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Serum interleukin-8 levels may predict relapse in brucellosis

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Aim: To investigate whether cytokines are effective in predicting relapses among patients with acute brucellosis.

Materials and methods: This trial was conducted in 42 patients who were being followed-up with diagnosis of acute brucellosis. Serum samples were obtained on days 0 and 45. In patients whose clinical symptoms recurred within a year of treatment and exhibited infectious parameters in compliance with brucellosis, a Rivanol standard tube agglutination (STA) test was performed and the diagnosis of relapse was based on brucella immunoglobulin M (IgM). Serum samples were evaluated for various parameters, namely tumor necrosis factor-alpha (TNF-α), interferon-gamma (IFN-γ), interleukin 2 (IL-2), IL-4, IL-6, IL-8, IL-10, and soluble IL-2 receptor (sIL-2R).

Results: Relapse was seen in 7 patients. No difference was found between relapsing patients (RPs) and fully recovered patients (FRPs) in terms of age, sex, leukocyte levels, or C-reactive protein (CRP) values. Comparison of TNFα, IFNγ, IL-2, IL-4, IL-6, IL-8, and IL-10 values on day 0 (day of enrollment) revealed 2-fold higher IL-8 values among RPs compared to FRPs. IL-8 was suggested as significant in terms of predicting relapse.

Conclusion: Diagnosis and treatment of relapsing cases in acute brucellosis have not yet been clarified. Predicting relapse by certain laboratory evaluations may be beneficial in preventing clinical relapses by rearranging treatment and monitoring strategies of patients.

Key words: Acute brucellosis, relapse, cytokines

Introduction

Brucellosis is a disease with low mortality but high morbidity rates, progressing with nonspecific clinical symptoms like fever, chills, malaise, myalgia, arthralgia, and headache (1,2). In Turkey, 1.4%-8.5% rate of seropositivity was determined, varying in different regions of the country (1,3-5).

Previous trials were not able to clarify the exact cause of treatment failures or relapse in brucellosis. Relapse was suggested to be associated with various factors like the type of the brucellosis bacteria, cellular immunity, localization of the infection, duration between the emergence of symptoms, and initiation of treatment (6).

Brucella stimulates both humoral and cellular immunity. Even though humoral antibodies are somewhat responsible for resistance against the infection, the main mechanism of recovery is cellular immunity. Elimination of the brucellosis occurs in conjunction with macrophage activation, which is, in turn, induced by T helper 1 (Th1) cell-mediated immunity. Cytokines released during this stimulation play a critical role in the pathogenesis of brucellosis (7-10).

Th1-Th2 balance is an important factor in the determination of disease liability. While Th1 lymphocyte response is a critical mediator in the development of resistance to intracellular pathogens, response to Th2 lymphocytes leads to serious complications in these types of infections (8-10).

The same treatment protocols are used in patients with brucellosis carrying similar features,

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and the majority of the patients recover but relapse is observed in 5%-8% of cases (1,2). Previous trials were not able to clarify the relation between clinical course/relapse and cytokines in acute brucellosis.

This study aimed to investigate whether cytokines are effective in predicting relapses among patients with acute brucellosis.

Materials and methods

This trial was conducted in 42 patients who were being followed-up with the diagnosis of acute brucellosis, at the outpatient clinics and inpatient wards of the Infectious Diseases and Clinical Microbiology Department of Meram Medical School, Selçuk University, between July 2007 and December 2008. Relapse was diagnosed in 7 patients.

Diagnosis of acute brucellosis was confirmed by serum agglutination titers (SATs) ≥ 1/160 or a 4-fold increase in SATs evaluated 2 weeks apart, and/or positivity in blood cultures, in addition to clinical symptoms. Patients under 18 years of age, pregnant or lactating, diagnosed with chronic and subacute brucellosis, with a described complication associated with brucellosis, with an immunosuppressive state, and patients with a history of allergy against the drugs to be used in the treatment were excluded from the trial. Patients who were considered to have acute brucellosis and met the inclusion criteria were included in the trial.

Before initiation, the local ethics committee approved the study protocol and all of the patients gave informed consent.

