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Original Article

IL-4, IL-10, TNF-α and IFN-γ Cytokine Levels in Patients with Cystic Echinococcosis and Fascioliasis

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Abstract

Background: Cytokines are protein substances involved in immune response and inflammation regulation, cell growth, tissue repair and natural and adaptive immunity events such as hematopoiesis. Changes in Th1 and Th2 immune responses provide information on the clinical presentation, pathology and diagnosis of diseases.

Methods: This study was conducted on 142 patients, aged 18-95 yr and applied to Parasitology Laboratory of Van Yuzuncu Yil Hospital between Aug 2018 and May 2019. Fifty-one of the patients were serologically diagnosed with CE, 53 with a diagnosis of fascioliasis, and 38 as the control group. Serum samples were taken from patients and levels of IL-4, IL-10, TNF α and IFN γ parameters were investigated by ELISA method. Results were evaluated by spectrophotometer and observed which immunologic parameters increased in which infections. Cytokine results were also evaluated according to patient age groups and genders. Chi-square and/or Fisher's exact test were used to evaluate the results. IL-4 response was detected in 50.9%; IL-10 in 44.2%; TNF-α in 43.3% and IFN-γ in 43.3% of CE seropositive patients.

Results: IL-4, IL-10, TNF-α and IFN-responses detected in 50.9%, 44.2%, 43.3% and 43.3% of CE patients, and in 43%, 39.2%, 34.4% and 40.6% of fascioliasis patients, respectively. The presence of Th1 and Th2 responses were detected in both infections. There was a significant relationship between fascioliasis and IL-4 response and between CE and IL-4, IL-10 and TNF-α responses ($P \leq .05$).

Conclusion: These cytokine levels may provide information about the immune response to infections, and may be useful for early detection of disease and recurrence and monitoring of treatment.

Keywords: Cystic echinococcosis; Fascioliasis; Cytokine; Interferons; Interleukins

Introduction

Cystic echinococcosis (CE) is a significant parasitic infection induced by *Echinococcus granulosus*. Adult parasites found in carnivores and larvae in humans and various mammalian animals. The infection is caused by the oral intake of parasite eggs excreted in the feces of the previous host, and the larval forms transform into cysts in organ tissues such as liver, lungs, kidney, spleen, brain and bone. The disease is a public health issue, as well as a source for serious economic losses in nations due to labor force losses, productivity losses in animals and health expenditures (1). Fascioliasis is a trematode infection that especially affects cattle and sheep and is caused by *Fasciola hepatica*. The infection is transmitted to humans via oral intake of

metacercaria forms of the parasite in contaminated water and food. It has two prognoses; acute (hepatic) and chronic (biliary). In the acute phase, symptoms such as hepatomegaly, abdominal pain, fever, eosinophilia and anemia could be observed, while in the chronic phase, symptoms such as cholestasis and cholangitis are seen (2, 3).

Cytokines are proteins that play a role in immune response and inflammation regulation, natural and adaptive immune system events such as cell growth, tissue repair and hematopoiesis. By playing a mediating role between the cells, they assume a significant function in determination of sustenance and level of the immune response. A cytokine could stimulate similar signal paths in cells or affect different signal paths in different cells (4). The cytokine signaling in a host could vary significantly and lead to both protective and destructive reactions. They could initiate a cascade, stimulate or inhibit the production of other cytokines when needed in immune response. The cytokines could be categorized into four groups based on their functions: Those that mediate natural immunity (Type I IFN, chemokines, TNF, IL-1, IL-6); those that regulate lymphocyte activation, growth and differentiation (IL-2, IL-4, TGF-β), those that regulate immune-mediated inflammation (IFN-γ, Lymphotoxin, IL-5, IL-10, IL-12) and those that stimulate hematopoiesis (i.e., IL-3). Today, cytokines are also used clinically in the treatment of various disorders (5).

