

A New Parameter in the Detection of Tuberculosis Activity: Reactive Oxygen Metabolites

Erkan Ceylan^a Abdullah Gülsün^c Mehmet Gencer^a Nurten Aksoy^b

Departments of ^aChest Diseases and ^bBiochemistry, Faculty of Medicine, Harran University, Sanliurfa, ^cDepartment of Chest Diseases, State Hospital of Inegöl, Bursa, Turkey

Key Words

Pulmonary tuberculosis · Sequel pulmonary tuberculosis · Detection of activity · Reactive oxygen metabolite

Abstract

Background and Objectives: In countries with a high frequency of tuberculosis, there are problems not only with active lung tuberculosis but also with past lung tuberculosis. Cases with sequel tuberculosis very frequently present with complaints like tuberculosis, and it is very hard to determine whether it is a sequel tuberculosis complication or reactivation of tuberculosis. In this study, we measured the serum reactive oxygen metabolite (ROM) levels of patients with active pulmonary tuberculosis and healthy controls, and investigated if these metabolites can be used as a criterion for differentiation between active pulmonary tuberculosis and sequel pulmonary tuberculosis. **Methods:** 40 patients with active tuberculosis, 35 patients with sequel pulmonary tuberculosis and 30 healthy control subjects with a similar age range and sex distribution were included in the study. Serum total ROM levels were detected in the patients and control group. **Results:** Mean serum ROM values were 994 ± 236 , 551 ± 135 and 236 ± 59 U/l among active lung tuberculosis cases, sequel lung tuberculosis cases and the healthy control group, respectively. As a result of these findings, serum ROM levels of active lung

tuberculosis cases and sequel lung tuberculosis cases were significantly higher than those of the control group (both $p < 0.001$). The serum ROM levels of active lung tuberculosis cases were also significantly higher than those of sequel lung tuberculosis cases ($p < 0.001$). **Conclusions:** In the light of our findings, it may be assumed that serum total ROM values can be used as an activity criterion in the differentiation of active lung tuberculosis and sequel lung tuberculosis.

Copyright © 2005 S. Karger AG, Basel

Introduction

Many complications, like secondary infection and secondary lung disease, may occur in patients with a history of tuberculosis and pulmonary sequelae. Cases with sequel tuberculosis very frequently present with complaints similar to tuberculosis, and it is not easy to determine whether it is a sequel tuberculosis complication or reactivation of tuberculosis. Because of chest X-ray findings and continuing symptoms, unnecessary antituberculous treatments may be given [1].

In normal conditions, during cellular metabolism, due to production of reactive oxygen metabolites (ROM) like superoxide anion and hydrogen peroxide, the lungs are exposed to a basal oxidative stress [2, 3]. If the antioxidant system is not adequate and the free radical level is greater than the antioxidant capacity of the organism, free

radical reactions become very toxic and may harm lungs. This will lead to acute and/or chronic pulmonary damage [2, 3].

Recently, many studies have focused on the free radical-scavenging antioxidants, which play an important role in the in vivo defense systems against oxidative damage initiated and promoted by ROM produced in various diseases such as diabetes mellitus, cardiovascular diseases and cancer [4–6]. However, there is no detailed study showing evidence of the presence of oxidative stress and of an imbalance of the oxidative-antioxidative status in tuberculosis. Therefore, the present study was designed to investigate this system by measuring and comparing total serum ROM levels among patients with active tuberculosis and sequel pulmonary tuberculosis and healthy controls.

Patients and Methods

40 patients with active pulmonary tuberculosis, 35 patients with sequel pulmonary tuberculosis (75 cases in total) and 30 healthy people as controls were included in our study. The patients included in the study had no secondary disease other than pulmonary tuberculosis. Among the tuberculosis cases, 41 were male and 34 were female. The average age of the tuberculosis patients was 36.43 ± 16.4 years. Of these patients, 31 (41.3%) were smokers and 44 (58.7%) were nonsmokers. In the healthy control group, there were 15 males and 15 females with an average age of 33.83 ± 9.51 years. In the control group, 12 subjects (40%) were smokers and 18 (60%) were nonsmokers.

Our patient group with sequel tuberculosis consisted of patients with inactive pulmonary tuberculosis whose diagnosis and treatments are undertaken in our clinic according to WHO criteria, negative sputum smears over 6 months of follow-up and chest X-ray findings unchanged or shrunken for 6 months. In particular those cases who had attended our clinic late after the onset of tuberculosis and therefore had had posttreatment sequelae on chest X-ray were chosen.

