ORIGINAL ARTICLE

SERUM LEVEL OF SOLUBLE INTERLEUKIN-2 RECEPTOR ALPHA AS A PREDICTOR OF TREATMENT RESPONSE IN BRUCELLOSIS

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Introduction: Iran is one of the endemic regions with high prevalence of brucellosis. Several serological markers for diagnosis and response to treatment are available. Serum level of Soluble Interleukin-2 Receptor alpha (SIL-2Rα) is a new marker to assess response to therapy and clinical relapse of brucellosis. This study intends to investigate the serum levels of SIL-2Rα before and after treatment, to evaluate this marker for patients responding to treatment of brucellosis. **Methods:** This study is an analytical cross-sectional study. Forty patients who had clinical signs of brucellosis and serological tests confirmed the disease have been treated with standard antibiotics for 6 weeks. 2ME and SIL-2Rα levels were measured before and after treatment and these values were compared. **Results:** Among the 40 patients, 27 patients (67.5%) had improvement in symptoms and 13 patients (32.5%) had no symptoms after treatment. In Comparing serum levels of SIL-2Rα and 2ME before and after treatment, decreasing of both markers after treatment was significant (p<0.001). In patients with false positive for 2ME, SIL-2Rα in 57% of patients had a reduction, but in patients with false negative for 2ME, SIL-2Rα in only 28% of patients increased. **Conclusion:** Not only is Serum level of SIL-2Rα useful for predicting response to treatment of brucellosis, but also in cases of false positive of 2ME can be helpful.

Keywords: Brucellosis, SIL-2Rα, 2ME

INTRODUCTION

Brucellosis is a common bacterial disease in human and animals, and it may be transferred to human from animal both directly and indirectly. For hard diagnosis, weak reports, and irresponsible supervising systems, Brucellosis outbreak in the world is unknown. Brucellosis frequency for both human and animals in recent twenty years has extended in Mediterranean Sea, Middle East, Western Asia, and Africa.[1,2] Today Brucellosis is a world crisis, and its outbreak in Iran is highly noticeable. According to the statistics of National Contagious Disease Organization, there are 50,000 reports every year. In 2004, 21,454 cases of malt fever were noticed, where the highest reports were respectively for Khorasan, Hamedan, West Azerbayejan, Kurdestan, Lorestan, Fars, and Eastern Azerbayejan.[1,3]

In the absence of positive culture results, serologic tests were taken to diagnose Malt Fever. Serum of Agglutination Test (SAT) is more extensively applied. Clinical diagnosis of Brucellosis is usually hard, which is mostly due to its similarity with infectious and non-infectious diseases.[3,4] One of the problems of Brucellosis diagnosis is cross reactions which occur between Brucellosis and other bacteria such as salmonella, Tularemia, and Cholera, and even numerous non-infectious diseases such as Lupus erythematosus, Multiple myeloma, Lymphoma. The other problem is that the Brucellosis diagnosis is not possible only by antibody test for healthy people in contact with humans can show a high amount of Brucellosis antibody. Cylinder Agglutination Test can have wrong negative results because of local phenomenon and antibody. Without additive test of 2ME, also, it is unable to diagnose acute cases from chronic ones.[5,6]

Brucellosis pathogens are intercellular which can live and reproduce in phagocytic, single-nucleus cells. Along with the infection, a dependent cell is created. Discharged cytokine macrophage like TNFα and IL-12 induce Lymphocyte Helper TH1 cells which produce INF and 2IL and that consequently results in activating macrophage and death of the cell.[6] Biologic activity of IL-2 depends on connection to the cellular receptor of IL-2R which contains 3 subunits α, β, and γ where a subunit of 5α appears in cell serum; therefore increase in level of Soluble Interleukin-2 Receptor alpha in biologic liquids is a factor in diagnosis of T cells.[7,8]

With respect to critical role of IL-2/il-2R system for immune response to Brucellosis, non-highly sensitiveness, false positive and negative serologic tests, it seems that the monitoring of this system can be useful to estimate the disease development and its treatment. Consequently, it can be a replacement for serologic tests like 2ME for investigation of disease.[9]

The objective of this study was to estimate the serum levels of SIL-2Rα before and after treatment, and to evaluate this marker for patients responding to treatment of brucellosis.

MATERIAL AND METHODS

This was a cross-sectional, analytical study. Samples were taken from the children under 18 years diagnosed with Malt fever who visited Amir Kabir and Valiasr hospitals in Arak in 2011.

The inclusion criteria were: children under 18 years old, Brucellosis diagnosis based on clinical symptoms and positive serologic test, no treatment prior to visit, no need to other types of treatment except treatments under this study, filling in a consent form by parents. Exclusion criteria were: No correct use of the drugs given, patient's resignation from the study, and persons quitting the study before full treatment.

