



Estimation of Some Immunological Parameters Levels in Giardiasis Patients in Al- Najaf Province

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Abstract

The current study was conducted during the period from August 2017 to April 2018 in the Department of Biology - Faculty of Education for Girls - University of Kufa to measure the level of some cytokines and immunoglobulins in patients with *Giardia lamblia* parasite in Al- Najaf Province. A total of 923 stool samples from patients, with diarrhea ranging in age from 1 to 60 years were examined with a direct smear and sedimentation methods. The results of the immunological examination were shown using the enzyme linked immunosorbent assay (ELISA) and single radial immunodiffusion (SRID) Plate. IgA, IgE, IgG and IgM concentrations were increased in patients with Giardiasis as 276 ± 32.3 mg / dl, 260 ± 39.3 IU / ml, 576 ± 42.4 mg / dL and 73 ± 17.8 mg / dL , respectively, compared with the control group of 65 ± 22.7 mg / dl, 175 ± 23.3 IU / ml, 365 ± 27.3 mg / dl and 47 ± 14.4 mg / dl, respectively, with a statistically significant difference $P < 0.05$. As well as an increase in concentrations of IFN- γ , IL-4 and TNF- α (284 ± 21.4 , 395 ± 27.1 and 443 ± 29.2) pg / mL respectively compared to control group (131 ± 18.7 , 345 ± 22.6 and 191 ± 26.6) pg / ml, respectively, with a statistically significant difference $P < 0.05$.

Keywords: *Giardia lamblia*; Cytokines; Immunoglobulins.

Introduction

Giardiasis is an important disease affecting a large number of people, estimated to be infected in millions caused by *Giardia lamblia* parasite, which is a parasitic protozoan, it spreads widely around the world and it is abundant and quality in developing areas and cause many of the problems of public health [1], estimating 280 million cases a year, most of them are from children, it is a common zoonosis and usually affects the area of duodenum and the upper part of the small intestine in humans and animals such as sheep, birds, goats, dogs and others, knowing that this parasite does not attack tissues.

Disease occurs by swallowing mature cysts with food and drink, and can be transmitted directly from one person to another by feco-oral route and this method is one of the causes of wide spread disease [2]. The main symptoms of this disease are diarrhea which is greasy and foul, bloating, abdominal pain, loss of appetite, nausea with or without vomiting, weight loss and rarely the patient

suffers from fever as well as to malabsorption in children and infants [3].

About 50-60% of the cases are asymptomatic and the children are more affected than adults. Symptoms do not usually appear two days before the infection and symptoms persist for two weeks or more [4]. The difference in clinical symptoms is the result of a number of factors including age, immunity, nutritional status, concurrent infections, and parasitic severity and pathogenicity [5].

G.lamblia parasites are traditionally detected by microscopic examination of cysts trophozoites of parasite in the stool and this is considered one of the first diagnostic tests performed on patients with giardiasis. The presence of cysts in the stool was clearly observed, while the presence of trophozoites was less. The work required time, intensive work and efficiency by the examiner, and the examination should be more than once.

The defense against the infection of *G. lamblia* is local because the parasite does not attack the epithelial layer. The immune response is important for the elimination of the parasite during the stage of infection and the development of protective immunity. Both cellular and humeral immunity have a significant and important role in the eradication of giardiasis. Cytokines are proteins produced by lymphocytes and macrophages, some of which are produced by CD cells of the Peyer's patches, or through the lymphatic tissues associated with the mucous layer due to long-term antigen stimulation or the encystation stage of the parasite (Scott et al., 2004). In the case of an inflammatory response in the case of parasitic diseases, it has been observed in several studies that IL-2, IL-6, IL-10 and TNF- α are more concentrated in the serum of *Giardia* patients compared to control.

The type and quantity of these cytokines is affected by parasitic infection as it is invasive or non- invasive [6]. Giardiasis is generally stimulated both cellular and humoral response. As a result of the release of parasite antigens almost every day, this stimulates T-cell. Thus, the immune response in the patient's body occurs and the secretion of certain antibodies such as IgA, IgE, IgG and IgM have an important role in the eradication of parasites. In addition to the secretion of some cytokines from human epithelial cells such as IL-6, IL-8, and tumor necrosis factor (TNF) [7]. The aim of the current study is to measuring the concentration of some cytokines and immunoglobulines in patients infected with *G.lamblia* in AL-Najaf Province.