In hospital, after a detailed history and physical examination, a chest X-ray and routine biochemical and hematological analyses of all cases were performed. *Mycobacterium tuberculosis* was looked for in sputum smears and submitted for tuberculosis culture in Lowenstein-Jensen media at least 3 times. On the patient's first morning in the hospital before breakfast, 10 ml of venous blood was drawn into tubes and kept at room temperature until it clotted. After that, the samples were centrifuged at 1,500 g for 10 min to separate the serum, which was then frozen at -40°C until the study was conducted. Serum ROM levels were measured by commercially available kits (Diacron ROM kit, Grosseto, Italy) in an RA-XT[®] autoanalyzer. Results were expressed as U/l.

Data were analyzed using SPSS 10.0 for Windows. Results were expressed as mean \pm SD. Levene's test for equality of variances was used. Student's t tests were used for pair-wise comparisons. Two-tailed significance values were used. A p value of 0.05 or less was considered to be significant.

This work was performed according to the European regulations under the supervision of a bioethics consultant.

Table 1. Characteristics of the patients with tuberculosis and the healthy controls

	Active tuberculosis	Sequel tuberculosis	Controls
n	40	35	30
Male	22	19	15
Female	18	16	15
Mean age, years	34.55 ± 18.15	38.57 ± 14.11	33.83 ± 9.51
Smokers	16	15	12
Pack-years	17 ± 15	19 ± 19	16 ± 11
Nonsmokers	24	20	18
Positive TB culture	40	35 ¹	–

The values represent the mean \pm SD. TB = Tuberculosis.

¹ Active-stage culture results before treatment.

Table 2. Serum ROM levels in the patients with tuberculosis and the healthy controls

Parameter	Controls	Active tuberculosis	Sequel tuberculosis
ROM, U/l	236 ± 59	994 ± 236	551 ± 135

The values represent the mean \pm SD. $p < 0.001$ comparing the control group and active tuberculosis patients; $p < 0.001$ comparing the control group and sequel tuberculosis patients; $p < 0.001$ comparing the active tuberculosis patients and sequel tuberculosis patients.

Results

Table 1 shows the social and demographic data (age, sex, etc.) of the patients with tuberculosis and the controls, and their laboratory findings. The serum ROM levels of patients with active lung tuberculosis and patients with sequel lung tuberculosis were significantly higher than those of the control group ($p < 0.001$ for both). The serum ROM values of patients with active lung tuberculosis were also significantly higher than those of patients with sequel lung tuberculosis ($p < 0.001$). The results are summarized in table 2.

There was no significant difference between mean serum ROM values of smoking and nonsmoking patients when they were evaluated separately or as a group ($p > 0.05$).

Table 3. Diagnostic values calculated according to ROM levels of patients with active and sequel pulmonary tuberculosis

ROM cutoff 800 U/l	Sensitivity %	Specificity %	Positive predictive value, %	Negative predictive value, %
Active-sequel	87.5	91.4	92	86.5

Diagnostic values in active and sequel pulmonary tuberculosis cases are shown in table 3. The mean cutoff serum ROM value was assumed to be 800 U/l.

Discussion

For the definitive diagnosis of tuberculosis, bacilli should be identified with microbiological procedures. Approximately 4–6 weeks should pass to demonstrate tuberculosis bacilli on classical culture media [7, 8]. Sometimes, despite all diagnostic studies, tuberculosis bacilli cannot be revealed; hence, the diagnosis of tuberculosis becomes more difficult. Various diagnostic parameters for fast and effective diagnosis of tuberculosis have been tried [9].

In cases with a history of tuberculosis, the secondary pulmonary disease symptoms and signs that develop hide clinically reactive tuberculosis and make it hard to diagnose. The symptoms and findings of nonspecific pulmonary infections in cases of past tuberculosis mask the reactivation of tuberculosis, and make it difficult to diagnose tuberculosis. For that reason, different parameters have been studied as activity criteria [10–12].

Many studies have focused on the role of free oxygen radicals in inflammation, tissue damage, acute respiratory distress syndrome (ARDS), aging and development of various diseases like diabetes and cancer [3, 13–18]. Although there are some studies on tuberculosis and free radicals, there is no detailed study evaluating the ROM levels in patients with sequel tuberculosis, and/or comparing them with those in patients with active pulmonary tuberculosis. Free radicals and ROM are generated throughout the human body. Enzymatic and nonenzymatic antioxidants such as superoxide dismutase (SOD), catalase and glutathione peroxidase, uric acid, bilirubin and albumin detoxify them and minimize damage to biomolecules. An imbalance between the production of ROM and free radicals and the antioxidant capacity leads to oxidative stress that contributes to the pathogenesis of

a number of human diseases. In this study, the presence of oxidative stress was shown by increased ROM levels in active and sequel pulmonary tuberculosis, and we investigated whether these ROM values can be used as a criterion for differentiation between active pulmonary tuberculosis and sequel pulmonary tuberculosis.

