QUANTITATIVE DETERMINATION OF SERUM IMMUNOGLOBULINS IN CHRONIC OBSTRUCTIVE PULMONARY DISEASES WITH SPECIAL REFERENCE TO COR PULMONALE

TORU YAMAMOTO, M.D., SHINJI FUKUTA, M.D., SETSUKO IWAMOTO, M.D.,
TAKANARI TOKUHISA, M.D., AND JUNICHI MISE, M.D.

ALTHOUGH several investigators¹⁻³ have studied on serum immunoglobulin levels in patients with chronic obstructive pulmonary disease, those in patients with chronic cor pulmonale are yet to be determined.

In previous studies⁴⁻⁸ we have reported that circulating anti-heart antibodies were found in almost the half of 29 patients with chronic cor pulmonale caused by pulmonary emphysema and that the anti-heart antibody titer indicated a good correlation with serum IgG levels. Furthermore, the mean value of serum IgG levels in the positive group for anti-heart antibodies was significantly higher than that in the negative group. These findings suggested that the antibody activity might arise in IgG and that the anti-heart antibodies contribute to elevate the serum immunoglobulin levels.

The present study was undertaken to compare serum levels of various immunoglobulin classes in chronic obstructive pulmonary diseases with an effort to clarify the etiology or pathogenesis of chronic cor pulmonale.

MATERIALS AND METHODS

One hundred and fifteen specimens of serum were collected from normal subjects and patients with chronic obstructive pulmonary disease. Samples involved 25 sera from patients with chronic cor pulmonale, 27 from emphysema and

31 from bronchial asthma. All these patients were clinically free from cardiac failure at the time of the study. Patients with chronic cor pulmonale selected here were caused by pulmonary emphysema and its diagnosis was established on the basis of findings of laboratory tests and heart catheterization, and clinical signs as well.⁹ As control, 32 normal persons were selected. They were free from any acute or chronic diseases, and no obvious abnormalities were detected by physical examinations. Table I shows the age of patients examined. Blood was obtained by phlebotomy and serum was separated within one hour. Sera were frozen at -20°C until use.

The quantitative determination of serum IgG, IgA and IgM was carried out by the single radial diffusion method using commercially prepared immunodiffusion plate and standard sera obtained from a commercial source (Tri-Partigen, Boehringerwerke). Each diffusion plate was incubated in a moist chamber at room temperature. Diffusion was allowed to progress 50 hours for IgG and IgA and 80 hours for IgM. Standard curves were made simultaneously with each group of determinations and were plotted on semilog paper, then the values of the sera in question were interpolated from this curve over the range covered.

The concentration of IgE in sera was determined by the radioactive single radial diffusion, one step method (*Arbesman and Ito*^{10,11}). Briefly, the gammaglobulin fraction of sheep antiserum against Fc fragment of IgE was labelled with ^{125}I and incorporated in agarose.

Key Words:

Cor pulmonale
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Department of Internal Medicine, Yamaguchi University School of Medicine, Ube, Japan

TABLE I MEAN VALUES OF EACH IMMUNOGLOBULIN CONCENTRATION IN DIFFERENT GROUPS. (CONCENTRATION IN MG. PER 100ML.; RANGE IN PARENTHESES)

	<i>Cor pulmonale</i>	<i>Emphysema</i>	<i>Asthma</i>	<i>Normal subjects</i>
<i>Age</i> (range)	63.1 ± 8.6SD (48-80)	62.0 ± 8.6SD (43-74)	45.3 ± 16.7SD (19-71)	21.0 ± 7.2SD (20-33)
<i>IgG</i> (range)	1631.7 ± 545.3SD (720-2700)	1400.8 ± 478.9SD (780-2520)	1257.7 ± 374.5SD (600-2000)	1213.8 ± 328.1SD (600-2000)
<i>IgA</i> (range)	246.4 ± 89.6SD (70-432)	241.8 ± 106.0SD (80-480)	212.2 ± 83.4SD (65-394)	173.6 ± 79.0SD (50-360)
<i>IgM</i> (range)	141.6 ± 61.6SD (50-330)	129.6 ± 74.5SD (40-400)	108.8 ± 55.9SD (40-255)	123.8 ± 46.3SD (46-258)

Fig.1. Serum IgG levels in cor pulmonale, emphysema, asthma and normal subjects. Bar indicates the mean value. NS: not significant

Small antigen wells, 5 mm in diameter, were cut in the agarose gel and filled with serial dilutions of a standard IgE solution in order to make the standard curve. Serum samples were placed in antigen wells made in the immunoplate. The immunoplate was incubated in a humid atmosphere and diffusion proceeded for 2 to 3 days.

