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The Effect of Levamisole Combined with the Classical Treatment in Chronic Brucellosis

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IRMAK, H., BUZGAN, T., KARAHOCAGİL, M.K., EVİRGEN, Ö., AKDENİZ, H. and DEMİRÖZ, A.P. *The Effect of Levamisole Combined with the Classical Treatment in Chronic Brucellosis.* Tohoku J. Exp. Med., 2003, **201** (4), 221–228 —— Levamisole is an immunopotenciator drug which is used as an antihelmintic drug as well as very effective remedy on cellular immunity compared with humoral immunity. A total 71 patients (37 men, 34 women) who referred to our department between March 1997 and December 2001, with a history of the disease for about 1 year, were diagnosed as having chronic brucellosis through those tests brucella serum agglutination test (SAT), SAT with Coombs and SAT with 2-mercaptoethanol. The patients were randomly divided into levamisole group (36 patients) and control group (35 patients). All patients were given rifampicin 600 mg/day + doxycycline 200 mg/day for 6 weeks as a standard classical combined therapy for brucellosis. In the levamisole group, oral levamisole 80 mg every other day for 6 weeks was added to the treatment. There was a statistically significant difference between two groups, in complaints of arthralgia, fatigue and sweats before and 6 months after treatment, as well as in erythrocyte sedimentation rate and C-reactive protein elevations and lymphomonocytosis finding. While it was provided both clinical and serological improvement in all patients in the levamisole group; 11 patients in the control group did not improve both clinically and in view of specific and nonspecific laboratory findings and a recurrence occurred in one case, in this group. In conclusion, levamisole added to classical antibiotic therapy in treatment of chronic brucellosis was found quite efficient in all patients in providing adequate clinical and laboratory response in comparison to classical antibiotic therapy alone. ———— chronic brucellosis; levamisole; treatment

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Brucellosis is a zoonosis, and virtually all infections derive directly or indirectly from exposure to animals (Corbel 1989). The disease is widespread especially in rural communities among the peasants who densely dealt with stockbreeding without paying any attention for milk hygiene. It is still extensively seen in Mediterranean and Middle-East countries meanwhile in Turkey (Akdeniz et al. 1998a; Gotuzzo 1998; Young 2000). It is more prevalent especially in Central, Eastern and Southeastern Anatolian regions of Turkey (Akdeniz et al. 1998b, c; Caksen et al. 2001, 2002). The clinical manifestation of the disease may vary from subclinical, acute, subacute to chronic levels (Moyer and Holcomb 1995; Young 2000). After the lapse of 8 weeks from the onset, it would be termed subacute, if more than a year it would be considered to be chronic (Gotuzzo 1998).

Levamisole is an immunopotenciator synthetic drug derived from phenylimidothiasole which is used as an antihelmintic against nematodes (Kayaalp 1998). The immunomodulating effect of levamisole, manifested in the process of treatment by a rise in the number of circulating lymphoid cells, their functional capacity, a decrease in the disproportion of immunoregulatory cells and the amount of circulating immune complexes, has been established (Mukovozova 1988). It has been shown that levamisole enhances peripheral blood monocyte phagocytosis and T lymphocyte number resulting in significant specific cellular immunity against brucella antigens (Boura et al. 1984). In a study with evidence of immunological findings, patients improved clinically, their E rosettes showed a significant increase and specific leukocyte inhibition migration factor was produced, antiglobulin titers were very low or negative (Raptopoulou-Gigi et al. 1980)

Acute brucellosis is treated with various antibiotic combinations. However, in spite of the best treatment choises, chronicity may occur in approximately 10% of the cases. The diagnosis and treatment of chronic brucellosis is very difficult and there is no standard treatment schedules as in acute brucellosis with proven efficacy. There are some studies performed by adding some other drugs to classical treatment with combined antibiotics.

This study was performed with the purpose of comparing the efficacies of antibiotic treatment alone and antibiotic treatment plus levamisole in chronic brucellosis.

Materials and Methods

A total of 71 patients (37 of them males, and 34 females) who referred to the Department of Infectious Diseases in the Medical Faculty of Yüzüncü Yıl University between March 1997 and December 2001, with a history of the disease for about 1 year or more, diagnosed with chronic brucellosis through brucella serum agglutination test (SAT) and SAT with Coombs and detection of IgG antibodies by SAT with 2-mercaptoethanol (2-ME SAT) were enrolled in the study. The patients were randomly divided into levamisole group (36 patients) and control group (35 patients). All patients were given rifampin 600 mg/day + doxycycline 200 mg/day for 6 weeks as a standard classical combined therapy for brucellosis. In the levamisole group, oral levamisole 80 mg every other day for 6 weeks was added to the treatment. The informed consent was taken from all the patients in levamisole group after they have been informed about the treatment based on the decision of the Infectious Diseases Academic Committee which approved the study (the decision date: 18 February 1997, number: 7). Clinical and laboratory controls of both groups were performed on at the end of 6 weeks, 3 months and 6 months.

