ORIGINAL RESEARCH ARTICLE

Tanaffos (2004) 3(11), 55-63

SICAM-1 as a Serum Marker for Follow-up of Pulmonary Tuberculosis Therapy

Majid Valiollahpour Amiri [1], Seyed Davood Mansoori [1], Mehdi Shekar-Abi [2], Seyed Mehdi Mirsaeidi [1], Soheila Zahirifard [3], Mehdi Kazempour Dizaji [1], Payam Tabarsi [1], Abolhassan Halvani [4], Seyed Djavad Tabatabaee [4], Mohammad Reza Masjedi [4]

[1] Department of Infectious Diseases, NRITLD, Shaheed Beheshti University of Medical Sciences and Health Services, [2] Department of Immunology, Iran University of Medical Sciences and Health Services, [3] Department of Radiology, [4] Department of Pulmonary Medicine NRITLD, Shaheed Beheshti University of Medical Sciences and Health Services, TEHRAN-IRAN

ABSTRACT

Background: *Tuberculosis (TB) is one of the commonest infectious diseases of our era; it is the second cause of death due to infectious diseases after AIDS. Studies have shown the significant effect of leukocyte integrins such as LFA-1and ICAM-1 on the function of macrophages against TB bacilli; increasing their activity during the process of TB infection.*

The objective of this research is to evaluate the changes observed in serum levels of SICAM-1 in pulmonary TB patients that had received treatment.

Materials and Methods: *All new pulmonary TB cases that had not received any treatment ,did not suffer from any kind of co-existing or underlying disorders such as hepatitis, sarcoidosis , lung cancer, HBV, HCV and HIV infections, chronic renal failure, cirrhosis, malnutrition, collagen vascular disorders and had not consumed immunosuppressive agents, were enrolled in this study. The SICAM-1 levels of the cases were measured by ELISA method before and 2 months after treatment with standard anti-TB drugs (Isoniazid, Rifampin, Ethambutol and Pyrazinamide) at the same time. T – test was used to compare the two sets of values of SICAM-1 levels before and 2 months after therapy.*

Results: *A total of 28 patients; 23 (82.1%) male and 5(17.9%) female cases were enlisted .Meanwhile, 50% of the patients were Iranian and the remaining had Afghan nationality. All of them were sputum smear and culture positive for Mycobacterium tuberculosis. Regarding the extent of pulmonary involvement as shown on lung CT-Scan, 68% demonstrated diffuse pulmonary involvement. The mean SICAM-1 level before the initiation of treatment was 554.17 ± 202.85 ng/ml. Considering age , sex ratio, ESR level, PPD test and severity of lung involvement, the SICAM-1 levels did not show any significant differences in different groups of patients. Among the patients enrolled in the study we were able to follow the seventeen patients (61%) who completed 2 months of treatment. The mean level of SICAM-1 before and after treatment in these patients were 573.9 ±204.4 and 481.2 ± 103.2 ng/ml, respectively (P<0.05).*

Conclusion: *SICAM-1 is considered as one of the inflammatory mediators that undergoes fluctuations during TB disease; its level is very much related to the extent of lung involvement. Since the level of this marker declines after therapy, it could be used as a "Serum marker "in evaluating the therapeutic response observed during the follow- up.*

Abbreviations: SICAM: Soluble Intercellular Adhesion Molecule, ICAM: Intercellular Adhesion Molecule, LFA: Leukocyte Function Antigen.

(Tanaffos 2004; 3(11): 55-63)

Key words: *Tuberculosis (TB), SICAM-1, Serum marker, Therapy*

Correspondence to: Valioallahpour Amiri M
Tel: +98-21-2803565
Email address: mamiri@nritld.ac.ir

INTRODUCTION

TB is one of the most common infectious diseases of our era. After AIDS, TB is considered as the second cause of death due to infectious diseases (1).

Today, about 2 billion people are infected with TB bacilli and each year 8.8 million people will be affected by this disease (2).

TB is highly prevalent in developing countries; being an important factor in health-related loss of man-power (3).

In our country, TB has an incidence of 18/100000 and is regarded as a "health obstacle" for the society (4).

