice) by dispensed 0.4 ml of washing solution into each well then the content of well was aspirated. 200 μ l of interleukin conjugate was added to each well. The covered plate was incubated for 2 h at room temperature 25°C and shaker at 500 \pm 50 rpm then washed as in up step, 50 μ l of substrate solution was added to each well, the covered plate was incubated for 1h at room temperature 25°C and shaker at 500 \pm 50 rpm. Then 50 μ l of amplifier

solution was added to each well, incubated for 30 minutes at room temperature. Then stop solution was added (50 μ l) to each well, the absorbance of each well was recorded by using ELISA microplate reader (Olympus/Japan) at 490 nm.

Detection of IL-10

Practical work was done following the instructions of US Biological IL-10 kit protocol. One hundred microliter from each standard and samples were added (in duplicate) into the antibody pre-coated microtiter plate, then incubated for 1 hour at room temperature. Without discarding standards or samples, solutions about 50 μ l biotin was added to each well, incubated for 1 hour at room temperature then the plate was washed to remove any unconjugated antibodies. The Avidin attached with HRP enzyme was added to all wells in quantity of 100 μ l, the plate was incubated in dark at room temperature for another 1 hour followed by washing step, finally 100 μ l substrate mixture was added for 15 minutes stand period in dark at room temperature then to stop their reaction, 100 μ l stop solution was added. At the end of experiment a standard curve for different standard concentrations verses their absorbance at 620 nm were plotted, then each IL-10 concentration was calculated.

Statistical analysis

Statistical analysis were carried out using SPSS statistical package (version 20). Analysis of variance (ANOVA) of the data was used to detect overall difference in group means. Data are presented as the mean \pm standard error. Differences among group means were assessed using least significance difference (LSD). Proportions were compared by Chi-square. $P \leq 0.05$ was considered statistically significant.

Results

The result of present study revealed that 24 patients from 375 were positive for *G. lamblia*. The

Table 1. Distribution of giardiasis according to gender

Gender	No. of examined sample	No. of positive sample	Infection rate (%)
Male	211	16	7.58%
Female	164	8	4.87%
Total	375	24	6.40%

Chi-Square=1.127, D.F =1, $P=0.395$, $P \leq 0.05$

Table 2. Distribution of giardiasis according to age group

Age group	No. of examined sample	No. of positive sample	Infection rate
1–14	73	5	6.84%
15–29	51	3	5.88%
30–44	142	10	7.04%
>45	109	6	5.05%
Total	375	24	6.4%

Chi-Square= 0.291, D.F =3, $P=0.962$, $P\leq 0.05$

Table 3. Comparison between infected patients and healthy control interleukins (IL-2, IL-4 and IL-10) concentration

Groups	Mean±standard error			
	No.	IL-2 (Pg/ml)	IL-4 (Pg/ml)	IL-10 (Pg/ml)
Infected patients	24	26.90±1.52 ^a	17.43±2.25 ^a	14.71±3.15 ^a
Healthy control	20	13.32±1.14 ^b	10.25±1.2 ^b	10.55±2.2 ^b
LSD		4.2	2.9	1.48

a,b – there are significant differences between groups

infection rate among patients was 6.40%. The positive samples included 16 male and 8 female. The rate of infection among patients according to gender was higher in males 7.58%, than females 4.87%. There is no significant difference in the rate of infection between genders (Tab. 1).

The influences of age on infection with *G. lamblia* was studied, a higher percentage in *G. lamblia* infection (7.04%) was found within the age group (30–45) years followed by (6.84) within the age group (1–14) and the lowest one was (5.05%) within the age group (>45). There is no significant difference shows in the infection of different ages under study ($P\leq 0.05$) (Tab. 2).

In this study, the amount of IL-2, IL-4 and IL-10 in infected patients was (26.90, 17.43 and 14.71), respectively, higher than those of controls group (13.32, 10.25 and 10.55), and it is significantly more than the healthy controls (Tab. 3).

Discussion

Giardiasis is an infectious disease that present all over the world but spread more in the third world countries like Iraq [14]. In Iraq, there is many studies on the prevalence of giardiasis had been

performed in North, in Baghdad and its vicinity as well as in the South [15–17]. These studies have shown variations depending on geographical regions and localities, sanitary environment and hygienic habits of people living there [18,19], this may be cause the contrast percentage.