Helminths possess complex defense strategies that could regulate host immune response. They produce various antigens that modulate the host immune response and promote the survival and growth of the parasite (6). In helminth infections, the simultaneous induction of Thl and Th2 responses are observed. There is a constant balance between Th1 and Th2 populations in CE and fascioliasis (7, 8). Th1 is associated with protective immunity and produces IL-2, IL-12, TNF, and IFN-γ, while Th2 is associated with susceptibility to infection, pathogenicity, and allows the survival of parasites in host tissues and expresses IL-4, IL-5, IL-6, IL-9, IL-10 and IL-13. In chronic extracellular parasite infections, Th2-based immune response is more prevalent (6, 9). Both Th1 and Th2 cytokines repair the damages induced by pathogens or the immune system, and the expression of Thl cytokines regulates the balance between fibrosis and injury repair (10). They are produced by TNF-α, active macrophages, NK cells and T lymphocytes. Assist neutrophil and macrophage migration to infection sites via endothelial cell stimulation and chemokine production. IL-10 is produced by activated macrophages and Th2 cells. It inhibits the production of IFN-γ production by Th1 cells, thereby shifting the immune response to Th2 type. It also inhibits cytokine production and class II MHC expression, leading to a decrease in immune response. INF-γ has several functions in both natural and adaptive immune systems. It inhibits Th2, B lymphocyte and humoral immunity. IL-4 is produced by macrophages and Th2 cells, inducing Th2 cell growth, leading to antibody response. It also plays an active role in the inflammatory reaction mediated by IgE and eosinophils (11, 12). In general, the variability and severity of the clinical expression of these infections are associated with variables such as the duration of the infection, the parasite intake quantity, the location and previous infection history. The postoperative relapses pose a serious risk for the patient and require long-term clinical, radiological and parasitological follow-up (9, 13). In fascioliasis, the sensitivity of parasitological diagnosis remains low due to the fact that parasite eggs could not be detected in stool and intermittent egg excretion in acute infections (2).

We aimed to determine the possible roles of IFN-γ, TNF-α, IL-4 and IL-10 cytokines by investigating the responses of these cytokines in CE and fascioliasis seropositive patients. Thus, the study attempted to identify immunological markers that may lead to early diagnosis of CE and fascioliasis infections, identification of the relapses and follow-up of therapeutic activities.

Materials and Methods

The present study was carried out on blood samples obtained during routine examination and treatment procedures of 18-95 yr old patients who applied to Parasitology Laboratory, Faculty of Medicine of Van Yuzuncu Yil University, Turkey between Aug 2018 and May 2019.

The patient blood samples were stored at -20 degrees until the tests were conducted. Overall, 142 patients were included in the study, 51 of serologically diagnosed with CE, 53 with a diagnosis of fascioliasis, and 38 as the control group. Some samples were ran out due to the low serum amount and the number of samples studied varies according to the test groups (Table 1).

Table 1: Infection, cytokine levels and gender of the patients

Variable	*Cytokines*			
Patient groups	IL-4	IL-10	TNF-α	IFN-γ
CE (female)	26	32	22	21
CE (male)	25	20	8	9
Fascioliasis (female)	20	32	21	21
Fascioliasis (male)	31	19	11	11
Control (female	20/23*	21/20*	18	16
Control (male)	15/13*	16	10	12

*CE/Fascioliasis

The seropositivity limit was the agglutination at 1/320 and above dilutions according to the IHA (Indirect Hemagglutination) (Fumouze Laboratories, France) test for CE and fascioliasis, and and higher values than the positive controls at 450 nm wavelength with the ELISA (R-Biopharm, Germany; IgG) method for CE. In addition, after serological diagnosis, radiologically or surgically confirmed patient samples for CE and clinically compatible and high serologic positive samples were selected to prevent cross-reactions for fascioliasis. Since some of the serum samples were low in volume, certain tests were conducted on serum samples obtained from different patients. IL-4, IL-10, TNF-α and IFN-γ levels were investigated with the commercial Human ELISA (Shanghai SunRed Biological Technology, China) kits based on the protocol published by the manufacturer. A total of six kits were used includes two IL-4 and 10 IL-10 kits, and one kit each for TNF-α and IFN-γ. Chi-square and/or Fisher's exact tests were used to analyze the correlation between the cytokine response and the disease, age and gender.

The cytokine levels of the patient serum samples were proportioned to the absorbance values of standard dilutions and presented in pg/ml. The of ELISA kit standard curve ranges were 5-1450 pg/ml for IL-4, 10-3000 pg/ml for IL-10, 3-900 pg/ml for TNF-α, and 2-600 pg/ml for IFN-γ. The kit sensitivities were 4.116 pg/ml, 9.012 pg/ml, 2.827 pg/ml and 1.706 pg/ml, respectively.

Ethical approval

Approval was obtained from Non-Interventional Clinical Research Ethics Committee (07.10.2018/07). Informed consent forms were signed by the patients for the procedure.

Results

IL-4 response was investigated in 86 patients for CE (51 CE seropositive and 35 control). It was positive 50.9% (26/51) of CE patients, 22.9% (8/35) of the control group (*P*=.032), 32.1% (18/56) of women and 53.3 (16/30) of men (*P*=.055).