Diagnosis of brucellosis was based on: a) an agglutination titer of 1/160 or higher than this and/or b) detection of brucella sp. from blood, bone marrow, cerebrospinal or other body fluids. Brucella abortus antigens were

used in the detection of brucella agglutinins. Brucella abortus 99s antigens (obtained from Cromatest-Linear Chemicals, Barcelona, Spain) was used in SAT. The antigens have been provided after heat inactivation of the bacteria obtained from colonies of B. abortus species, by suspending the bacteria with phenol.

In serum agglutination test procedure, venous blood samples taken from the patients were centrifuged at 2000 rpm/min for 5 minutes and sera were collected. Serum dilutions of 1/20, 1/40, 1/80, 1/160, etc., were prepared in twofold increments within appropriate tubes. Antigen was added onto serial dilution tubes and the tubes were incubated at 37°C in water bath for 48 hours, after that titration was evaluated.

For serum agglutination test with 2-ME; 0.1 molar 2-ME was prepared, and this solution was kept in dark color bottle at 4°C to be used within 15 days. SAT test with 2-ME was applied to all sera which are positive at titer of 1/160 or higher than this. Two-ME solution was mixed with patients' sera in the manner that to be 3 units 2-ME to 1 unit patient's serum in the solution and the final solution were kept at room temperature for 15 minutes. After that, mixtures were centrifuged at 2000 rpm/min for 10 minutes and SAT was applied to the samples taken from supernatants.

After completion of medical history, physical examination, routine laboratory tests, SAT, SAT with Coombs, SAT with 2-ME, and brucella cultures, the patients were given treatment. In all the patients, initially and if required, during their controls, complete blood count (CBC), erythrocyte sedimentation rate (ESR), peripheral blood smears, routine biochemical tests (glucose, alanine aminotransferase [ALT], aspartate aminotransferase [AST], alkaline phosphatase, gamma glutamil transpeptidase [GGT], blood urea nitrogene [BUN], creatinine), C-reactive protein (CRP), rheumatoid factor (RF) and serologic tests for brucella were achieved. Cultures for brucella were performed when the patient had fever. Radiological examinations were made if necessary.

RESULTS

Age range of the patients in levamisole group was 18–58, mean age 35, and 20 of whom were males, and 16 females (totally 36 patients). Age range of the patients in control group was 21–55, mean age 37, and 18 of whom were males, and 17 females (totally 35 patients). Symptoms and findings of the patients before and after treatment in the both groups are shown in Table 1.

SAT titrations of totally 71 patients who

TABLE 1. *Symptoms and signs before and 6 months after treatment*

Symptoms and signs	Levamisole Group ($n=36$)		Control Group ($n=35$)	
	Pre-treatment n (%)	Post-treatment n (%)	Pre-treatment n (%)	Post-treatment n (%)
Fever	23 (63.9)	0 (0)	21 (60)	3 (8.5)
Arthralgia	24 (66.7)	7 (19.4)	20 (57)	11 (31.4)
Fatigue	26 (72.2)	0 (0)	21 (60)	6 (17.1)
Sweating	21 (58.3)	0 (0)	15 (42.9)	5 (14.3)
Weight loss	9 (23.1)	0 (0)	7 (20)	0 (0)
Hepatomegaly	5 (13.8)	1 (2.8)	5 (14.3)	2 (5.7)
Splenomegaly	4 (11.1)	0 (0)	3 (8.6)	1 (2.8)
Peripheral arthritis	5 (13.8)	0 (0)	5 (14.3)	0 (0)
Spondylitis	4 (11.1)	0 (0)	3 (8.6)	0 (0)

TABLE 2. *Distribution of patients according to SAT titers pre and posttreatment*

SAT	Levamisole Group ($n=36$)				Control Group ($n=35$)			
	Pre-treatment	Post-treatment			Pre-treatment	Post-treatment		
		6th w	3rd m	6th m		6th w	3rd m	6th m
1/1280	6	2	0	0	5	1	1	2
1/640	10	5	4	2	9	7	6	4
1/320	9	8	5	3	9	6	4	3
1/160	11	11	9	5	12	8	7	6
1/80	0	6	6	9	0	7	8	9
1/40	0	4	5	9	0	6	6	6
Negative	0	0	7	8	0	0	3	5

SAT, Serum agglutination test; w, week; m, month.