Despite the availability and presence of standard diagnostic procedures for TB, we face serious barriers in diagnosing TB in many patients.

In developing countries like Iran, advancement of basic sciences is of great importance for clinical diagnosis and management of this threatening disease.

Leukocyte integrins such as Leukocyte Function Antigen-1 (LFA-1) and intercellular Adhesion Molecule-1 (ICAM-1) along with the complement receptors (CR3 and CR4) have major role in the adhesion process of macrophages as well as phagocytosis.

Researches have shown that during the process of TB infection, LFA-1 and ICAM-1 levels are increased in the *M. tuberculosis* infected macrophages, increasing their adhesiveness.

However, the number of the phagocytic receptors (e.g CR3, CR4) decreases, which would weaken the phagocytic activity of the infected macrophages. As a consequence, the bacilli will remain alive in the infected macrophages (5). ICAM-1 is present on the surfaces of the endothelial, and epithelial cells as well as macrophages and fibroblasts (6, 7). Some of the cytokines such as TNF-α, IL-1 B, and IFN-γ that are present in the inflammatory areas, increase expression of ICAM on the surfaces of these cells (8,9).

Also, these cytokines increase the soluble form of ICAM via enzymatic transformation of the membrane ICAM (MICAM-1) (10, 11, 12).

Recent studies have demonstrated that similar to endothelial cells, epitheloid and giant cells which are present in the granuloma and are derived from monocytes and macrophages, have MICAM-1 on their surfaces (13, 14).

Based on this information, many researches have been conducted studying the SICAM-1 level and severity of TB disease as well as reviewing the changes observed in SICAM-1 level after therapy (15, 16).

In this research we have evaluated the levels of serum SICAM-1 in drug sensitive pulmonary tuberculosis, before and 2 months after the treatment.

The main aim was evaluating the serum level changes of this marker after two months of standard anti-TB therapy.

MATERIALS AND METHODS

New case pulmonary TB patients that had relevant positive radiological findings, positive sputum smears (for AFB) and had not received any anti-TB drugs were enrolled in the study.

Meanwhile, the exclusion criteria included: having history of hepatitis, sarcoidosis, lung cancer, chronic renal failure, cirrhosis, malnutrition, collagen vascular diseases, consumption of immunosuppressive agents and / or being infected with HBV, HCV and / or HIV.

All routine hematological and biochemical tests were performed for all patients.

According to radiological manifestations (chest X-ray and CT- scan), lung involvement is divided into two forms:

- Cavitary form
- Non – cavitary form.

Also the extent of pulmonary involvement, demonstrated as a radiological feature, is of two types:

- Limited (single lobe is involved)
- Extensive (bilateral and/or multiple lobes are affected).

Sputum smear was performed for all patients using Ziehl-Neelsen stain, while sputum culture was carried out in Lowenstein Jensen medium.

Before the initiation of therapy 5cc of blood was obtained from basilic vein and kept at -20ºC.

The standard therapeutic regimen that was given to all patients included:

- Isoniazid (5mg/kg, maximum dose 300 mg)
- Rifampin (10mg/kg, maximum dose 600 mg)
- Pyrazinamide (30mg/kg, maximum dose 2 mg)
- Ethambutol (20 mg/kg, maximum dose 1200 mg)

After two months of directly observed treatment, 5cc of blood was once again collected from the basilic vein and stored at -20°C.

SICAM-1 level in both blood samples (before and 2 months after the treatment) was measured by ELISA method (using Bendermed kit with a sensitivity degree of 2.3 ng/ml) at the same time.

Using the PPD solution (5 TU), mantoux test was performed in all patients and the induration was measured after 48-72 hrs.

Demographic data of the patients, radiological manifestations as well as SICAM-1 levels were recorded in special forms.

It is notable that the procedure was completely explained to all patients and written consent was obtained from them.

STATISTICAL ANALYSIS

Descriptive analyses of quantitative (mean, standard deviation) and qualitative variables (percentage, frequency) were performed.

The paired t-test was used to compare the means of the quantitative variables (SICAM-1) before and 2 months after the treatment. Meanwhile to compare the means of the quantitative variables of the two groups in regard to sex, racial group, radiological manifestations, ESR level and PPD test, t-test and/or Mann-Whitney U test were used.