The results of our study reported that there are differences to the infection rate between genders as in table 1 where the males were highly than females. This result agreed with several studies such as [20–24], which pointed out that there is an increase in number of infected male with *G. lamblia* than female. The cause of increase in male infected rate belong to the males more active and contact with environment than females [25].

The age influences the rate of infection *G. lamblia* revealed that most cases of giardiasis occurred in the (30–45) years age group followed by the 1–14 years age group. This could be due to a number of factors such as poor health hygiene, overcrowding, low socioeconomic status and climatic conditions [26]. As shown in table 2, the result shown there is no significant difference between groups. These results agreed with [27,28] who illustrated that there were no significant differences between age groups. While it was

disagreed with [20,23,29], who showed that the infection was significantly associated with age.

Although the immune response in parasitic infection has been well characterized, there is limited literature and few clinical works on importance of interleukins in giardiasis. This study was addressed the issue by evaluation the levels of IL-2, IL-4 and IL-10 in patients comparing with healthy control group. Several mechanisms have been proposed to explain the immune response against the parasitic infections, including the production of IL-2, IL-4 and IL-10.

Results revealed increase in the concentration of interleukins (IL-2, IL-4 and IL-10) in patients comparing with healthy control group (Tab. 3). The increase ratio of IL-2, IL-4 and IL-10 was statistically significant ($P \leq 0.05$).

The results of the present study was in agreement with several studies [31–34]. Other studies demonstrate that T-helper 1 (Th1) pro-inflammatory cytokines such as IL-2 may play an important role in the elimination of protozoa from the human host [35,36,38].

T-helper 2 (Th2) anti-inflammatory cytokines such as IL-4 promote the activation of mast cells, eosinophils, basophils, and B-cell-mediated immunoglobulin and IgG production leading to increased levels of the regulatory cytokines, transforming growth factor (TGF) and IL-10 [38,39], therefore leading to eradication of parasitic infection [40].

IL-10, a product of innate immune cells, regulatory T cells (Tregs) and B cells, is a potent anti-inflammatory cytokine IL-10 has been shown to inhibit IFN- γ , induced killing potential of macrophages against both intra- and extracellular parasites as well as prevent immune-mediated host tissue destruction in response to infectious agents [41].

However, this finding was different with the results obtained by Mitra et al. [12] who approved that the level of IL-4 was decreased in patients with giardiasis. We also found disagreement with authors [42,43] who approved that there was no significant difference in the level of IL-10 in patients with giardiasis compared with control group.

In animal model giardiasis, most of the interleukins are produced by CD4⁺ of Peyer's patches or generated from the mucosa associated lymphoid tissue as a result of long duration antigenic stimulation via or cystic stage of *G. duodenalis* [44]. Type and amount of these

cytokines responses may be affected by the infecting parasite whether it is invasive or non-invasive [45].

References

- [1] Mohammed B.A., Rasheed Z.K., Jihad L.J., Abass K.S. 2020. Frequency of *Giardia lamblia* among Iraqi children in Kirkuk governorate. *Systematic Reviews in Pharmacy* 11: 1909–1911.
- [2] Savioli L., Smith H., Thompson A. 2006. *Giardia* and *Cryptosporidium* join the 'Neglected Diseases Initiative'. *Trends in Parasitology* 22: 203–208. doi:10.1016/j.pt.2006.02.015
- [3] Feng Y., Xiao L. 2011. Zoonotic potential and molecular epidemiology of *Giardia* species and giardiasis. *Clinical Microbiology Reviews* 24: 110–140. doi:10.1128/CMR.00033-10
- [4] Barwick R.S., Levy D.A., Braun G.F., Beach M.J., Calderon R.L. 2000. Surveillance for waterborne-disease outbreaks – United States, 1997–1998. *Morbidity and Mortality Weekly Report, Centers for Disease Control and Prevention, Surveillance Summaries* 49: 1–21.
- [5] Carranza P.G., Lujan H.D. 2010. New insights regarding the biology of *Giardia lamblia*. *Microbes and Infection* 12: 71–80. doi:10.1016/j.micinf.2009.09.008
- [6] Nash S.F., Patel R. 2010. The *Giardia lamblia* vsp gene repertoire: characteristics, genomic organization, and evolution. *BMC Genomic* 11: 424. doi:10.1186/1471-2164-11-424
- [7] Ensink J.H., van der Hoek W., Amerasinghe F.P. 2006. *Giardia duodenalis* infection and wastewater irrigation in Pakistan. *Transactions of The Royal Society of Tropical Medicine and Hygiene* 100: 538–542. doi:10.1016/j.trstmh.2005.08.014
- [8] Gottstein B., Harriman G.R., Conrad J.T., Nash T.E. 1990. Antigenic variation in *Giardia lamblia*: cellular and humoral immune response in a mouse model. *Parasite Immunology* 12: 659–673. doi:10.1111/j.1365-3024.1990.tb00995.x
- [9] Islam A., Stoll B.J., Ljungstrom I., Biswas J., Nazrul H., Huldt G. 1983. *Giardia lamblia* infections in a cohort of Bangladeshi mothers and infants followed for one year. *The Journal of Pediatrics* 103: 996–1000. doi:10.1016/s0022-3476(83)80739-2
- [10] Baqai R., Kazmi S.U., Qureshi H. 2000. Role of cytokines in giardiasis. *Journal of The Pakistan Medical Association* 50: 113–115.
- [11] Faubert G. 2000. Immune response to *Giardia duodenalis*. *Clinical Microbiology Reviews*. 13: 35–54. doi:10.1128/CMR.13.1.35
- [12] Mitra Z., Nasrin D., Nahid E., Arezoo J. 2012. [Evaluation of cytokines changes in patients infected with *Giardia lamblia* in comparison with healthy

