

Detection of Serum Cytokines Before and After Pharmacological and Surgical Treatment in Patients with Cystic Echinococcosis

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ABSTRACT

BACKGROUND: Human cystic echinococcosis (CE), caused by *Echinococcus granulosus*, is one of the most important and widespread parasitic zoonoses in the developing world. Postsurgical relapses or treatment failure is a frequent risk and a long-term clinical and serological follow-up is required to evaluate the success and failure of therapy. Therefore, the present study was aimed to identify the immunological markers that correlate with the effectiveness of pharmacological and surgical treatment.

METHODS: The relation of serum cytokine levels and outcome of chemotherapy and surgery was evaluated in 50 patients with cystic echinococcosis (CE). Serum IL-4, IL-10 and interferon-gamma (IFN- γ) concentrations were determined by ELISA before and after pharmacological and surgical treatment.

RESULTS: The serum cytokine levels of IL-4, IL-10 and IFN- γ were elevated in significant proportion of patients during the active stage of disease. IL-4, IL-10 and IFN- γ were measurable in 41 (82%), 37 (74%) and 25 (50%) patients before the treatment. Clinical and radiological assessment of patients at 2 years, after the pharmacological and surgical treatment, showed that 48 of the 50 patients had responded to the treatment. IL-4 and IL-10 levels were decreased significantly ($P < 0.05$) in these patients. Conversely, of the patients who did not respond had high levels of IL-4 and IL-10 and undetectable levels of IFN- γ .

CONCLUSION: In conclusion, the results indicate that the serum IL-4 and IL-10 cytokines are useful immunological markers demonstrating the effectiveness of surgical and pharmacological treatment in the follow-up of patients with cystic echinococcosis.

Keywords: Cystic Echinococcosis; Th1 Serum Cytokines; Th2 Serum Cytokines; Interleukins IL-4, IL-10; IFN- γ

INTRODUCTION

Cystic echinococcosis (CE) is a chronic infection caused by the tapeworm *Echinococcus granulosus*. In humans, the larval stage of the parasite can develop and form cysts in almost any organ, especially the liver and the lungs [1]. *Echinococcus granulosus* in humans triggers a humoral and cellular response characterized by elevated serum antibodies and by concurrent intervention of T helper cell 1 (Th1) and T helper cell 2 (Th2) cytokines [2]. The variability and severity of the clinical expression of the disease probably also reflects the variety of human immunological responses to the parasite. Therapy of CE is primarily surgical, even though

pharmacological treatment with benzimidazole carbamates is nowadays an effective alternative [3-7]. Clinical evaluation of the outcome of the disease is difficult and relies on combined imaging methods and serological techniques. Because specific antibodies persist in patient's serum for several years after recovery, a long-term clinical and serological follow up is required to evaluate the success or failure of therapy. New and interesting research in the immunosurveillance of CE includes cytokines possibly associated with the outcome of the disease. Most studies on CE cytokines are mainly based on in vitro experiments, i.e. determination of cytokine production following stimulation of peripheral blood mononuclear cell or T helper

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cells of patients with crude and B hydatid antigen [8-10]. These studies demonstrated an increase in the production of some cytokines such as IFN- γ , IL-4 and IL-5. Others demonstrated simultaneous involvement of the Th1 and Th2 cells in CE patients [11-13]. Although two studies have reported the presence of cytokines in sera of surgically treated CE patients, neither of them evaluated the association between cytokines and the clinical outcome of the disease [14, 15]. In particular, Torcal et al reported a close relationship of IL-1, IL-2 and IL-4 with the number, characteristics and location of cysts within the liver; Touil-Boukuffa et al postulated that after surgical removal of hydatid cysts, measurement of serum cytokine levels might allow early detection of relapse [14, 15]. Continuing our research programme into the identification of immunological markers indicating the effectiveness of pharmacological and surgical treatment, in the present study we have monitored serum IL-4, IL-10 and IFN- γ concentrations in 50 pharmacologically and surgically treated CE patients to evaluate, *in vivo*, the association of cytokine production with the outcome of disease.