P-value less than 0.05 was regarded as significant. The data were analysed using SPSS version 11.5 software.

RESULTS

A total of 28 new case pulmonary TB patients were enrolled in the study.

There were 23 male (82.1%) and 5 female (17.9%); 50% had Afghan nationality and the remaining were Iranian.

The mean age of the patients was 41.5 ± 22 with minimum and maximum ages of 16 yrs. and 85 yrs, respectively.

In 19 patients (64.9%) the induration of the PPD test was less than 10mm, while in 9 (32.1%) the test was positive.

Chest X-ray was performed in all patients; cavitation and infilteration without cavitation were detected in 20 (71.5%) and 8 patients (28.5%) respectively.

Pulmonary CT-scan was carried out in 10 patients that showed cavitation in 80% of them and the remaining demonstrated alveolar infilteration.

In regard to the extent of lung involvement, 19 (67.9%) had extensive lung involvement (shown by chest X- ray and/or CT scan), while in 9 patients (32.1%) it was limited.

The mean SICAM-1 level before therapy was 554.17±202.85ng/ml (min: 220ng/ml, max: 1080ng/ml). Levels of SICAM-1 in different groupings of sex, nationality, PPD test, ESR, and types of lung involvement are shown in Table1.

Table 1. Levels of SICAM -1 in different groups of patients before the initiation of therapy.

Group of patients	Number(%)	Mean SICAM-1(ng/ml)	P-value
Sex			
Male	23(%82.1)	561. 9±219.1	0.062
Female	5 (%17.9)	518.6±108.4	
Nationality			
Afghan	14 (%50)	585.9±236.4	0.072
Iranian	14 (%50)	522.4±165.5	
PPD test			
induration ≥ 10mm	9 (%32.1)	506.8 ±188.2	0.092
induration ≤ 10mm	19 (%67.9)	576.6±210.5	
CT Scan of lung			
Cavitary	8 (%80)	475.3±137	0.068
non – cavitary	2 (%20)	705±261.6	
CXR			
Cavitary	20 (%71.5)	552.8±209.5	0.087
non cavitary	8 (%28.5)	557.8±199	
Extent of lung involvement			
Limited	9(%32.1)	474.2±219.7	0.086
Extensive	19(%67.9)	592.1±188.6	
ESR level			
<50	8 (%28.6)	422.3±166.3	0.058
>50	20 (%71.4)	624±201.8	

It is notable that only 17 patients gave consent to the follow up and measurement of SICAM-1, two months after the treatment.

The mean SICAM-1 levels of this group (17 patients) before and after the treatment are shown in Table 2.

Table 2. SICAM-1 levels in 17 patients before and 2 months after the treatment.

Time of measurement	Number of patients	SICAM-1 level (ng/ml)	P-value
Before therapy	17	573.9±204.4	P <0.05
2 month after therapy	17	481.2±103.2	

The differences in SICAM-1 levels before and after initiation of treatment were calculated by paired sample test. The p-value of this difference was less than 0.05 that means statistically significant.

DISCUSSION

ICAM, as an adhesion molecule has significant role in the accurate functioning of the cellular immunity and T-lymphocytes (17,18,19,20,21).

ICAM-1 exists in two types of membrane (MICAM-1) and soluble (SICAM-1) forms.

MICAM-1 by undergoing proteolysis, produces SICAM-1 (22).

SICAM-1 can be considered as a serum marker for the diagnosis of inflammatory processes (23).

SICAM-1 levels are increased in type II diabetes mellitus, chronic hepatitis B, AIDS and SLE (23, 24, 25).

Thus, all those patients that had the above mentioned diseases and other rheumatic and granulomatous diseases were excluded from this study.

Meanwhile, different studies have demonstrated that SICAM-1 levels are elevated in infectious diseases including pulmonary infections (5, 6, 21, 26, 27, 28).

The SICAM-1 level of TB patients when compared with healthy control group shows a significant increase ($p<0.05$); a fact which is pointed out in most of the studies (6, 12, 28, 29).

IL-1, TNF-α and IFN-γ along with increased levels of MICAM-1 raise SICAM-1 (10, 11, 12, 30, 31, 32).

