

Role of IL-2, IL-4 and IL-10 in Patients Infected with *Giardia lamblia*

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SUMMARY: A total of 116 sera collected from 86 persons infected with *G. lamblia* (40 males, 46 females) and 30 healthy controls (12 males, 18 females) were tested in order to determine the levels of some cytokines. Serum levels of IL-2, IL-4, and IL-10 were measured by the enzyme linked immunosorbent assay. The cytokine that increased most was IL-2 which had an elevated level of 46.5% and was followed by IL-4 (2.3%). IL-10 had not increased at all. The elevated ratio of IL-2 levels showed significant difference ($p<0.001$). From these results, it can be concluded that Th1 immune response seems to be predominant in subjects infected with *G. lamblia*.

Key Words: Giardia infection; IL-2, IL-4, IL-10

Giardia Enfeksiyonu olan Hastalarda IL-2, IL-4 ve IL-10 Rolü

ÖZET: *Giardia* enfeksiyonu bulunan 86 hasta (40 erkek, 46 kadın) ile 30 kişilik (12 erkek, 18 kadın) sağlıklı kontrol grubundan toplanan 116 serum örneğinde, serum sitokin seviyeleri çalışıldı. IL-2, IL-4 ve IL-10 seviyeleri ELISA yöntemiyle ölçüldü. IL-2 en fazla artan sitokin olarak bulundu. (%46,5), bunu %2,3 artışla IL-4 takip etti, IL-10 seviyesinde artış gözlenmedi. IL-2 seviyesindeki artış oranı, istatikselsel olarak anlamlı bulundu ($p<0.001$). Bu verilerle, *G. lamblia* ile infekte kişilerde Th1 immün cevabın baskın görüldüğü sonucuna varılabilir.

Anahtar Sözcükler: *Giardia* enfeksiyonu, IL-2, IL-4, IL-10

GİRİŞ

Giardia lamblia (synonymous with *Giardia intestinalis* and *Giardia duodenalis*) is one of the most common intestinal parasites endemic in Turkey and sometimes referred to as *Lamblia intestinalis* (5). Children are more frequently affected than adults (9). In some infected persons, the parasite remains asymptomatic. Clinical features associated with infection range from acute, short-lasting diarrhoea to chronic syndromes such as metabolic disorders, weight loss and retardation of growth especially in infants and children less than 3 years old (6). Differences in clinical manifestations may be due to a number of factors, including host age, immune and nutritional status, concurrent infections, as well as the virulence and pathogenicity of the *Giardia* strain (1, 2, 20). Pathophysiological changes include disaccharidase insufficiencies and malabsorption and maldigestion of electrolytes, nutrients, and water due to diffuse intestinal microvillous alterations (15).

Immunodeficiency states particularly malnutrition and hypogammaglobulinemia increase susceptibility to giardiasis (2, 13). Potent immune response is important for eradication of the parasite from the intestine during infection and in development of protective immunity (10). Although there is some evidence about development of protective immunity against *G. lamblia*, multiple exposures are necessary for this specific immunity (9). Previous investigations have demonstrated the importance of both cellular and humoral mechanisms in the development of protective immunity and resistance to giardiasis (7, 10, 11, 13). Recently, it has been demonstrated *Giardia* antigens induces a significant increase in the total number of CD8⁺ and CD4⁺ lymphocytes in intestinal wall and Peyer's patches proliferation (11, 15).

Although the role of cytokines in parasitic infections has been widely investigated in animal models, there is limited literature and few clinical works on the importance of Th₁ (IL-2,) and Th₂ (IL-4, IL-10) in human giardiasis. Thus, the present work was undertaken to study the levels of these cytokines in the sera of subjects infected with *G. lamblia*.

MATERIALS AND METHODS

A total of 86 persons infected with *G. lamblia* (40 males and 46 females) and 30 healthy controls (12 males and 18 females) were recruited for this study. The ratio of males to females was 0.87. Patients were selected among the individuals attending Clinical Microbiology Laboratory, Turgut Ozal Medical Center for parasitological examination. The ages of the subjects with *Giardia* infection ranged from 2.5 to 59 years with a mean age of 12.3 ± 11.3 years (median=7 years). Healthy controls were between 16 and 47 years old with a mean age of 27.7 ± 9.7 years (median=24.5 years). The diagnosis of *G. lamblia* infection was made by three consecutive stool examinations using wet mount with normal saline and Lugol iodine in direct slide method Floatation stool examination was made to check presence of other parasites.