METHODS AND MATERIALS

Blood samples were obtained from 50 patients (16 males and 34 females; mean \pm SD age was 31.1 ± 11.2 years, range 9-69 years) with clinically/radiologically diagnosed hydatidosis and 10 sex and age-matched healthy controls. Samples from all subjects were centrifuged at $2000 \times g$ for 10 minutes at $40^\circ C$ to obtain the serum. The lipemic or hemolyzed sera were discarded. The sera was divided in to 3 tubes for each subject and stored immediately at $-700^\circ C$ until cytokine analysis. All 50 patients received antihelminthic treatment of albendazole, 400 mg twice a day for 3 months, plus praziquantel, 40 mg/kg/day for two weeks as per standard guidelines [7] and underwent appropriate surgical procedures. The effectiveness of pharmacological and surgical treatment was evaluated by objective criteria mainly based on imaging techniques and cytokine determination at six months, one year and two years following pharmacological and surgical treatment. The long-term outcome of therapy was assessed by the presence or the lack of relapse 3 years after the pharmacological and surgery treatment. All procedures were approved by the local Ethical Committee and all subjects gave their informed consent to the study.

Cytokine assays: Serum cytokine concentration of IL-4, IL-10 and IFN- γ were determined by ELISA commercial kits (Human IL-4, IL-10 and IFN- γ high sensitivity ELISA kits, Gen-Probe Diacclone, France) according to the manufacturer's instructions. The ranges of the sensitivity standard curve of the ELISA kits were 1-35pg/mL for IL-4, 12.5-400pg/mL for IL-10 and 0.78-25pg/mL for IFN- γ . All serum samples were tested in duplicate.

Statistical analysis: Results of cytokine detection were expressed as arithmetic means with standard deviation. The results were compared as healthy control group v/s pre-operative group; and also between pre-operative v/s post-operative groups. The results were evaluated using Student's 't' test. A P-value of less than 0.05 was considered statistically significant.

RESULTS

In the present study, the age of patients varied between 9 and 69 years. The mean \pm SD age of the patients was 31.12 ± 11.24 years. The highest incidence of disease was recorded among patients between 20 to 49 years (41). Hydatidosis was found to be predominant in females (68%) than in males (32%). Thirty four (34) of the radiologically and surgically confirmed cases had hepatic cysts while 16 had extrahepatic cysts (12-lung cysts, 3-liver and lung and 1-thigh cyst). All patients responded to pharmacological and surgical treatment except for two women (32 and 36 years old) in whom multiple cysts (12 and 7 cysts) were detected in liver and lung two years after the first operation.

Detection of serum cytokines: Serum cytokine levels of IL-4, IL-10 and IFN- γ were detected in patients' sera at the time of surgery (pre-surgery), and post-surgery i.e., 6, 12, 24 months.

IL-4 levels (Table 1) were significantly higher ($P < 0.0001$) in patients (pre-operative) v/s the healthy controls, the same data clearly shows that post surgery at the indicated time points (6, 12, 24 months), there was a significant decrease ($P = 0.001$) in IL-4 levels when compared to the pre-operative levels. From this data it becomes obvious that IL-4 can serve as a reliable marker for the success of such surgery.

The IL-10 levels were significantly higher ($P = 0.010$) in patients (pre-surgery) v/s the healthy controls, although there was a significant decrease ($P = 0.037$) in the post-surgery, but the trend is not as marked as seen in IL-4 ($P = 0.010$).