IL-4 levels was investigated in the serum samples of 87 patients for fascioliasis (51 fascioliasis seropositive and 36 control). It was positive 43% (22/51) of the fascioliasis patients, 27.8% (10/36) of the control group (*P*=.016) (Fig. 1), 48.8% (21/43) of women and 25% (11/44) of men (*P*=.116).

IL-10 response was investigated in 89 patients for CE (52 CE seropositive patients, 37 control group members). It was positive 44.2% (23/52) of CE patients, 16.2% (6/37) of the control group (*P*=.005), 30.2% (16/53) of women and 14.7% (13/36) of men (*P*=.558).

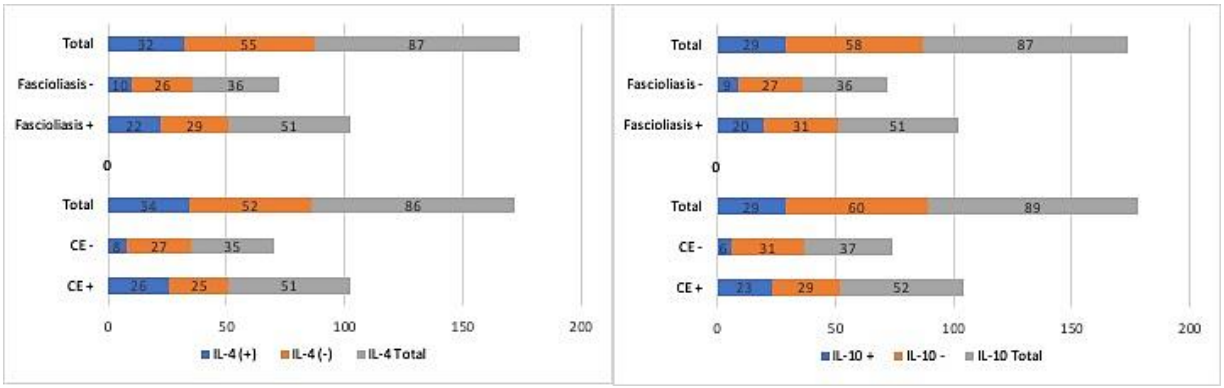

Fig. 1: IL-4 and IL-10 results according to infections and control groups

IL-10 positivity was investigated in 87 serum samples (51fascioliasis seropositive and 36 control group). It was determined 39.2% (20/51) of fascioliasis patients and 25% (9/36) of the control group (*P*=.166) (Fig. 1), 17 (32.7%) of women and 12 (34.3%) of men (*P*=.194).

TNF-α response was investigated in 90 patients for CE (30 CE patients, 32 fascioliasis patients and 28 control group). It was determined 43.3% (13/30) of CE seropositive patients, 34.4% (11/32) of fascioliasis patients, 17.9% (5/28) of the control group (*P*=.110) (Fig. 2), fifteen (24.5%) of 61 female patients and 14 (48.3%) of 29 male patients.

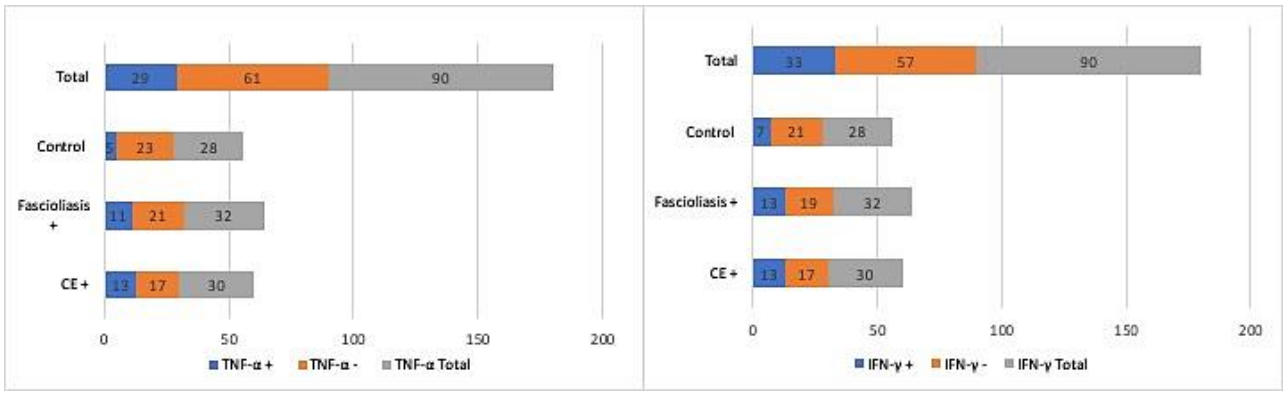

Fig. 2: TNF-α and IFN-γ results according to infections and control groups

The IFN-γ response was investigated in 90 serum samples (30 patients with CE, 32 patients with fascioliasis and 28 control group members). It was determined 43.3% (13/30) of CE patients, 40.6% (13/32) of fascioliasis patients and 25% (7/28) of the control group (*P*=. 297) (Fig. 2).