In a study which compared tuberculosis pleurisy cases with malignant pleurisy, parapneumonic pleurisy and a healthy control group, serum ROM levels of tuberculosis pleurisy cases were found to be significantly higher compared to the other groups [18]. Jack et al. [19] measured lipid peroxidation end products in sera of patients with active pulmonary tuberculosis and sequel pulmonary tuberculosis. They found that the levels of 9,11-linoleic acid and the percentage molar ratio of 9,11-linoleic acid to 9,12-linoleic acid in active pulmonary tuberculosis cases were higher than those in inactive pulmonary tuberculosis cases. They also showed that free radical activity increased in active pulmonary tuberculosis cases, which may play a role in the development of resultant fibrosis [19]. Additionally, in the same study, in active pulmonary tuberculosis patients who had started to receive antituberculosis treatment, repeated measurements of lipid peroxidation end products in the following months showed a proportional decrease. Makinskii et al. [20] showed that high pretreatment levels of free radicals had decreased to normal at the end of treatment in active pulmonary tuberculosis cases. Kwiatkowska et al. [21] showed that malondialdehyde and lipid peroxidation product levels in sputum smear-positive patients with advanced pulmonary tuberculosis and sputum smear-negative patients with small radiographic changes were significantly higher than those of a healthy control group. Similarly, in our study, in the cases with active pulmonary tuberculosis, serum ROM levels were found to be significantly higher than those of sequel pulmonary tuberculosis cases, and both were much higher than controls. Although lipid peroxidation end products like malondialdehyde have been studied as an indicator of oxidative stress in some studies, it is well known that free radicals and/or ROM directly cause oxidative damage and that elevation of their serum levels implies oxidative stress [16–18, 21]. Our results indicate that elevated serum ROM levels provide evidence for the presence of oxidative stress in tuberculosis and that they may imply the activity of pulmonary tuberculosis.

To investigate the relation between free oxygen radicals and tuberculosis, different antioxidants have been studied in tuberculosis cases. Durak et al. [22] studied total cytoplasmic Cu, Zn-SOD and mitochondrial Mn-

SOD activities in serum and pleural fluids from patients with carcinoma of the lung, tuberculosis or chronic heart failure and a control group. SOD activities were found to be higher in all the patient groups compared to the control group, and these values were the highest in the tuberculosis group. They concluded that this enzyme activity might be used as a nonspecific prognostic marker in assessing cellular and mitochondrial tissue destruction [22]. Safarian and Karapetian [23] studied the sera of 77 primary tuberculosis patients and 20 healthy people as a control group. They observed increased SOD activity depending on the stage of disease in nodular tuberculosis patients, evidently decreased SOD in the disseminated stage of miliary and infiltrative tuberculosis, and again increased SOD activity when there is no dissemination [23].

All these studies revealed that reactive oxygen products increase in proportion to the presence of pulmonary tuberculosis and the spread of disease activity. These re-

active oxygen products decrease in the course of treatment in proportion with the reduced activity. We found that, in sequel pulmonary tuberculosis cases, ROM levels are evidently lower than those in active pulmonary tuberculosis cases, but higher than in a healthy control group. Although there are studies showing that this increment in sequel cases is due to fibrosis, comprehensive studies should be performed. However, extreme elevations in ROM values may provide an important indication that these patients might have an active tuberculosis lesion.

In conclusion, we found that serum ROM levels in patients with active pulmonary tuberculosis were significantly higher than those in patients with sequel pulmonary tuberculosis, and in patients with sequel tuberculosis, they were significantly higher than in the control group. In the light of these findings, we concluded that ROM levels could be used as activity criteria with other parameters in the differentiation of active and sequel pulmonary tuberculosis.