- subjects]. *Payavard Salamat* 6: 4–11 (in Persian with summary in English).
- [13] Paniker C.K. 1989. Textbook of medical parasitology. 2nd ed. Joypee Brothers, Daryaganj, New Delhi, India.
- [14] Al-Sabbawi M.H. 2007. Giardiasis in the Third World Countries. *Journal of Chinese Clinical Medicine* 2: 539–540.
- [15] Al-hanoon Z.A., Mukhlis S. 1982. Prevalence of intestinal parasites among secondary school students in Mosul, Iraq. *Journal of the Faculty of Medicine Baghdad* 24: 225–230.
- [16] Jassan B.A., Al-Dujaily A.A., Saleh M.M. 1986. Prevalence of intestinal parasite school children of Kirkuk city, Iraq. *Journal of Biological Sciences Research* 7: 119–125.
- [17] Al-Aboody B.A., Al-Rumaidh Sh.Z., Abdul-Allah 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.
- [18] Gleason N.N., Horwitz M.S., Newton L.H., Moore G.T. 1970. A stool survey for enteric organisms in Aspen, Colorado. *The American Journal of Tropical Medicine and Hygiene* 19: 480–484. doi:10.4269/ajtmh.1970.19.480
- [19] Abu-Zeid H.A., Khan M.U., Omar M.S., Al-Madani A.A. 1989. Relationship of intestinal parasites in urban communities in Abha to socioenvironmental factors. *Saudi Medical Journal* 10: 477–480.
- [20] AL-Shaheen Z., AL-Maki A.K., Hussein K. 2007. A study on prevalence of *Entamoeba histolytica* and *Giardia lamblia* infection among patient attending Qurna hospital in Basrah. *Basrah Journal of Veterinary Research* 6: 30–36.
- [21] AL-Killaby K.K.A. 1999. Epidemiological study for common intestinal pathogens and related with acute diarrhea in children in Najaf governorate. MSc thesis. College of Education for Women, University of Kufa, Iraq.
- [22] AL-Mashhadani W.S.H. 2000. Isolation and diagnosis for some microbial causes to diarrhea and resistant bacterial isolations to antibiotic and product it of betalactamase enzyme. MSc thesis. College of Science, University of Al-Mustansiriya, Iraq.
- [23] Al-Saeed A.T., Issa S.H. 2006. Frequency of *Giardia lamblia* among children in Dohuk, Northern Iraq. *Eastern Mediterranean Health Journal* 12: 555–561.
- [24] Salman A.O. 2002. Epidemiology study to intestinal parasites in infective children with diarrhea and intended two children hospital in Baghdad city. MSc thesis. College of Education, University of Baghdad, Iraq.
- [25] Mahdi N.K., AL-Sadoon I., Mohamed A.J. 1996. First report of cryptosporidiosis among Iraqi children. *Eastern Mediterranean Health Journal* 2: 115–120.
- [26] Ulukanligil M., Seyrek A. 2004. Demographic and socio-economic factors affecting the physical development, haemoglobin and parasitic infection status of school children in Sanliurfa province, Turkey. *Public Health* 118: 151–158. doi:10.1016/j.puhe.2003.06.003
- [27] Kia E.B., Hosseini M., Meamar A.R., Rezaein M. 2008. Study of intestinal protozoan parasites in rural inhabitants of Mazandaran Province, Northern Iran. *Iranian Journal of Parasitology* 3: 21–25.
- [28] Mohammed M.T., Zeib I.A., Shwick R.S. 2009. Epidemiological study of giardiasis among children under five years of age Al-Maammil city. *Journal of Techniques* 22: 128–135.
- [29] Saail Y.K. 2009. Prevalence of intestinal parasites in children in Baghdad City. *Al-Taqani Journal* 22: 32–37.
- [30] Ouattara M., N'guéssan 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
- [31] 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.
- [32] Shakir M.J., Hussein A.A. 2014. Assessment of serum interleukin-2, 4 and C-reactive protein levels in patients with giardiasis and cryptosporidiosis. *Journal of the Faculty of Medicine Baghdad* 56: 313–317.
- [33] Bayraktar M.R., Mehmet N., Durmaz R. 2005. Serum cytokine changes in Turkish children infected with *Giardia lamblia* with and without allergy: effect of metronidazole treatment. *Acta Tropica* 95: 116–122. doi:10.1016/j.actatropica.2005.05.006
- [34] Mahmuod 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. *Journal of Parasitology* 11: 75–81.
- [35] Cox F.E. 2001. Concomitant infections, parasites and immune responses. *Parasitology* 122 (Suppl. S1): S23–S38. doi:10.1017/s003118200001698x
- [36] Lopez-Romero G., Quintero J., Astiazarán-García H., Velazquez C. 2015. Host defenses against *Giardia lamblia*. *Parasite Immunology* 37: 394–406. doi:10.1111/pim.12210
- [37] Redpath S.A., Fonseca N.M., Perona-Wright G. 2014. Protection and pathology during parasite infection: IL-10 strikes the balance. *Parasite Immunology* 36: 233–252. doi:10.1111/pim.12113
- [38] Rafi W., Ribeiro-Rodrigues R., Ellner J.J., Salgame P. 2012. Co-infection helminthes and tuberculosis'. *Current Opinion in HIV and AIDS* 7: 239–244.
- [39] Sanchez A.L., Mahoney D.L., Gabrie J.A. 2015. Interleukin-10 and soil-transmitted helminth infections in Honduran children. *BMC Research Notes*