TNF-α (*p*=.02) and IFN-γ (*P*=.001) responses were significantly higher among male patients. The highest levels of cytokines were observed in the 18-30 age group.

An increase was observed in all cytokine types and Th1 and Th2 responses were determined. Moreover, there were significant correlations between CE and IL-4, IL-10 and TNF-α responses and between fascioliasis and IL-4 response (*P*≤.05). While no correlation was

detected between cytokine responses depending on the age group, the correlation between TNF-α and IFN-γ responses was significant in male patients.

Discussion

Th1 and Th2 responses coexist in helminth infections and cytokines inhibit each other (7). In CE and fascioliasis, balanced immune response occurs due to the production of regulatory cytokines that induce mutual inhibition (8). Although simultaneous involvement of both cell responses is observed in helminth infections, animal model experimental studies demonstrated that cytokine profile mostly varied based on the duration of the infection (7). Contrary to this idea, cytokine response was not associated with the clinical stage of the disease (14). In general, the Th0 or Th1 response is dominant in early infections, and the Th2 response is dominant in late and chronic infections. The Th1 is associated with protective immunity, resistance and parasite destruction, and the Th2 is associated with disposition to the disease. The immune response is known to be polarized towards Th2 in CE and fascioliasis (7).

Immunomodulatory mechanisms that could inhibit host immune and inflammatory responses are required for the survival of both the parasite and the host and for the interaction between the parasites with the vertebrate host. Various strategies developed by the parasite to adopt to the host include modulation of Th2 cell response and the production of cytokines responsible for the pathophysiology of the infection (15).

In a study on peripheral blood mononuclear cell, increased IL-4 and IL-10 concentrations were found to be consistent with Th2 cell activation, while the presence of IFN-γ production indicated the intervention of the Th1 or Th0 cell subset (14). The significance of IL-4 in CE cases was demonstrated and found the most increased cytokine (68.7%) (16). In addition, an increase of 46.8% in IL-2 and 40.6% in IL-10 was determined. It was demonstrated the coexistence of Th1 and Th2 cytokines in CE, while the Th2 cytokines were more dominant.

The clinical condition of the infection, whether it is a primary or relapse infection, and the number of factors affect the immunological response to the parasite as well (17). Cytokines play a role in both active and inactive stages of the CE infection. In an in vitro study on T cell stimulation, patients with inactive cysts exhibited a Th1 profile, and those with an active and transitive cyst exhibited both types of immune responses (18). Similarly, there was a significant increase in IFN-γ (Th1) and IL-4, IL-10 (Th2) levels in patients in the active stage of the disease, and cytokine responses were identified in 50%, 82% and 74% of the patients, respectively. In patients who responded two years after the pharmacological and surgical treatments, IL-4 and IL-10 levels decreased significantly, while in those who did not respond to the treatment, these levels increased and IFN-γ was not detected (19). In another study, IL-4 response was positive in 87% of the patients who received CE treatment, IL-10 response was positive in 33% of the patients and IFN-γ response was positive in 13% of the patients. Patients who responded to the treatment after a year reported decreased IL-4 and IL-10 levels, while those with a resistant active disease exhibited increases in the same cytokine levels (20). When a cyst dies with natural causes or due to chemotherapy or when it is surgically removed, Th2 responses drop rapidly and Th1 responses become dominant (7). Patient follow-up is important since CE could relapse. IL-4 and IL-10 cytokines were important markers that reflect the effectiveness of surgical operation and pharmacological treatment and may be beneficial in early detection of relapses. IL-10 could help parasite survival by disrupting the protective Th1 response (7, 21). Increased IL-6, IFN-γ and IL-17A activities were observed in serum samples and in patients tested for cytokine production in antigen-5 induced peripheral blood mononuclear cell cultures; however, it was found that the same cytokine levels were lower in relapsed patients. IL-17A production was shown in during human CE infections, which contributed to the host defense

mechanisms against the parasite via immune-protective activities (22).