Sixty patients were selected. All attendants were confirmed to be diagnosed with Brucellosis by both clinical symptoms like fever, arthralgia etc. and serologic test (SAT≥1/80).

Prior to treatment, venous blood samples were taken for tests of 2ME and SIL-2Rα. Then all patients were treated with antibiotics for 6 weeks. To patients above 8 years Doxycycline plus Rifampin, and for the patients below 8 years Cotrimoxazole plus Rifampin or Gentamicin was given. During treatment, use of drugs was checked through calls and sometimes in person. After treatment venous samples were tested again in the same lab. 2ME level was tested by serologic titration using antigen of Pastor Institute. Serum level of SIL-2Rα was measured by ELISA using the kit of Biovendor Co.

Data were analysed using SPSS-16. Results were expressed in percentage of frequency, average, and standard deviation. For analytical data, parameters distribution was normalised by logarithm diversion. Pearson's Correlation Coefficient was used to determine the correlation of parameters before and after the east. Paired *t*-test was applied to compare the means before and after the test.

RESULTS

Out of 60 patients, 12 did not visit for the primary tests, and 8 did not visit for the treatment or did not finish it; thus 40 patients, 25 boys and 15 girls, were included. Primary symptoms were fever, chills, no appetite, arthritis, and myalgia. Table-1 illustrates the frequency of each symptom.

Table-1: Frequency of symptoms and signs of brucellosis in this study

Signs and symptoms	Frequency (%)
Fever	29 (72.5)
Arthritis	17 (42.5)
Arthralgia	15 (37.5)
Anorexia	13 (32.5)
Myalgia	8 (20)
Chills	6 (15)

The mean serum SIL-2Rα before treatment was 5.1408±3.93 ηg/l (Range: 0.81–15.96 ηg/l). The mean serum SIL-2Rα after the treatment was 2.7157±1.53 ηg/l (Range: 0.8–8.98 ηg/l). Range of serum 2ME level before treatment was 0–1.1280, and after treatment it was 0–1.640. There were significant differences between values of 2ME and SIL-2Rα before and after treatment ($p<0.001$).

Symptoms disappeared after 6 weeks of treatment in 27 (67.5%) patients, and 13 (32.5%) still had clinical symptoms (no response to the treatment). Mean serum level of 2ME and SIL-2Rα before and after treatment were significantly different ($p<0.001$). In the uncured patients, mean serum level of 2ME and SIL-2Rα before and after treatment were statistically different ($p=0.003$ for SIL-2Rα and $p=0.02$ for 2ME).

Cured patients who had increased or constant serum level of 2ME (false positive) were 7, out of whom 4 (57%) had a decreased serum level of SIL-2Rα, and 3 (43%) had an increased serum level of SIL-2Rα. Uncured patients who had a decreases serum level of 2ME (false negative) were 7, out of whom 5 (71.4%) had a decreased serum level of SIL-2Rα and 2 (28.57%) had an increased serum level of SIL-2Rα.

In 28 (70%) patients 2ME had decreased, in 10 (25%) it had remained constant, and in 2 patients (5%) it had increased. Serum SIL-2Rα level after treatment in 31 patients (77.5%), 2ME had decreased while in 9 (22.5%) it had had increased.

Out of 15 patients who had positive 2ME, 9 had clinical symptoms, and 6 had no symptoms. Out of 9 patients who had increased SIL-2Rα, 3 had clinical symptoms, and 6 had no symptoms.

DISCUSSION

In our study, same as most studies done in Iran, the main symptoms in brucellosis patients were fever and arthralgia. Also in cases with relapse of the disease or no response to the treatment these two symptoms were common.[2,5]

In Research Centres, using immunology serum markers for investigating the consequences of brucellosis treatment and also prediction of relapse of the disease is increasing, but practical results have not yet been achieved, and there are few studies about it.[9]

In a study in 2004 on 20 children for diagnosis of brucellosis, serum level of IL-2 and SIL-2Rα before and after treatment was measured and compared with 20 healthy children. In IL-2 serum level, there was no significant difference, but there was such difference higher than the control group before the treatment. After the treatment SIL-2Rα serum level had decreased.[8] In our study, the decrease in serum level of SIL-2Rα and 2ME was significant.

In Alexander's study, in three patients, who had relapse of disease, the SIL-2Rα serum level had no increase compared to ones before treatment, yet higher

than the control group. However this decrease was observed after another treatment period. This study showed that the decrease in the serum level of SIL-2Rα is a reliable predicting marker for responding to relapse or cur of brucellosis.[11] Unlike the above study, the serum level of SIL-2Rα in our study did not decrease in all cases. In 9 (22.5%) patients this level even showed an increase, although it was small.