Thus, in TB patients with long-term interaction between bacilli and host, the cytokines load is high and SICAM-1 level is increased.

In studies conducted by Baumer et al. (28) and Demir et al. (6) the mean serum levels of SICAM-1 in pulmonary TB patients before treatment were: 496.9±497 ng/ml and 436.2±124.2 ng/ml respectively.

In our research, this value was 554.2±202.9 ng/ml; being higher than those reported in other investigations.

The reason for this difference could be due to the large number of patients with extensive lung involvement in this study.

In a study conducted by Demir (6) the SICAM-1 level was determined in 3 groups of 10 pulmonary TB patients suffering form mild, moderate and severe types of disease.

According to the results of the above mentioned study the level of SICAM–1 was significantly higher ($P<0.0l$) in patients having the severe form of pulmonary involvement as compared to the other two groups (mean± SD 562.4±173.9).

This value was similar to our rate of SICAM-1 level. In the present study, out of 28 patients, 19(67.9%) suffered from extensive pulmonary TB (multiple lobes and/ or bilateral involvement) and only 9 patients (32.1%) had limited type of the disease (single lobe involvement). Mean SICAM-1 levels in extensive and limited groups of pulmonary TB patients were 592.05±188.6 ng/ml and 474.2±219.7 ng/ml respectively ($P > 0.05$).

Thus, the difference observed in the SICAM-1 level of this study with that of other researches is explained by the presence of large number of patients with extensive form of the disease in this study.

In Demir study (6) the level of SICAM-1 in severe cases was greater than pulmonary TB patients having mild or moderate type of disease ($P<0.01$).

Also, it was higher in the moderate form as compared to the mild type.

However, it was not statistically significant ($P>0.05$).

The results of the study conducted by Shijubo et al. (29) showed that the level of SICAM-1 was significantly higher in patients suffering from miliary and far advanced pulmonary TB, than those with mild to moderate form of disease.

Additionally they demonstrated that increased levels of SICAM-1 are significantly associated with increased levels of TNF-α and IFN-γ.

Meanwhile, another study (12) showed the significant increase of SICAM-1 level in miliary and far advanced TB as compared to pulmonary TB that has fewer chest X-ray manifestations.

The same condition exists in our research, which illustrates that patients with extensive form of disease have mean SICAM-1 levels greater (i.e about 188 ng/ml) than those with limited form of

disease.

Although this difference was not statistically significant ($p>0.05$), together with other researches (6, 12, 29) point towards the fact that SICAM-1 level is higher in that form of TB disease which has extensive tissue damage.

In several studies, SICAM-1 has been considered as a serum marker for measuring the activity of inflammatory diseases (26, 27).

Shijubo et al. (29) demonstrated that raised SICAM-1 levels gradually decrease with clinical improvement in signs, symptoms, and radiological manifestations of miliary and far advanced pulmonary TB.

Additionally Demir et al. (6) illustrated that one month after initiation of treatment in mild, moderate and severe forms of pulmonary TB, SICAM-1 levels significantly decreased ($p<0.01$).

In our research, out of 28 patients, only 17(61%) were ready to be followed-up.

Thus, we could only measure the SICAM-1 levels before and 2 months after initiation of treatment in seventeen patients.

Accordingly, the levels of SICAM-1 in these cases before and 2 months after treatment were: 573.9±204.4 ng/ml and 481.1 ± 103.1 ng/ml respectively.

As it is seen, SICAM-1 level had shown a decrease of 92.7 ng/ml after therapy. From the statistical point of view and paired-sample test, the p-value was calculated less than 0.05 which was significant.

CONCLUSION

SICAM-1 as a marker of inflammatory process undergoes changes during the pulmonary TB disease; the level of which is very much related to the extent of the lung involvement. Since its value declines with appropriate treatment, SICAM-1 could be considered as a serum marker for the follow– up and detecting the response to the treatment.