Serum samples were obtained on days 0 and 45. The patients were followed-up for 1 year, with 3-monthly control visits. In patients whose clinical symptoms recurred within a year of treatment and exhibited infectious parameters in compliance with brucellosis, a Rivanol standard tube agglutination (STA) test was performed and the diagnosis of relapse was based on brucella immunoglobulin M (IgM). Serum samples were obtained on the day the diagnosis of relapse was confirmed. Blood samples of 10 mL were centrifuged at 5000 cycles for 3 min and the serum was separated. Serum samples were kept frozen at –80 °C.

Serum samples were evaluated for various parameters, namely tumor necrosis factor-alpha (TNF-α), interferon-gamma (IFN-γ), interleukin 2 (IL-2), IL-4, IL-6, IL-8, IL-10, and the soluble IL-2 receptor (sIL-2R). During the evaluation of cytokines, human TNFα, IFNγ, IL-2 IL-4, IL-6, IL-8, IL-10, and sIL-2R (Bender MedSystems GmbH, Austria) kits were used. The kits were utilized in compliance with the manufacturer's instructions, and absorbance values were read at 450 nm using a microplate reader (BioTek Microplate Instruments, USA) enzyme-linked immunosorbent assay (ELISA) reading instrument. Respective sensitivity values for TNF-α, IFNγ, IL-2, IL-4, IL-6, IL-8, IL-10, and sIL-2R were as follows: 1.65 pg/mL, 0.66 pg/mL, 2.3 pg/mL, 0.6 pg/mL, 0.92 pg/mL, 1.3 pg/mL, 0.66 pg/mL, and 0.21 ng/mL.

Data were evaluated using SPSS 13.0 for Windows (SPSS Company, Chicago, IL, USA). Statistical analysis was carried out using the same program. For data analysis, the Mann-Whitney U test with Bonferroni correction and Wilcoxon signed-rank test were used and the Friedman test was utilized for repetitive measurements.

This trial (Project no: 07102016) was supported by the Selçuk University Scientific Research Project Division.

Results

This trial was conducted in 42 patients, with 7 (16.6%) relapsing patients (RPs) and 35 fully recovered patients (FRPs). Of the patients, 28 (66.7%) were women and 14 (33.3%) were men. No difference was found between RPs and FRPs in terms of age, sex, leukocytes, sedimentation, C-reactive protein (CRP), or standard tube agglutination (STA) values (Table 1).

In 28 of the 42 patients (66.6%), treatment was initiated with doxycycline + rifampicin (D+R) and, in 14 patients (33.3%), treatment was initiated with doxycycline + streptomycin (D+S). Four of the 7 RPs received D+R treatment while 3 received D+S treatment. No difference was determined between these 2 treatment groups in terms of treatment efficacy, side effects, or relapse.

Table 1. Descriptive features and certain clinical characteristics of patients with brucellosis.

	FRPs (n = 35)	RPs (n = 7)	P value
Age	38.9 ± 14.7	43.7 ± 15.3	>0.05
Leukocytes	7329.5 ± 1830.3	7428.5 ± 1359.7	>0.05
Lymphocytes	2828.5 ± 1439.9	3342.8 ± 1448.9	>0.05
Sedimentation	27.4 ± 21.5	28.1 ± 13.1	>0.05
CRP	17.4 ± 17.5	14.0 ± 13.6	>0.05
STA*	1/320	1/320	>0.05

*median value

Upon comparison of TNFα, IFNγ, IL-2, IL-4, IL-6, IL-8, and IL-10 values of acute brucellosis on day 0, the IL-8 values were higher in RPs compared to those in FRPs (P = 0.004) (Table 2).

No significant difference was observed between the 7 RPs and 35 FRPs in terms of TNFα, IFNγ, IL-2, IL-4, IL-6, IL-8, IL-10, or sIL-2R values on day 45 (Table 3).

A Friedman test revealed an impact of relapse upon differences observed in IL-8 values on days 0 and 45 (P = 0.048), while no such effect was determined regarding the remaining cytokines.

Discussion

Trials to date were not able to clarify the exact cause of treatment failure or relapse in brucellosis. Alavi et al. found an association between relapse and lymphopenia, high erythrocyte sedimentation rate (ESR), and CRP duration between the onset of the symptoms and initiation of the treatment, male sex, and age, in a trial conducted in 115 patients. He reported that the relapse rate was significantly higher among male patients over 50 years of age, with lymphopenia, high ESR, and CRP values, and with a long interval time between the onset of the symptoms and the treatment (6).

