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Serum Lysozyme Levels and Clinical Features of Sarcoidosis

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Abstract. Serum lysozyme is used as a marker of sarcoidosis disease activity. In this study we examined the association between lysozyme levels and the clinical features of sarcoidosis and thus the clinical usability of this parameter in a large population. One hundred ten sarcoidosis patients from central Japan were examined for clinical features and serum lysozyme level at the first visit to our hospital and on a regular basis thereafter. The sensitivity of lysozyme for predicting sarcoidosis was 79.1%, whereas that of serum angiotensin-converting enzyme (ACE) was 59.0%. Even in the cases without an elevated serum ACE level, a value of 72.1% was obtained. The serum lysozyme level demonstrated a significant tendency to increase with the number of organs involved ($p < 0.01$). There were significant differences among the four radiographic stages ($p < 0.05$). The maximum serum lysozyme levels of patients without a disappearance of abnormal shadows on chest radiography within 5 years were significantly greater than those of individuals with a disappearance ($p < 0.05$). A positive correlation between serum lysozyme and serum ACE levels was observed. Because serum lysozyme is much less specific for sarcoidosis than serum ACE, its diagnostic value may be limited. However, the sensitivity was high even when serum ACE levels were within normal limits and correlated well with clinical features in sarcoidosis. Therefore, this parameter seems suitable for disease monitoring in proven cases.

Key words: Lysozyme—Sarcoidosis—Angiotensin-converting enzyme—Activity—Serum marker.

Introduction

Sarcoidosis is a multisystem granulomatous disorder characterized by enhanced immune processes at the sites of disease activity [18]. Circulating monocytes that are

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Table 1. Subjects

	Number	Sex (F/M)	Mean age (years ± S.D.)
Sarcoidosis patients	110	74/36	54.8 ± 16.6
Disease controls	30	12/18	52.0 ± 18.1

attracted to disease sites are subjected to the influence of local proinflammatory signals, resulting in phenotypic and functional changes as they undergo maturation and activation. One of the activation products of stimulated mononuclear phagocytes is lysozyme (muramidase, *N*-acetylmuramide glycanohydrolase, 1,4-β-*N*-acetylmuramidase; EC 3.2.1.17) [11]. Serum lysozyme is elevated in a number of conditions [8, 10, 14] and is often raised in active sarcoidosis in a manner similar to that of serum angiotensin-converting enzyme (ACE), levels tending to alter with the clinical state [1, 12, 15]. However, there have been only few reports of the comparative advantages of the two parameters for diagnostic purposes. In the present study, using a large population, we investigated the association of both lysozyme and ACE levels with the clinical features of sarcoidosis.

Materials and Methods

Subjects

One hundred ten sarcoidosis patients from central Japan were examined (Table 1). Sarcoidosis was diagnosed based on the clinical picture and the presence of epithelioid cell granulomas in biopsy specimens from the lung, skin, or lymph nodes. Thirty-six of the patients were male, and 74 were female. They had a mean age of 54.8 ± 16.6 years (mean ± S.D.) at the first visit to our hospital. There were 12 patients at radiographic stage 0, 78 at stage I, 15 at stage II, and 5 at stage III, according to the classification system defined by DeRemee [3]. The extent of the disease was assessed by chest radiography, high resolution CT scans, bronchoalveolar lavage, ^{67}Ga lung uptake, ^{201}Tl myocardial scintigraphy, echocardiography, abdominal ultrasonography, and Holter ECG. As disease controls, serum lysozyme values were evaluated in a series of 30 patients with other granulomatous diseases, comprising 7 cases of summer-type hypersensitivity pneumonitis, 20 of pulmonary tuberculosis, and 3 of pulmonary aspergillosis. Eighteen patients were male, and 12 were female. They had a mean age of 52.0 ± 18.1 years. All subjects had normal renal function and gave informed consent for the study.

Serum Lysozyme and ACE Assays

Serum lysozyme activity was assayed by a turbidimetric method, in which a continuous rate reaction under conditions of optimum substrate concentration, pH, and ionic strength is measured [9, 20]. This is based on the spectrophotometric measurement of lysis of *Micrococcus lysodeikticus*. Twenty-five-μl aliquots of serum was added to 1.5 ml of a working substrate solution made up of 240 mg of *M. lysodeikticus* liter in an M/15 pH 6.6 phosphate-sodium buffer, followed by incubation at 37°C for 10 min. The decrease in absorbance at 600 nm was recorded by spectrophotometer (Hitachi 7050, Hitachi Corporation, Tokyo). Enzymatic activity was determined from the standard curve in egg white lysozyme equivalents. The serum lysozyme mean level ± S.D. of 39 healthy subjects was 7.4 ± 1.8 μg/ml. A serum lysozyme level greater than 2 S.D. (>11.0 μg/ml)

Table 2. Serum parameters and organ involvement.
The maximum serum lysozyme levels had a tendency of increase significantly ($p < 0.01$) with the number of organs involved. There was no significant difference in the maximum serum ACE levels ($p = 0.24$).