REFERENCES

1. Dye C, Scheele S, Dolin P, Pathania V, Raviglione MC. Consensus statement. Global burden of tuberculosis: estimated incidence, prevalence, and mortality by country. WHO Global Surveillance and Monitoring Project. ***JAMA*** 1999; 282 (7): 677-86.
2. Kamholz SL. Drug resistant tuberculosis. ***J Assoc Acad Minor Phys*** 2002; 13 (2): 53-6.
3. Sudre P, ten Dam G, Kochi A. Tuberculosis: a global overview of the situation today. ***Bull World Health Organ*** 1992; 70 (2): 149-59.
4. Report of Iranian center for diseases control in 2003.
5. DesJardin LE, Kaufman TM, Potts B, Kutzbach B, Yi H, Schlesinger LS. Mycobacterium tuberculosis-infected human macrophages exhibit enhanced cellular adhesion with increased expression of LFA-1 and ICAM-1 and reduced expression and/or function of complement receptors, FcgammaRII and the mannose receptor. ***Microbiology*** 2002; 148 (Pt 10): 3161-71.
6. Demir T, Yalcinoz C, Keskinel I, Demiroz F, Yildirim N. sICAM-1 as a serum marker in the diagnosis and follow-up of treatment of pulmonary tuberculosis. ***Int J Tuberc Lung Dis*** 2002; 6 (2): 155-9.
7. van de Stolpe A, van der Saag PT. Intercellular adhesion molecule-1. ***J Mol Med*** 1996; 74 (1): 13-33. Review.
8. Fattal-German M, Le Roy Ladurie F, Cerrina J, Lecerf F, Berrih-Aknin S. [Expression of intercellular adhesion molecule 1 (ICAM-1) on human alveolar macrophages]. ***Ann Pharm Fr*** 1995; 53 (3): 97- 110. French.
9. Geissler D, Gaggl S, Most J, Greil R, Herold M, Dietrich M. A monoclonal antibody directed against the human intercellular adhesion molecule (ICAM-1) modulates the release of tumor necrosis factor-alpha, interferon-gamma and interleukin 1. ***Eur J Immunol*** 1990; 20 (12): 2591-6.
10. Leeuwenberg JF, Smeets EF, Neefjes JJ, Shaffer MA, Cinek T, Jeunhomme TM, et al. E-selectin and intercellular

adhesion molecule-1 are released by activated human endothelial cells in vitro. ***Immunology*** 1992; 77 (4): 543-9.