- 8: article number 55. doi:10.1186/s13104-015-1019-x
- [40] De-Waal M., Moree K.M. 1988. The cytokine handbook. 3rd ed. Thompson, Academic Press, London.
- [41] Redpath S.A., Fonseca N.M., Perona-Wright G. 2014. Protection and pathology during parasite infection: IL-10 strikes the balance. *Parasite Immunology* 36: 233–252. doi:10.1111/pim.12113
- [42] Ahmed N.Sh., AL-Khayat F.A., Abdullah F.T. 2015. Interleukins IL-6, IL8, IL10 and tumor necrosis factor TNF expression in human infected with *Giardia duodenalis*. *American Journal of Medicine and Medical Sciences* 5: 15–19. doi:10.5923/j.ajmms.20150501.04
- [43] Joe D.K., Steven M.S. 2009. Phosphoinositide 3-kinase-dependent inhibition of dendritic cell interleukin-12 production by *Giardia lamblia*. *Infection and Immunity* 77: 685–693. doi:10.1128/IAI.00718-08
- [44] Scott K.G., Yu, L.C., Buret, A.G. 2004. Role of CD8+ and CD4+ T lymphocytes in jejunal mucosal injury during murine giardiasis. *Infection and Immunity* 72: 3536–3542. doi:10.1128/IAI.72.6.3536-3542.2004
- [45] Jung H.C., Echmann L., Yang S.K., Panja A., Flerer J., Morzycka W.E., Kagnoff M.F. 1995. A distinct array of proinflammatory cytokines is expressed in human colon epithelial cells in response to bacterial invasion. *The Journal of Clinical Investigation* 95: 55–65. doi:10.1172/JCI117676

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