TABLE 3. *Laboratory findings before and 6 months after treatment*

Laboratory Findings	Levamisole Group ($n=36$)		Control Group ($n=35$)	
	Pre-treatment *n* (%)	Post-treatment *n* (%)	Pre-treatment *n* (%)	Post-treatment *n* (%)
Anemia (Hb<10 g/100 ml)	10 (27.8)	0 (0)	10 (28.6)	3 (8.6)
Leukopenia (<4000/mm³)	4 (11.1)	0 (0)	3 (8.6)	1 (2.9)
Trombocytopenia (<150.000/mm³)	3 (8.3)	0 (0)	2 (5.7)	0 (0)
Pancytopenia	2 (5.5)	0 (0)	1 (2.9)	0 (0)
Lymphomonocytosis	13 (36.1)	0 (0)	14 (40)	5 (14.3)
High ESR (>20 mm/hours)	23 (63.9)	0 (0)	19 (54.3)	4 (11.4)
Elevated transaminase levels	7 (19.4)	0 (0)	5 (14.3)	0 (0)
CRP Positivity (>5 mg/100 ml)	21 (58.3)	0 (0)	20 (57.1)	3 (8.6)
Positivity of SAT with 2-ME	36 (100)	0 (0)	35 (100)	5 (14.3)
Positivity of culture in bone marrow	5 (13.9)	0 (0)	3 (8.6)	0 (0)

ESR, Erythrocyte sedimentation rate; CRP, C-reactive protein; SAT, Serum agglutination test; 2-ME, 2-mercaptoethanol.

were considered to be chronic brucellosis with findings of medical history, physical examination and serologic tests were in the range of 1/160 and 1/1280. SAT with Coombs resulted positive in one patient (1.4%), while SAT resulted negative. SAT titration results in pre and posttreatment periods are shown in Table 2.

Brucella melitensis was isolated in bone marrow samples of 5 patients in levamisole group and of 3 patients in control group.

Laboratory findings of both groups in pretreatment and posttreatment periods are shown in Table 3. While anemia, leucopenia, lymphomonocytosis, high ESR, elevated transaminase and CRP positivity rates were respectively as 27.8%, 11.1%, 36.1%, 63.9%, 19.4% and 58.3% in levamisole group in pre treatment period; these tests improved in all patients by the end of 3 months of therapy. In the control group, who received classical treatment alone; the rates aforementioned were respectively as 28.6%, 8.6%, 40%, 54.3%, 14.3% and 57.1% in pretreatment period; and these tests did not improve in some patients by the end of 3 months of therapy.

In chi-square test performed in view of symptoms such as fever, arthralgia, fatigue and sweats before treatment and 6 months after

treatment was found a statistically significant difference between two groups (levamisole and control group) in favor of levamisole group in especially 3 parameters (arthralgia, fatigue and sweating) ($p<0.05$). Hematologic complications were seen in 28 cases (39.4%) and hepatic complications in 12 cases (17%) among our patients. In chi-square test which we compared findings of ESR and CRP elevations and lymphomonocytosis in levamisole and control group, before treatment and 6 months after treatment, superiority of levamisole group was significantly noticed ($p<0.05$).

DISCUSSION

Brucellosis constitutes an important therapeutic problem in underdeveloped countries where agriculture and stockbreeding are the main source of living. Physical disability, loss of productive effort and economic losses, permanent sequels, and even death may be resulted from human infection. Immune mechanisms differ in miscellaneous forms of brucellosis and clinical differentiation between these forms that is to determine subclinical, acute, subacute and chronic course is difficult (Moyer and Holcomb 1995; Gotuzzo 1998).

First IgM antibodies and after 7–14 days IgG antibodies are produced in human body against the bacteria, like in other infections (Hall 1991; Baldwin 1996). Specific IgM antibodies for brucella appear within the first week of the disease, make summit in 3 months, and then slowly decrease and disappear. Even if IgM antibodies are progressively reduced and vanish from the blood, they sometimes may persist in low titers for a long time, being rarely in high titers.

IgG antibodies begin to rise approximately 3 weeks after onset of the disease and make a peak at 6–8 weeks, they exist in significant levels in blood during a chronic infection. Persistence of IgG antibodies in high titers after brucellosis treatment shows that the case has become chronic and treatment should be continued (Pellicer et al. 1987; Araj and Kaufmann 1989; Ariza et al. 1992).

In a study, 52 patients diagnosed with brucellosis through blood culture were followed up for 13 months (Gazapo et al. 1989). Their serum anti-brucella IgG and IgM levels were measured by enzyme immunoassay (EIA) in various times and it was seen that IgM levels obtained from treated patients did not correlate with the treatment of infection. It was reported that IgG levels of the patients markedly decreased in clinically recovered patients after treatment, but elevated IgG levels persisted in patients with focal infection. In patients in which recurrence occurred and blood culture resulted positive again, was established a second peak of IgG.

In other studies were also demonstrated to develop IgG, IgM and IgA antibodies by EIA. But the antibody model seen in acute and chronic brucellosis patients differed. While in acute brucellosis patients, every 3 types of Ig had significantly increased, in chronic brucellosis patients, only IgG and IgA type antibodies had been found so (Pellicer et al. 1987; Araj et al. 1989).