Materials and Methods

Samples Collection

The present study was conducted during the period from August 2017 to April 2018 in the Department of Biology - Faculty of Education for Girls - University of Kufa. A total of (932) stool samples were collected from patients aged (1-60) years who had attended hospitals of Najaf suffering from diarrhea and gastrointestinal disorders who were visiting hospitals in Najaf, including: Al-Zahra Hospital for Childbirth and Children, Al-Furat Middle Teaching hospital, Al-Sadr Teaching Hospital, Al-Hakim Hospital General Hospital and AL-Sajad Hospital, as well as some health centers in the

governorate, including the health center in Al-Wafaa, primary health care in Maysan district, health center in Al-Askari district, 15 Shaban health center, Al-Ansar health center. Samples of stool were collected in sterile plastic containers.

Which contains patient-specific information and stool samples examined in the hospital's parasite laboratory for the purpose of investigating the cysts and trophozoite of the *Giardia* parasite, at the same time , blood samples were collected from patients with giardiasis after confirming their infection with *G. lamblia* parasites, which took about (3-5)ml of venous blood from patients and placed in the Gel tubes and it is left for a period of time in the laboratory to after stabilize the blood sample is subjected to centrifugation (2000 cycle/5 minutes) we get the serum necessary for the immunological tests where.

The resulting serum is distributed by affine Pipette on Eppendorf tubes and according to the number of immunological tests required in the study. The serum samples were frozen at -20°C until they were used in the immunological tests.

Diagnosis of *G.lamblia* Parasite

Cyst and trophozoite of *G.lamblia* parasites were identified in stool samples method prepared with normal saline solution (0.9%) and lugol's iodine solution and by sedimentation method [8].

Immunological Tests

The concentration of IgE was determined by the enzyme linked immunosorbent assay (ELISA) and according to the manufacturer's instructions of the kit and based on the Vercelli [9]. The concentration of IgA, IgG and IgM was determined by the Single Radial Immunodiffusion Assay (SRID) as instructed by the manufacturer's of the kit. The concentration of the cytokines (IFN-g, IL-4 and TNF- α) were determined by the ELISA technique and according to the manufacturer's instructions for the kit.

Statistical Analysis

The results were statistically analyzed using SPSS version (22) and the t-test was used in the immunological study to determine the differences between treatments at probability level of $P < 0.05$ [10].

Results and Discussion

The results of the present study showed an increase in the concentration of antibodies IgA, IgE, IgG and IgM (276±32.3 mg/dl, 260 ± 29.3 mg/ dl, 576±42.2mg /dl and 73±17.8mg

/dl).respectively, compared to the control (65± 22.7 mg /dl, 175±23.2 IU /ml, 365 ± 27.3mg /dl and 47±14.4 mg /dl) respectively .with statistical significance differences at probability level P<0.05, as in a Table (1).

Table 1: Concentration of antibodies IgA,IgE,IgG and IgM in the serum of studied groups

Antibodies	Control M± SD	Patients M± SD	Calculated T	Table T	Results of statistical analysis
IgA (mg /dl)	65 22.7	276±32.3	18.4	2.32	Significant differences P<0.05
IgE (IU /ml)	175±23.2	260 ± 29.3	12.7	2.32	
IgG (mg/ dl)	365±27.3	576±42.2	19.6	2.32	
IgM (mg/dl)	47±14.4	73±17.8	7.3	2.32	

The results of the present study were consistent with the results of Faubert [11] and AL-Anbaki [12], while the results of Yanke [13] and Hussain (2013) showed a significant increase in the level of antibodies IgA, IgG while the level of antibody IgM was non-significant increase in the level of IgA antibody during the stage of parasitic infection. Several studies have documented that this antibody is linked to the resistance of a number of mucous pathogens [14].Valenzuela *et al* [15].Reported that the increase in the level of IgM antibody occurs in the first three months of infection.The IgG antibody remains several years after infection.

Several studies have shown that antigens of *Giardia* parasites can be distinguished by T-cell, thus stimulating the immune response, which is limited and plays an important and effective role in controlling *Giardia* infection [16]. The present study contrasted with Zarebavani *et al.* (2012), there was no statistically significant difference in the level of IgA, IgM, in patient and control groups.