A study in 2007 showed a decrease in percentage of CD-4 blood lymphocytes which appears SIL-Rα is related with the relapsing brucellosis.[12] In our study, the correlation coefficient of serum levels of 2ME and SIL-2Rα before and after treatment were statistically different.

A significant decrease in average level of serum of SIL-2Rα and 2Me after treatment (compared to before treatment) was observed in the present study; thus this serum level of SIL-2Rα is a predicting marker to response to treatment of brucellosis.

In false positive responses, patients with cured clinical symptoms but increased or constant serum level of 2Me, measurement of the SIL-2Rα can be useful, because the serum level showed a decrease more than half of the patients. In false negative responses, patients with constant symptoms but decreased 2ME, SIL-2Rα cannot be useful.[13,14] Consequently, SIL-2Rα serum level of patients should be checked for the patients with the wrong positive response of 2ME test.

Generally, it seems the serum level of SIL-2Rα can alone be helpful to predict the response to the brucellosis and also this level can assist in cases with the wrong positive responses.

CONCLUSION

Serum level of SIL-2Rα is useful to predict the response to brucellosis treatment and in false positive 2ME test, serum level of SIL-2Rα can be used to evaluate the response to treatment. Further studies to investigate the usefulness of serum level of SIL-2Rα are suggested.

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REFERENCES

1. Longo DL, Fauci AS, Kasper DL. Harrison's Principles of Internal Medicine, McGraw-Hill Professional; 18th ed., 2011.
2. Galanakis E, Bourantas KL, Leveidiotou S, Lapatsanis PD. Childhood brucellosis in north – western Greece : a retrospective analysis. Eur J Pediatr 1996;155:1–6.
3. Hadadi A, Rasoulinezhad Mehrnaz, Afhami Sh, Mohraz M. Epidemiological, Clinical, Para clinical Aspects of Brucellosis in Imam Khomeini and Sina Hospital of Tehran (1998–2005). J Kermanshah Univ Med Sci, Iran 2006;10(3):242.
4. Baldwin CL, Winter AJ. Macrophages and Brucella. Immunol Ser 1994;60:363–80 .
5. Shapoury R, Imani Fooladi AA, Rahnama M, Izadi M. Designing and validation of indirect and competitive ELISA for diagnosis of Brucellosis in human. J Military Med. 2009;11(1):17–8.
6. Zaitseva MB, Golding H, Betts M. Human peripheral blood CD4C and CD8CT-cells express Th1-like cytokine mRNA and proteins following *in vitro* stimulation with heat-activated Brucella abortus. Infect Immun 1995;63:2720–8 .
7. Zhan Y, Liu Z, Cheers C. Tumor necrosis factor alpha and interleukin-12 contribute to resistance to the intracellular bacterium Brucella abortus by different mechanisms. Infect Immun 1996;64:2782–6.
8. Kamal Esalatmanesh, Zahra Soleimani, Abbas Arj, Hossein Akbari, Mansour Salesi. Diagnostic value of ELISA (IgG and IgM) test in brucellosis patients in Kashan during 2004. Feyz 2008;3:54–9.
9. Agranovich I, Scott DE, Terle D, Lee K, Golding B. Down regulation of Th2 responses by Brucella abortus, a strong Th1 stimulus, correlates with alterations in the B7.2-CD28 pathway. Infect Immun 1999;67:4418–26.
10. Saleh P. Diagnostic value of brucella (IgM and IgG) in patients with brucellosis in Kashan. Infect Dis 1999;44:59–63. [Article in Persian]
11. Alexandros C. Makis, Emmanouil Galanakis, Eleftheria C. Hatzimichael, Zoe L. Papadopoulou, Antigone. Serum level of soluble interleukin-2 receptor alpha (SIL-2Rα) as a predictor of outcome in brucellosis J Infect 2005;51:206–10.
12. Skendros P. Diminished Percentage of CD4+ T-lymphocytes expressing interleukine-2receptor alpha in chronic brucellosis. J Infect 2007;54(2):192–7.
13. Honda M, Kitamura K, Takeshita T, Sugamura K, Tokunaga T. Identification of a soluble IL-2 receptor beta-chain from human lymphoid cell line cells. J Immunol 1990;145:4131–5.
14. Galanakis E, Makis A, Bourantas KL, Papadopoulou ZL. Interleukin-3 and interleukin-4 in childhood brucellosis. Infection 2002;30:33–4.

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