Decision was made whether control subjects were healthy according to results of blood examination (routine parasitological, biochemical and haematological tests) in addition to apparent healthy status with no history of current illness and pathological condition. Venous fasting blood samples were collected in sterile tubes. Blood samples were centrifuged as soon as possible at $2000 \times g$ for 10 minutes to obtain the serum. The lipemic or haemolysed sera were discarded. The other sera were divided into 3 tubes for each subject and stored immediately at -20°C until measurement. For each determination, a new aliquot was used since freezing and thawing may partially destroy its biological reactivity. Permission for all procedures was taken from the local Ethical Committee and all subjects gave their informed consent to the study.

Statistical analysis was made using Chi-square, Fisher's and Mann-Whitney U tests. Pearson's linear correlation test was used for assessment of correlation between parameters. The level of significance was set at $P < 0.05$ in all analyses. All analyses were performed using the SPSS 9.0 software.

RESULTS

According to kit guides, the normal serum concentration of IL-2 and IL-10 were less than 5 pg/ml and the mean level of IL-4 was <0.4 pg/ml. The cytokine values of individuals having giardiasis higher than those of controls were considered as increased. The mean levels of studied cytokines were shown in table 1. As shown in table 2, the most increased cytokine was IL-2 (46.5%) followed by (2.3%) IL-10 was not increased at all. The increased ratio of IL-2 were statistically significant ($P < 0.001$). No statistically significant correlation was detected between any of the studied cytokine levels.

DISCUSSION

Much knowledge on the immune response in giardiasis has been originated from *in vitro* studies involving the challenge of immune cells removed from a variety of hosts with *G.*

duodenalis trophozoites, and animal models infected with *G. duodenalis* or *G. muris* (10, 12, 16, 18). The release of exogenous IL-4, IL-5 and IFN- γ by spleen and Peyer's patches cells removed from BALB/c mice infected with *G. muris* was demonstrated (8). However, results obtained using animal models may not represent the conditions of human giardiasis. The difference in performed method, type and immune status of laboratory animal, strain of the parasite as well as natural pathogens in laboratory animals may all affect the research results and interpretation of the assays (4, 10). However, results of animal studies may be extremely informative and useful. An ideal method is to assay cytokines in cell culture supernatants, derived from animals or humans, which can be regarded as negative control in comparing with results obtained after specific antigenic stimulation (8, 18).

Table 1. Comparison of serum cytokine levels (Mean \pm SD) in patients with giardiasis and healthy controls.

Parameter	Healthy controls (n=30)	Patients with infected <i>G. lamblia</i> (n=86)	P value*
IL-2 (pg/mL)	2.8 ± 0.6	7.4 ± 5.1	<0.001
IL-4 (pg/ml)	0.14 ± 0.11	0.26 ± 0.49	>0.05
IL-10 (pg/mL)	0.18 ± 0.10	0.21 ± 0.10	>0.05

*Mann-Whitney U test

Table 2. The numbers and percentages of persons infected with *G. lamblia* showing increased cytokines.

Cytokines	Increased ^a		Normal ^b		Total	P-Value ^c
	n	%	n	%		
IL-2 (pg/mL)	40	46.5	46	53.5	86	<0.001
IL-4 (pg/ml)	2	2.3	84	97.7	86	$>0.05^d$
IL-10 (pg/mL)	0	0	86	100	86	>0.05

a: *G. lamblia* infected persons with increased cytokines levels;

b: *G. lamblia* infected persons with normal cytokines levels,

c: Chi-Square test; d: Fisher's test

In giardiasis, most of the cytokines are produced by CD4+ of Peyer's patches or generated from the mucosa associated lymphoid tissue (MALT) as a result of long duration antigenic stimulation via trophozoites or cystic stage of *G. lamblia* (13,15). Type and amount of these cytokine responses may be affected by the infecting parasite whether it is invasive or non-invasive. In an experimental study by Jung et al (14), it was reported that colon epithelial cells challenged with non-invasive *G. lamblia* showed no expressed mRNA for IL-2, IL-4, IL-6, IL-12, and INF- γ but showed significant IL-1 and IL-10 expression, which appears the general property of colon epithelial cells.

In the present study, IL-2 was increased only in 46.5%. The lower percentage of IL-2 may be due to its very short life span (19). The increase of IL-4 in few cases and no increase in IL-10 serum level in giardiasis may indicate a minor role of Th₂ cytokines in immune response. In a similar study, IL-4 and IL-10 was present in 33.3%, 12% respectively (3). As reported in a previous study, these cytokines may have no influence on immunity to giardiasis (18). This could mean the weak immune response to *G. lamblia* that explain the chronic nature of this disease. The weak immune response may be in part due to the fact that this parasite does not invade intestinal mucosa but locally stimulates mucosa associated lymphoid tissue (1, 10). Nevertheless, the exact immunological mechanism in combating giardiasis particularly self-limited cases is not completely understood (16). However, in agreement with previous comments (13, 18), our results can confirm that the immune response to giardiasis may predominantly depends on the Th₁ type of cytokines.

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