Parameters	1 organ ($n = 33$)	2 organs ($n = 55$)	3 organs ($n = 18$)	4 organs ($n = 4$)
Serum lysozyme (μg/ml)	16.5 ± 4.7	18.0 ± 7.8	20.8 ± 7.1	28.1 ± 12.5
Serum ACE (IU/liter)	26.1 ± 9.2	26.2 ± 11.1	30.9 ± 12.3	32.4 ± 8.3

was considered to be elevated. Serum ACE activity was measured by a colorimetric method (colorimetric assay kit, Fujizoki Assay, Tokyo) using *p*-hydroxyhippuryl-L-histidyl-L-leucine as the substrate [7].

Statistical Analysis

Values are expressed as means ± S.D. Analysis of serum lysozyme levels was performed using the Mann-Whitney U-test for comparison of the two groups, and the Kruskal-Wallis test for three or more groups. Correlation coefficients were used to assess the correlation between serum lysozyme and ACE levels [21]. A p value < 0.05 was considered significant.

Results

Sensitivity

The mean serum lysozyme value for all 110 patients at the first visit to our hospital was 15.7 ± 7.0 (μg/ml) and at the maximum was 18.4 ± 7.7. At the first visit to our hospital the sensitivity for sarcoidosis was 79.1% (87/110), whereas that for serum ACE was 59.0% (62/105). Thirty-one of the 43 patients (72.1%) whose serum ACE levels were not elevated had elevated serum lysozyme levels. In disease controls, the mean serum lysozyme value was 11.0 ± 6.6 (μg/ml), and in 12 of 30 patients (40.0%) elevation was evident. However, the serum lysozyme values of the sarcoidosis patients were significantly greater than those of the disease controls ($p < 0.0001$).

Organ Involvement

The maximum serum lysozyme level had a tendency to increase significantly according to the number of organs involved ($p < 0.01$) (Table 2). However, there was no significant difference in the maximum serum ACE level ($p = 0.24$).

Radiographic Stage

Concerning the radiographic stage, the mean values for maximum serum lysozyme levels at stages 0–III were 15.0 ± 4.7, 18.4 ± 8.3, 20.0 ± 6.4, and 21.4 ± 4.9, respectively. The variation was significant ($p < 0.05$), again in contrast to the maximum

Table 3. Serum parameters and roentgenographic stage.
There was a significant difference in the maximum serum lysozyme levels among the four groups ($p < 0.05$), whereas no such significant difference was evident for maximum serum ACE levels ($p = 0.29$).

Parameters	Stage 0 ($n = 12$)	Stage I ($n = 78$)	Stage II ($n = 15$)	Stage III ($n = 5$)
Serum lysozyme (μg/ml)	15.0 ± 4.7	18.4 ± 8.3	20.0 ± 6.4	21.4 ± 4.9
Serum ACE (IU/liter)	26.3 ± 9.2	26.6 ± 10.8	28.4 ± 11.1	35.4 ± 11.6

serum ACE ($p = 0.29$) (Table 3). Two or more specialists evaluated the disappearance of abnormal shadows on chest radiography 5 years after the disease onset. Forty-one of the total 110 patients had been observed for 5 or more years, and abnormal shadows on chest radiography disappeared in 15 of these. The maximum serum lysozyme levels of patients with and without disappearance were 16.7 ± 4.2 and 23.4 ± 10.7, respectively, with values at the first visit to our hospital of 13.4 ± 4.2 and 18.4 ± 10.0. There was a significant difference in the maximum serum lysozyme level between the two groups ($p < 0.05$) but only a trend at the first visit to our hospital ($p = 0.096$) (Table 4).

Association with Serum ACE Level

The maximum serum lysozyme level correlated with the maximum serum ACE level ($r = 0.548$, $p < 0.0001$) (Fig. 1). A similar association was observed for these levels at the first visit to our hospital ($r = 0.455$, $p < 0.0001$).