Solera et al. found that body temperature over 38.3 °C, positive blood culture, and symptom duration of less than 10 days were associated with relapse. However, sex, patient age, ESR, alanine transaminase (ALT), leukocyte, hemoglobin, platelet, serum albumin, and alkaline phosphatase (ALP) levels were found not to be associated with relapse (11). Ariza et al. showed that inefficient antibiotic treatment, prolonged blood culture positivity, pretreatment illness duration shorter than 10 days, and platelet count less than 150,000/mm^3 were predictive factors of relapse (12). In our trial, no difference was found between RPs and FRPs in terms of age, sex, or leukocyte, ESR count, CRP, or STA values.

Table 2. Day 0 TNFα, IFNγ, IL-2, IL-4, IL-6, IL-8, IL-10, and sIL-2R values in patients with brucellosis in terms of relapse status.

	FRPs (n = 35)	RPs (n = 7)	P value
TNFα	45.8 ± 61.9	43.8 ± 26.3	>0.05
IFNγ	12.6 ± 9.7	14.6 ± 9.4	>0.05
IL-2	33.3 ± 24.9	38.9 ± 36.1	>0.05
IL-4	27.0 ± 38.94	17.4 ± 10.5	>0.05
IL-6	6.1 ± 6.4	5.0 ± 2.6	>0.05
IL-8	32.8 ± 17.3	60.1 ± 21.3	<0.004
IL-10	9.9 ± 6.6	8.3 ± 5.3	>0.05
sIL-2R	3.0 ± 4.5	2.3 ± 1.4	>0.05

Table 3. TNFα, IFNγ, IL-2, IL-4, IL-6, IL-8, IL-10, and sIL-2R values of patients with brucellosis on day 45 in terms of relapse status.

	FRPs (n = 35)	RPs (n = 7)	P value
TNFα	37.5 ± 21.8	31.6 ± 23.0	>0.05
IFNγ	11.0 ± 9.2	11.5 ± 10.4	>0.05
IL-2	28.2 ± 23.4	45.8 ± 40.6	>0.05
IL-4	28.0 ± 35.1	16.7 ± 19.2	>0.05
IL-6	7.1 ± 7.9	3.5 ± 1.5	>0.05
IL-8	39.9 ± 37.2	48.7 ± 27.7	>0.05
IL-10	9.2 ± 5.7	7.8 ± 5.8	>0.05
sIL-2R	2.7 ± 3.1	1.7 ± 0.8	>0.05

The leading defensive response against intracellular pathogens like *Brucella* spp. is cellular immunity (1,2). Elimination of brucella is mediated by macrophage activation, which is, in turn, induced by Th1 cell-mediated immunity. Cytokines released during this stimulation phase play a very critical role in the pathogenesis of brucellosis (7-10).

A new cytokine family with chemotactic activity on leukocytes and fibroblasts was described recently. They are activated through binding to specific transmembrane receptors at a concentration of 10^{-8} -10^{-11} M. IL-8 is also a member of the chemokine family (10,13).

The sources of IL-8 are monocytes, macrophages, fibroblasts, keratinocytes, hepatocytes, chondrocytes, and epithelial and endothelial cells. Chemokines influence the function of target cells, rather than producing dominant growth in these cells. They play a critical role in tissue damage, and the migration of specific cells to inflammation sites. They target the cells of IL-8 neutrophil T cells. They provide mobilization, activation, and degranulation of neutrophils and play a role in angiogenesis. They are one of the most potent chemotactic factors for neutrophils (10,13).

In the pathogenesis of brucellosis, an increase in IL-8 level stimulates the migration of leukocytes and chemotaxis. In a trial conducted by Akbulut et al. on acute brucellosis, the IL-8 level was reported to be unchanged, while, in the trial performed by Refik et al., high IL-8 levels were observed in the patient group compared to the controls (9,14). Currently, available trials showing the association between cytokines and relapse in brucellosis are scarce.

In various trials, no correlation was reported between relapse and IL-8. We demonstrated that IL-8 was twice as high among RPs compared to FRPs (P = 0.004). In this study, we found that serum end-of-treatment IL-8 levels were elevated in RPs when compared to FRPs. The levels of serum inflammatory response mediators such as CRP, IL-2, IL-2R, TNF-α, and IFN-γ were twice as high among RPs. Delpino et al. have recently shown that *Brucella* spp. infected hepatocytes released IL-8 and triggered immune responses (15). Intracellular survival of brucella leads to IL-8 secretion before cytotoxic immune response is initiated. Our findings and Delpino et al.'s results have led us to hypothesize that the intracellular survival of brucella is the keystone of the relapse mechanism. Relapse can be predicted with high IL-8 levels before immune activation occurs.