Table 1: Cytokine levels in healthy controls and hydatid patients before and after treatment

Cytokine (pg/ml)	Healthy controls (n=10)	Patients with CE(n=50)					P-value
		Pre-surgery	P-value	Post-surgery			
				Six month follow-up	One year follow-up	Two year follow-up	
				Mean conc.(range)	Mean conc.(range)	Mean conc.(range)	
IL-4	3.28 (1-3.5)	15.09 (5-26)	<0.0001	11.15 (4-18)	7.75 (4-18)	8.02 (4-18.5)	0.001
IL-10	13.88 (13-18)	73.24 (25-195)	0.010	49.83 (18-138.2)	31.22 (18-55.4)	55.77 (45-176)	0.037
IFN- γ	3.63 (1-4)	8.34 (4-14)	0.011	7.16 (5-12)	7.37 (4.4-12)	7.73 (5-18)	0.261

The high mean concentration of IL-4 and IL-10 levels at two year follow-up was due to the relapse of disease in two patients. But still at that time it is significantly less ($P=0.037$) than pre-surgery value. Hence, this cytokine also decreases post successful surgery, and therefore could serve as additional adjunct marker (Table 1).

The IFN- γ levels (Table 1) were also elevated significantly ($P=0.011$) in patients as compared to healthy controls, however post-surgery there was no significant ($P=0.261$) change in this cytokine levels.

The success of therapy was evident through clinical and imaging assessment at 2 years, after the end of albendazole therapy that showed 96% patients had responded clinically.

DISCUSSION

One of the most immediate needs in the post-surgical or post-pharmacological treatment immunosurveillance of CE patients is to identify markers indicating the effectiveness of treatment. The frequent relapse of disease (25%) after an initial therapeutic success is a serious problem in the pharmacological treatment of CE [3]. The results of the current study revealed that, there is a considerable increase in both Th1 (IFN- γ) and Th2 (IL-4, IL-10) cytokine in CE patients. The cytokine levels of IL-4, IL-10 and IFN- γ were detected in 82%, 74% and 50% hydatid patients at the time of diagnosis. These results are well supported by previous investigations [2, 16, 17]. Elevated cytokine levels were also measurable in the sera from hydatid disease patients with lung and liver involvement [15]. The coexpression of both Th1 and Th2 cytokines is in agreement with most of the previous studies [18-21]. The coexistence of significantly high IL-4 production with high IL-10 concentrations observed in most of the hydatid patients supports Th2 cell activation in human hydatidosis. Despite producing IL-10, a known inhibitor of Th1 cytokine production [22, 23], the patients also produced IFN- γ , suggesting that the immune

response to *E. granulosus* infection is regulated also by Th1 (or Th0) cell subset. In human hydatidosis Th1- and Th2-type cytokine patterns probably coexist, each T cell subset recognizing distinct epitopes on the parasite antigen.

The assessment of changes in the serum cytokine levels of IL-4, IL-10 and IFN- γ in six month, one year and two year follow-up samples clearly demonstrate, the IL-4 and IL-10 levels were considerably decreased in patients who responded to treatment. The results showed an association between IL-4 and IL-10 cytokine levels in patients with cystic echinococcosis and outcome of pharmacological and surgical treatment. The high levels of IFN- γ and low levels of IL-4 and IL-10 (Th2) in patients who had responded successfully to treatment are in agreement with many studies [16, 24, 25]. Ortona et al [12] observed the decrease in IL-4 and IL-10 (Th2) and increase in IFN- γ (Th1) response in patients who responded successfully to treatment and concluded that high IFN- γ (Th1) levels and low IL-4 and IL-10 (Th2) are the markers for successful treatment. Mezioug and Touil-Boukoffa [21] reported that Th1 response is more related to resistance, protective immunity and killing the cyst, whereas Th2 response is associated with susceptibility to disease and escapes immunity. Zhang and McManus [26] stated that when a cyst dies naturally, is killed by chemotherapy treatment or is removed by surgery, Th2 responses drop rapidly and Th1 responses become dominant. This can be interpreted as Th1 lymphocytes contribute significantly to the inactive stage of hydatid disease, with Th2 lymphocytes being more important in the active and transitional stages.

CONCLUSION

In conclusion, the results point to the presence of both Th1 and Th2 cytokine profile in CE with Th2 predominance at the active stage of disease and significant decrease of Th2 (IL-4, IL-10) cytokines in patients who had responded to treatment. Thus, the results overall, indicated the

usefulness of cytokine monitoring in the clinical follow-up of patients after treatment and underline the need to search for more sensitive techniques to identify patients at risk of relapse.

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