Essentially, the structure of the antigen and the amount of antigen released from the cyst also play an important role in the cytokine response. AgB, one of the two major components of the cyst fluid, affects the Thl / Th2 cytokine response favoring the Th2 in chronic infections (23). Cytokine release is also associated with the localization of the agent, agent count and fertility of the cyst (24). IL-1, IL-2 and IL-4 were closely associated with the count, features and location of liver cysts (21). Mice infected with 500 protoscoleces led to the production of significant levels of type 2 cytokines (IL-4, IL-5, IL-10 and IL-6) as well as type 1 (IFN-γ) response; thus, early IFN-γ release by *E. granulosus* in experimental infections was dependent on the dose of parasites that the host was exposed to (25).

In a study conducted on in vitro cytokine production in CE patients, high IL-2, IFN-γ and IL-5 production levels were observed, while there were no changes in IL-10 levels (17). IFN-γ and IL-10 cytokines increased mostly during the first trimester of infection. An in vivo mouse experiment demonstrated that IL-6 and TNF-α expressions were normal and then significantly decreased, while IL-10 and TGF-β levels gradually increased (26). Such a response would help the parasite to escape the harmful response of the host (27). In Algeria, serum IL-12 and IL-8 levels were significantly high in CE patients. IL-12 is an important clinical marker for disease activity and protective Thl response in CE. Along with its role in downregulating Th2 responses associated with the pathology, it is a powerful cytokine that manipulates the immune system. Therefore, IL-12 may possess clinical benefits in evaluating CE treatment in humans (24). Similar to CE, the involvement of cytokines in immune responses favors both Th1 and Th2. Although the increase in Th1 and Th2 cells leads to a conventional Th1–Th2 balance with mutual negative effects, T cell induction in fascioliasis is polarized towards the type 2 (7, 8, 28).

Patients infected with *F. hepatica* experience immunosuppression during the acute and chronic phases. Immunosuppression could allow parasites to survive a continuous immune response (29). Cytokine response changed with the infection duration and reported high levels of Th1-related IFN-γ, IL-12 and TNF-α cytokines in acute infection, high IL-4 and IL-5 levels and low Th1 cytokine levels in chronic infection (30). In another study, on the seventh day of *F. hepatica* infection, IFN- γ, Th2-related IL-10 and IL-4 levels increased and Th1 related IL-2 levels decreased. IL-4 and IL-10 could have played a role in the escape of the parasite from the protective immune response and in the reduction of cellular proliferation in early stages of liver penetration (31). The TNF-α levels increased in the infection group and were involved in the hepatic injury process induced by *F. hepatica* (32). High IL-4 and IL-10 levels in the chronic phase of the infection suggested that Th1 cell responses; and thus, IFN-γ expression were inhibited, in addition to a synergism between these cytokines (28). In a study on experimental *F. hepatica* infection and culture, the infection-specific IL-10 levels increased as the infection became chronic, and the inhibition of IL-10 led to an increase in parasite-specific and nonspecific IFN-γ release, however, IL-4 response could not be renewed. IFN-γ production suppression was induced by the parasite during infection and could mediate the parasite survival (29). *F. hepatica* induced a Th2 response characterized by IL-4, IL-5 and IL-10 production, and the parasite suppressed the production of proinflammatory mediators such as Thl-related IL-12, IFN-γ and nitric oxide (33). High levels of IFN-γ production could activate macrophages and induce NO production (31). In a pervious in-vivo study, the relatively low parasite count determined in the IFN-γ administered group supported this approach (34).

However, serum cytokine levels do not only change in the presence of infectious agents but they could be affected by several factors such as autoimmune diseases and the type of consumed food (15). Studies demonstrated increases in certain parameters such as IL-1β, IL-6, IL-8, IL-10 and TNF-α in smoking patients (35). The proliferative response in CE and fascioliasis

infections is closely associated with the number of agents, and location, duration and characteristics of the infection. The high parameter levels obtained in the study in certain control group members also suggested that the control group may include smokers or individuals with a bacterial or viral infection. Moreover, the weakness of the present study was the lack of information on whether fascioliasis was acute or chronic and on whether CE cases were primary or relapse infections.

Understanding Thl and Th2 immune responses is important for the development of new vaccines or therapeutic approaches against infections. The efficiency of future vaccines could be affected by parasitic immunomodulation. Activation of T cells that determine the cytokine profile is a critical event that could affect the outcome of the infections (29).

Conclusion

There were significant correlations between CE and IL-4, IL-10 and TNF-α responses and between fascioliasis and IL-4 response. The changes in these cytokine responses provide information about the immune response development against CE and fascioliasis and may be beneficial markers for early detection of infection and relapses and in treatment follow up.

Journalism Ethics considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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Conflict of interest

The authors declare no conflict of interest

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