However differentiation of acute and chronic cases may be difficult, it may be possible by evaluation of duration of the disease, and clinical and laboratory findings. In laboratory assessment, 2-ME test and enzyme-linked immunosorbent assay (ELISA) may be used (Buchanan and Faber 1980; Young 1991). We used 2-ME SAT as a laboratory parameter in differentiation of acute and chronic cases.

Chronic form is seen in 10% of the patients, very rarely seen in children, but frequently in adults above 40 years of age (Gotuzzo 1998).

Chronic brucellosis may appear in 4 types:

a) The disease may show an insidious course.
b) Recurrent attacks may be seen following acute disease.
c) The disease may show localized organ involvements.

d) The disease may not respond to antibiotic treatment.

Eighty-five percent of chronic cases are of asymptomatic course. These patients are noticed when investigated some pathological findings found during their physical examination. In symptomatic cases, findings are generally nonspecific, such as weakness, fatigue, irritability, insomnia, lability, vague extremity pains and headache simulating depression findings. Although many patients diagnosed with chronic brucellosis have sense of fever, measurable fever is generally not determined (Gotuzzo 1998).

An antihelmintic drug levamisole directly stimulate lymphocytes, macrophages and granulocytes; enhance their releases, movements and/or proliferation. It is more efficient in cellular immunity than humoral immunity because of more stimulation on T lymphocytes than B cells. However, it may sometimes stimulate supressor T lymphocytes more than other subtypes of lymphocytes and cause immunosupression paradoxically. It requires to be given in accompaniment of a primary stimulus such as antigen in order to make apparent its immunostimulating activity. Therefore, it is immunopotentiator rather than being immunostimulating. Its stimulating effect, which is weak in general decreases in conditions that immune system is excessively insufficient. Therefore, it is used in conditions that immune system has not excessively been depressed and an antigen source is present in the body such as cancer, recurrent viral diseases, some chronic bacterial diseases rather than pure immune insufficiency syndromes. It has been reported that in some cases has limited advantage, and in some cases was found to be inefficient (Moyer and Holcomb 1995).

However the recurrence rate in using levamisole alone, in cases of acute brucellosis is unacceptable such as 50%, its combination with antibiotics (Gotuzzo 1998) has been found promising in prevention of the disease to be chronic (Mukovozova 1986). This effect of levamisole is probably associated with enhancement of both T-cell function and of phagocytosis capacity of monocytes (Boura et al. 1984; Mukovozova 1987). The clinical efficacy of levamisole has been compared to that of vaccine in a controlled trial of a combined treatment performed in 311 patients with brucellosis (Mukovozova 1986). As compared with vaccine, levamisole appeared more effective in reducing the percentage of the chronic forms of the disease.

Boura et al. (1984) have reported in a series of 32 chronic brucellosis cases that 6 months' levamisole treatment has a good therapeutic efficacy. Raptopoulou-Gigi et al. (1980), in their study consisting of a series of 10 cases, have found clinical and laboratory improvement in patients treated by administration of levamisole at a dose of 150 mg/day, 3 times a week, for 6 months and followed up to another 6 months after treatment. However, in all of the studies which have found in the literature, the results are either based on clinical observations, or levamisole was used as a single agent in the treatment, or such as in forementioned studies, the comparison has been made between different situations. As in our study which was compared classical combined treatment and levamisole added to classical treatment especially in view of clinical outcome and laboratory response of the patients has not been encountered in the literature.

In our study, levamisole 80 mg/every other day for 6 weeks which was added onto a standard combined therapy (rifampin 600 mg/day + doxycycline 200 mg/day for 6 weeks) provided both clinical and serologic improvement in all patients. However complaints continued, they decreased in 7 of 24 patients with arthralgia. On the other hand, 11 of 35 patients in the control group did not improve both clinically and in view of specific and nonspecific laboratory findings. SAT values in 9 of these 11 patients remained unchanged in the course of 6

months, with a little decrease in one patient in whom SAT positivity persisted by the end of 6 months. In the last case was firstly seen a titer decrease, contrary to re-increase at the end of 6 months, and from clinical evaluation along with positive laboratory tests was judged a recurrence in the patient.

In student t test (paired samples t-test) performed in view of SAT values before and 6 months after treatment in both levamisole and control groups was found statistically significant improvement ($p < 0.05$). In comparison of the cases in levamisole and control groups (of SAT results 6 months after treatment) with student t-test (independent samples t-test), statistically more significant SAT titer decreases were determined ($p < 0.05$).

In conclusion, levamisole added to classical antibiotic therapy in treatment of chronic brucellosis was found quite efficient in all patients in providing adequate clinical and laboratory response in comparison to classical antibiotic therapy alone.

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