Discussion

Lysozyme or muramidase, first discovered in 1922 by Fleming [4], is a low molecular weight lysosomal cationic enzyme with bactericidal activity, cleaving β 1–4-glycosidic bonds in cell walls of certain bacteria. It is normally present in granules of monocytes, macrophages, and polymorphonuclear leukocytes and is released constantly into various body fluids, e.g. saliva, tears, and airway secretions [2, 13]. In sarcoidosis, lysozyme has been identified mainly in macrophages and the epithelioid cells of fresh granulomas but not in older lesions [16]. Lysozyme filters through renal glomeruli and is reabsorbed and metabolized in the proximal tubules. Decreases in glomerular filtration lead to increased serum levels, and disturbed tubular function leads to increased urinary excretion. Therefore it is necessary to take into account renal function when evaluating serum lysozyme levels. There are no differences in serum lysozyme levels between men and women, smokers and nonsmokers, with age and in healthy volunteers during a 7-day intake of 10 mg of prednisolone [16].

Elevated serum levels of this enzyme have been found useful in the diagnosis of monocytic and myelomonocytic leukemias and histiocytic medullary reticulosis [10, 16]. Measurement of lysozyme concentrations has also been used to provide an index of active sarcoidosis [2, 12, 15]. The sensitivity of this parameter in detecting activity

Table 4. Comparison of serum lysozyme and ACE levels between patients with and without disappearance of abnormal shadows on chest radiography.
There was a significant difference in the maximum serum lysozyme level between the two groups ($p < 0.05$), whereas there was only a trend in serum lysozyme level at the first visit to our hospital ($p = 0.096$). No significant differences were found in serum ACE levels at the maximum ($p = 0.091$) and at the first visit to our hospital ($p = 0.13$) between the two groups.

	Serum lysozyme level (μg/ml)		Serum ACE level (IU/liter)	
	At first visit	At maximum	At first visit	At maximum
Patients with disappearance	13.4 ± 4.2	16.7 ± 4.2	20.6 ± 4.8	25.4 ± 9.4
Patients without disappearance	18.4 ± 10.0	23.4 ± 10.7	25.3 ± 9.7	32.0 ± 12.9

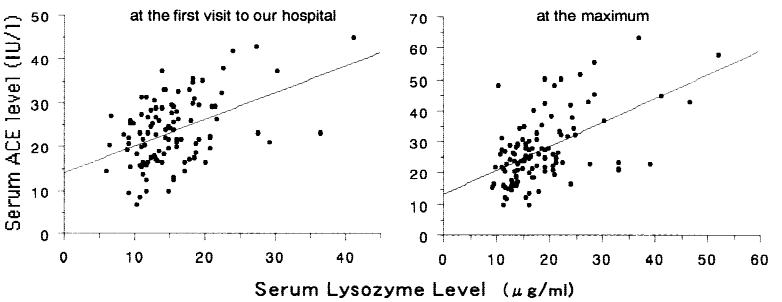

Fig. 1. Correlation between serum lysozyme and serum ACE levels. The maximum serum lysozyme correlated with the maximum serum ACE level ($r = 0.548$, $p < 0.0001$). A similar association was observed for levels at the first visit to our hospital ($r = 0.455$, $p < 0.0001$).

in sarcoidosis was found in the present report to be even superior to that of ACE, in line with the literature [6]. However, the specificity is low because abnormal values have been reported in many other diseases, including those similar to sarcoidosis, such as tuberculosis, silicosis, asbestosis, and berylliosis [5, 8, 20]. In our series, 40% of patients with other granulomatous disease showed elevated serum lysozyme values. Until now the determination of serum lysozyme has been considered to have no diagnostic value [2]. However, Romer et al. proposed that serum lysozyme measurement may be preferential for monitoring disease activity because serum lysozyme is often elevated even when serum ACE is within normal limits [15]. The sensitivity of serum ACE was 59.0% in the present study and 60.8% in our previous study [19]. On the other hand, the sensitivity of lysozyme was 79.1%. Especially in those patients without an elevated serum ACE level, the sensitivity of serum lysozyme was very high at 72.1%. Our results may support their proposal. Prior et al. mentioned that differences in sensitivity between serum lysozyme and serum ACE might be related to variation in the rates of diffusion [13], that of lysozyme from granulomas into the blood stream being facilitated by its low molecular weight (14,500). ACE has a molecular weight about tenfold higher (150,000).

The serum lysozyme level has been considered to reflect the total body mass of

biological active granulomas like the serum ACE [17, 18]. Our finding of a correlation with the number of organs involved and the radiographic stage provide further support for this conclusion. If the serum lysozyme level is higher than expected from the detected lesions, it may thus be necessary to examine other organs. Because the maximum serum lysozyme level of patients without a disappearance of abnormal shadows on chest radiography was significantly greater than in those with disappearance, elevated values in sarcoidosis patients may indicate a likelihood for persistence.

In conclusion, because the sensitivity of serum lysozyme was high even when serum ACE level was within normal limits and because serum lysozyme levels demonstrated better correlation with the clinical features of sarcoidosis than the serum ACE level, this parameter appears particularly suitable for disease monitoring in proven cases.

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