11. Giavazzi R, Chirivi RG, Garofalo A, Rambaldi A, Hemingway I, Pigott R, et al. Soluble intercellular adhesion molecule 1 is released by human melanoma cells and is associated with tumor growth in nude mice. ***Cancer Res*** 1992; 52 (9): 2628-30.
12. Tsujisaki M, Imai K, Hirata H, Hanzawa Y, Masuya J, Nakano T, et al. Detection of circulating intercellular adhesion molecule-1 antigen in malignant diseases. ***Clin Exp Immunol*** 1991; 85 (1): 3-8.
13. van Dinther-Janssen AC, van Maarsseveen TC, Eckert H, Newman W, Meijer CJ. Identical expression of ELAM-1, VCAM-1, and ICAM-1 in sarcoidosis and usual interstitial pneumonitis. ***J Pathol*** 1993; 170 (2): 157-64.
14. Shijubo N, Hirasawa M, Inuzuka M, Asakawa M, Abe S, Imai K. [Intercellular adhesion molecule 1 antigen in tuberculosis]. ***Kekkaku*** 1994; 69 (7): 471-4. Japanese.
15. Shijubo N, Imai K, Nakanishi F, Yachi A, Abe S. Elevated concentrations of circulating ICAM-1 in far advanced and miliary tuberculosis. ***Am Rev Respir Dis*** 1993; 148 (5): 1298-301.
16. Lai CK, Wong KC, Chan CH, Ho SS, Chung SY, Haskard DO, et al. Circulating adhesion molecules in tuberculosis. ***Clin Exp Immunol*** 1993; 94 (3): 522-6.
17. Moreno C, Mehlert A, Lamb J. The inhibitory effects of mycobacterial lipoarabinomannan and polysaccharides upon polyclonal and monoclonal human T cell proliferation. ***Clin Exp Immunol*** 1988; 74 (2): 206-10.
18. Marlin SD, Springer TA. Purified intercellular adhesion molecule-1 (ICAM-1) is a ligand for lymphocyte function-associated antigen 1 (LFA-1). ***Cell*** 1987; 51 (5): 813-9.
19. Altmann DM, Hogg N, Trowsdale J, Wilkinson D. Cotransfection of ICAM-1 and HLA-DR reconstitutes human antigen-presenting cell function in mouse L cells. ***Nature*** 1989; 338 (6215): 512- 4.
20. Van Seventer GA, Shimizu Y, Horgan KJ, Shaw S. The LFA-1 ligand ICAM-1 provides an important costimulatory signal for T cell receptor-mediated activation of resting T cells. ***J Immunol*** 1990; 144 (12): 4579- 86.
21. Melis M, Pace E, Siena L, Spatafora M, Tipa A, Profita M, et al. Biologically active intercellular adhesion molecule-1 is shed as dimers by a regulated mechanism in the inflamed pleural space. ***Am J Respir Crit Care Med*** 2003; 167 (8): 1131- 8. Epub 2003 Feb 5.
22. Budnik A, Grewe M, Gyufko K, Krutmann J. Analysis of the production of soluble ICAM-1 molecules by human cells. ***Exp Hematol*** 1996; 24 (2): 352- 9.
23. Ceriello A, Falleti E, Bortolotti N, Motz E, Cavarape A, Russo A, Gonano F, Bartoli E. Increased circulating intercellular adhesion molecule-1 levels in type II diabetic patients: the possible role of metabolic control and oxidative stress. ***Metabolism*** 1996; 45 (4): 498- 501.
24. Doi T, Yamada G, Mizuno M, Tsuji T. Immunohistochemical study of the distribution of intercellular adhesion molecule-1 and lymphocyte function-associated antigen-1 in chronic type B hepatitis. ***J Gastroenterol*** 1994; 29 (2): 164- 71.
25. Bohnet S, Braun J, Dalhoff K. Intercellular adhesion molecule-1 (ICAM-1) is upregulated on alveolar macrophages from AIDS patients. ***Eur Respir J*** 1994; 7 (2): 229- 34.
26. Sari RA, Taysi S, Erdem F, Yilmaz O, Keles S, Kiziltunc A, Odabas AR, Cetinkaya R. Correlation of serum levels of soluble intercellular adhesion molecule-1 with disease activity in systemic lupus erythematosus. ***Rheumatol Int*** 2002; 21 (4): 149- 52.
27. Burns AR, Takei F, Doerschuk CM. Quantitation of ICAM-1 expression in mouse lung during pneumonia. ***J Immunol*** 1994; 153 (7): 3189- 98.
28. Baumer I, Zissel G, Schlaak M, Muller-Quernheim J. Soluble intercellular adhesion molecule 1 (sICAM-1) in bronchoalveolar lavage (BAL) cell cultures and in the circulation of patients with tuberculosis, hypersensitivity pneumonitis and sarcoidosis. ***Eur J Med Res*** 1998; 3 (6): 288- 94.
29. Shijubo N, Imai K, Nakanishi F, Yachi A, Abe S. Elevated concentrations of circulating ICAM-1 in far advanced and miliary tuberculosis. ***Am Rev Respir Dis*** 1993; 148 (5): 1298- 301.

30. Couffinhal T, Duplaa C, Labat L, Lamaziere JM, Moreau C, Printseva O, et al. Tumor necrosis factor-alpha stimulates ICAM-1 expression in human vascular smooth muscle cells. ***Arterioscler Thromb*** 1993; 13 (3): 407- 14.
31. Combe C, Duplaa C, Couffinhal T, Moreau C, Bonnet J. Induction of intercellular adhesion molecule-1 by monocyte adhesion to endothelial cells in human culture system. ***J Cell Physiol*** 1995; 164 (2): 295- 303.
32. Fais S, Burgio VL, Silvestri M, Capobianchi MR, Pacchiarotti A, Pallone F. Multinucleated giant cells generation induced by interferon-gamma. Changes in the expression and distribution of the intercellular adhesion molecule-1 during macrophages fusion and multinucleated giant cell formation. ***Lab Invest*** 1994; 71 (5): 737- 44.