IFNγ is required for the elimination of brucella and for the survival of the host that encounters this microorganism. IFNγ is the most important product of Th1 cells and diverts immune response toward the Th1 genotype (9). Akbulut et al., Rafiei et al., Demirdag et al., and Ahmed et al. also determined high IFNγ levels in brucellosis cases compared to the control group (9,16-18). In trials conducted to date, no relation was shown between relapse and IFNγ.

Similarly, we did not detect any differences between RPs and FRPs in terms of IFNγ levels.

In trials conducted on brucellosis cases, TNFα levels were investigated. Refik et al., Palmer et al., and Ahmed et al. reported no significant increases in TNFα levels among patients with brucellosis (14,18,19). On the other hand, Akbulut et al. and Demirdag et al. found significantly high TNFα levels among the patient group compared to the control group (9,17). Insignificant differences in TNFα levels compared to the control group were related to a short half-life of TNFα, while high TNFα levels were associated with proinflammatory mediating features of this cytokine and with the increase in IFNγ levels (9,17,18). However, no relation was shown between relapse and TNFα in trials conducted to date. In the current trial, we also did not demonstrate any differences between RPs and FRPs in terms of TNFα levels.

Makis et al., Akbulut et al., and Ahmed et al. investigated IL-2 levels among brucellosis cases and found no difference between the patient group and the control group (9,18,20). No relation has been shown between relapse and IL-2 to date. Similarly, we did not find any differences between RPs and FRPs in terms of IL-2 levels. Refik et al. reported that sIL-2R is increased in brucellosis (14). On the other hand, Makis et al. found an increase in soluble interleukin-2 receptor α (sIL-2Rα) in brucella cases (20). He observed relapse in patients with high sIL-2Rα levels at the end of the treatment and reported that sIL-2Rα was a reliable parameter in predicting relapse. In our trial, no association was found between relapse and sIL-2Rα.

IL-4 naive CD4+ T lymphocyte Th2 stimulates cell growth and acts as an autocrine growth factor for differentiated Th2 lymphocytes. Therefore, it is responsible for stimulation and increase in the IL-4 and Th2 subgroup. Moreover, IL-4 antagonizes the macrophage activation characteristic of IFNγ and suppresses cell-mediated immune response (21). Akbulut et al. and Pasquali et al. reported low IL-4 levels among acute brucellosis in the patient group compared to the control group (22,23). In another trial, Akbulut et al. did not observe any differences between patient and control groups in terms of IL-4 levels (9). On the other hand, high IL-4 levels were reported in brucellosis in a few trials (24). In trials conducted to date, no relation was shown between relapse and IL-4. In our trial, no difference was found between the study groups in terms of IL-4 levels.

Interleukin-6 is a cytokine with biological activity on a number of cells that plays a role in innate and acquired immune response. It stimulates T cells, acting synergistically with IL-1 and TNF-α. IL-6 plays a role in the differentiation, activation, and growth of T cells, including a differentiation into cytotoxic T cells (25). Akbulut et al., Refik et al., and Durand et al. reported high IL-6 levels in the patient group compared to the control group (9,14,26). However, no relation was shown between relapse and IL-6 in trials performed to date. We also did not demonstrate any differences between RPs and FRPs in terms of IL-6 levels.

IL-10 is an inhibitor of activated macrophages. By acting on macrophages, they inhibit both cytokine release and expression through co-stimulators (27). In a trial conducted by Rafiei et al., the IL-10 level was similar in the patient and control groups ($P > 0.05$) (16). Trials performed to date did not show a correlation between relapse and IL-10. In the current trial, no difference was found between RPs and FRPs in terms of IL-10 levels.

Conclusion

Currently, few studies are available indicating a correlation between cytokines levels and relapse in brucellosis. The diagnosis and treatment of relapsing cases in acute brucellosis are not clarified yet. Predicting relapse by certain laboratory evaluations may be beneficial in preventing clinical relapses by rearranging treatment and monitoring strategies of the patients. Based on the results of the current trial, we suggest that a high IL-8 value may be influential in indicating relapse, and we think that our findings should be supported by further trials conducted on larger patient groups.

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