The presence of antibodies to *Giardia* in serum, especially IgA, can help to track and diagnosis of *G.lamblia* [17]. *Giardia* infection in children stimulates the production of antibodies secreted in saliva, stool and Salivary response (IgA) and serum (IgG) against the disease are clearly high in Patients compared to control. This observation can be used to develop the mechanisms in which these antibodies are measured when they are infected with *G.lamblia* parasites, where B-cells and IgA antibodies are important factors for parasite elimination and expulsion outside the intestines. IgA antibody can be detected in breast milk [18].

Special studies of host defense mechanisms show that individuals who have a low of immunoglobulin IgD, IgE, IgG, IgM may be more susceptible than others to infection [19], High levels of IgE rate are evidence of increased immunization in host organs when infected with *Giardia* parasites, where the level of IgE in infected people is high compared to control and this was also observed in the current study, and according to many studies conducted by researchers showed that in the case of infection *G.lamblia*, the concentration of IgE becomes high [20], and the presence of IgG antibodies in more than 80% of *Giardia*-infected patients, according by symptoms possibly because of the highest titer of antibodies in patients in areas where the disease is present, as well as the role played by antibodies in the serum of the patient and IgA antibodies in localized immunity [21]. Studies of the specific immune response in the experimental subjects of *G.lamblia* showed that immunoglobulin IgA, IgG and IgM (100, 70, 60) of patients with high rates and respectively [22].

IgE antibody activates platelets and promotes cytotoxicity against parasites. The high antibody ratio was recorded in patients with parasites and a significant difference, including *G.lamblia* [23]. IgE antibody is the initial exposure of the parasite antigens or in the case of allergies. The high level of IgE antibody in patient serum occurs during the stage of parasite use to tissue (Vieira- Silva *et al.*, 2012). The Current study was shown by measuring the concentration of cytokines IFN- γ , IL-4 and TNF- α in the serum of patients with giardiasis, there is an increase in their rates (284± 21.4,395 ±27.1, 443± 29.2) pg/ml respectively, compared with the

control group (131± 18.7, 345± 22.6 and 191 ±26.6) pg/ml respectively and with a

statistical significance difference at a probability level $P < 0.05$ as in a Table (2).

Table 2: Concentration of cytokines, IFN- γ , IL-4 and TNF- α in serum of studied groups

cytokines	Control M \pm SD	Patients M \pm SD	Calculated T	Table T	Results of statistical analysis
IFN- γ pg/ml	131± 18.7	284± 21.4	13.3	2.32	Significant differences $P < 0.05$
IL-4 Pg/ml	345± 22.6	395 ±27.1	9.9	2.32	
TNF- α Pg/ml	191 ±26.6	443± 29.2	17.8	2.32	

Parasitic infections in the body are the source of foreign antigens and external toxins that are a local stimulant or systemic inflammatory processes [11]. Cytokines are a small protein that mediate the process of differentiation, activation and proliferation of cells in the body and is produced by different types of cells, where it is involved in the regulation of cellular and humoral immunity when the body of the organism is exposed to a foreign body [24].

The broad range of cytokines is produced by the CD T-cells found in the spleen and mesenteric lymphatic nodes of the mice following the infection with *G.lambli*a parasite [25], The host control on the infection of *G.lambli*a parasite requires a process of activation of cellular and humoral immune response in the body, such as the balanced response of CD T-cells specialized in antigen, which release cellular including, IFN- γ , IL-4, IL-6 and TNF- α as well as the secretion of antibodies IgA and IgG against

parasite antigen in the host's body after the infection [26], where the high rates of IFN- γ and IL-4, in the serum are associated with the long period of infection and this helps to eliminate the parasite naturally in the bodies of infected people without the need for medicines to eradicate parasites (Long *et al.*, 2010), and through the study of [27].it was found that IFN- γ is produced by activation of Th1 cells to build and release IgE.

It was found that the IFN- γ stimulates the internal killing of parasites by macrophages [28]. The toxicity of a eosinophils increases under the effect of IL-5 and TNF- α produced by mast cells, lymphocytes, and Macrophages, so the neutrophils, macrophage and platelets appear to be toxic against parasites by surrounding them with antibodies [29]. TNF- α is a pro inflammatory cytokines which is secreted during the infection and play a major role in the elimination of *G.lambli*a parasite [27, 30].

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