References

- Dye C, Scheele S, Dolin P, Pathania V, Raviglione MC: Consensus statement. Global burden of tuberculosis: Estimated incidence, prevalence and mortality by country. World Health Organization Global Surveillance and Monitoring Project. *JAMA* 1999;18:677-686.
- Bast A, Haenen G, Doelman C: Oxidants and antioxidants: State of the art. *Am J Med* 1991; 91(suppl 3C):S2-13S.
- Barber DA, Harris SR: Oxygen free radicals and antioxidants: A review. *Am Pharm* 1994;NS34:26-35.
- Vural H, Sabuncu T, Arslan OS, Aksoy N: Melatonin inhibits lipid peroxidation and stimulates the antioxidant status of diabetic rats. *J Pineal Res* 2001;31:193-198.
- Abe M, Reiter RJ, Orhii PB, Hara M, Poeggeler B: Inhibitory effect of melatonin on cataract formation in newborn rats: Evidence for an antioxidative role for melatonin. *J Pineal Res* 1994;17:94-100.
- Barlow-Walden LR, Reiter RJ, Abe M, Pablos M, Menendez-Pelaez A, Chen LD, Poeggeler B: Melatonin stimulates brain glutathione peroxidase activity. *Neurochem Int* 1995;26:497-502.
- Rieder HL: Epidemiology of tuberculosis; in Gibson GJ, Geddes DM, Costabel U, Sterk PJ, Corrin B (eds): *Respiratory Medicine*, ed 3. London, Saunders, 2003, pp 938-975.
- Schluger NW, Rom WN: Current approaches to the diagnosis of active pulmonary tuberculosis. *Am J Respir Crit Care Med* 1994;149: 264-267.
- Tahhan M, Ugurman F, Gozu A, Akkalyoncu B, Samurkasoglu B: Tumour necrosis factor- α in comparison to adenosine deaminase in tuberculous pleuritis. *Respiration* 2003;70:270-274.
- Juffermans NP, Verbon A, van Deventer SJH, van Deutekom H, Speelman P, van der Poll T: Tumor necrosis factor and interleukin-1 inhibitors as markers of disease activity of tuberculosis. *Am J Respir Crit Care Med* 1998;157: 1228-1231.
- Abe Y, Nakamura M, Oshika Y, Hatanaka H, Tokunaga T, Ohkubo Y, Hashizume T, Suzuki K, Fujino T: Serum levels of vascular endothelial growth factor and cavity formation in active pulmonary tuberculosis. *Respiration* 2001;68:496-500.
- Emad A, Rezaian GR: Lactate dehydrogenase in bronchoalveolar lavage fluid of patients with active pulmonary tuberculosis. *Respiration* 1999;66:41-45.
- Winrow VR, Winyard PG, Morris CJ, Blake DR: Free radicals in inflammation: Second messengers and mediators of tissue destruction. *Br Med Bull* 1993;49:506-522.
- Holley AE, Cheeseman KH: Measuring free radical reactions in vivo. *Br Med Bull* 1993;49: 494-505.
- Conner EM, Grisham MB: Inflammation, free radicals, and antioxidants. *Nutrition* 1996;12: 274-277.
- Vural H, Aksoy N, Arslan SO, Bozer M: Effects of vitamin E and selenium on lipid peroxidation and antioxidant enzymes in colon of methylazoxymethanol treated rats. *Clin Chem Lab Med* 2000;38:1051-1053.
- Aksoy N, Vural H, Sabuncu T, Aksoy S: Effects of melatonin on oxidative-antioxidative status of tissues in streptozotocin-induced diabetic rats. *Cell Biochem Funct* 2003;21:121-125.
- Gürler B, Vural H, Yılmaz N, Oguz H, Satıcı A, Aksoy N: The role of oxidative stress in diabetic retinopathy. *Eye* 2000;14:730-735.
- Jack CI, Jackson MJ, Hind CR: Circulating markers of free radical activity in patients with pulmonary tuberculosis. *Tuber Lung Dis* 1994; 75:132-137.
- Makinskii AI, Baikuev RF, Zaliyev RA: Markers of alteration of tissue structures as indicators of pulmonary tuberculosis activity (in Russian). *Probl Tuberk* 2002;(7):39-42.
- Kwiatkowska S, Piasecka G, Zieba M, Piotrowski W, Nowak D: Increased serum concentrations of conjugated dienes and malondialdehyde in patients with pulmonary tuberculosis. *Respir Med* 1999;93:272-276.
- Durak İ, Canbolat O, Kavutçu M, Öztürk HS, Yurtarslan Z: Activities of total, cytoplasmic and mitochondrial superoxide dismutase enzymes in sera and pleural fluids from patients with lung cancer. *J Clin Lab Anal* 1996;10:17-20.
- Safarian MD, Karapetian ET: Dynamics of the activity of antioxidant enzymes in the blood of patients with pulmonary tuberculosis (in Russian). *Probl Tuberk* 1990;(8):60-61.