Fig.2. Serum IgA levels in cor pulmonale, emphysema, asthma and normal subjects. Bar indicates the mean value. NS: not significant

Afterwards the plate was washed exhaustively with the borate buffered saline. The gel layer was dried and exposed to x-ray film for 48 hours.

RESULTS

Mean values of serum IgG, IgA and IgM concentrations are illustrated in Table I. Figures 1, 2 and 3 show statistical comparison of serum immunoglobulin concentrations among three groups of patients and normal subjects.

Serum IgG Level:

Fig.3. Serum IgM levels in cor pulmonale, emphysema, asthma and normal subjects. Bar indicates the mean value. NS: not significant

The mean value of serum IgG concentration was 1631.7 (± 545.3 SD) mg/100ml in 25 cor pulmonale patients, 1400.8 (± 478.9 SD) mg/100ml in 27 emphysema and 1257.7 (± 374.5 SD) mg/100ml in 31 asthma patients, while that in 32 normal subjects was 1213.8 (± 328.1 SD) mg/100ml. There was a statistically significant difference of the serum IgG level between the group of cor pulmonale and emphysema ($p < 0.05$), between cor pulmonale and asthma ($p < 0.01$) and between cor pulmonale and normal subjects ($p < 0.01$). No significant difference was found in the mean value of serum IgG between the groups of emphysema and asthma, between emphysema and normal subjects, and between asthma and normal subjects.

Serum IgA Level:

The mean value of serum IgA was 264.4 (± 89.6 SD) mg/100ml in 25 cor pulmonale patients, 241.8 (± 106.0 SD) mg/100ml in 27 emphysema and 212.2 (± 83.4 SD) mg/100ml in 31 asthma patients, while that in 32 normal subjects was 173.6 (± 79.0 SD). There was a significant difference of the serum IgA level between groups of cor pulmonale and normal subjects ($p < 0.01$) and between the groups of emphysema and normal subjects ($p < 0.01$). No significant difference was detected in the mean value of serum IgA between groups of cor pulmonale and emphysema, between cor pul-

Fig.4. Serum IgE levels in asthma, emphysema, cor pulmonale and normal subjects. Asthma patients classified into allergic, non-allergic and questionable group (concentration in U per ml.).

monale and asthma, and between asthma and normal subjects.

Serum IgM Level:

The mean value of serum IgM was 141.6 (± 61.6 SD) mg/100ml in 25 cor pulmonale, 129.6 (± 74.5 SD) mg/100ml in 26 emphysema and 108.8 (± 55.9 SD) mg/100ml in 31 asthma patients, while that in 32 normal subjects was 123.8 (± 46.3 SD) mg/100ml. There was a statistical difference in the serum IgM only between groups of cor pulmonale and asthma. No significance was found in the mean value of serum IgM between three groups of patients and normal subjects.

Serum IgE Level:

Serum concentrations of IgE in asthma, emphysema, cor pulmonale and normal subjects are presented in Figure 4. The mean value of serum IgE was 679.4 (± 79.5 SD) U/ml in 31 asthma patients, while that in normal subjects was 110.9 (± 17.8 SD) U/ml. The mean value of serum IgE in the group of asthma was higher than that in normal subjects ($p < 0.01$). 300 U/ml was selected as the arbitrary limit from the figure. Fourteen of 31 (45.2 per cent) samples of asthma were found to have an elevated level (exceeding the arbitrary limit), while 3 of 12 (25 per cent) of emphysema, 4 of 9 (44.4 per cent) of cor pulmonale and 1 of 32 (3.1 per cent) of normal subjects had the IgE level exceeded over

300 U/ml. Patients with asthma were subdivided into allergic and non-allergic types. Patients assigned to the allergic group were those with a positive skin test and a positive reaction to certain allergens on inhalation provocation. In some patients who had a definite allergy history, provocation was omitted. Ten of 15 sera (66.7 per cent) from allergic asthma raised more than arbitrary limit in the IgE level, compared to 1 of 7 sera from patients with non-allergic asthma.

DISCUSSION

Results of this study revealed higher serum IgG levels in the group of cor pulmonale than that in groups of emphysema, asthma and normal subjects. The mean value of serum IgA levels was significantly higher in two groups of cor pulmonale and emphysema than that in the groups of asthma and normal subjects. However, there was no significant difference of the serum IgM levels between normal subjects and other patient groups.

Biegel *et al.*¹ reported that the IgM and IgG levels in the group of emphysema patients were of the same order as those of healthy control group, but the IgA level in the patient group was significantly higher than that of the same aged control group. Satake *et al.*² and Kubo³ also reported an elevated IgA and IgG levels and, in contrast, a low IgM level in sera from patients with chronic pulmonary emphysema. In this study serum IgA levels were significantly higher in emphysema patients than the normal subjects. Serum IgG and IgM concentrations were almost same in both groups. This finding was also present in patients with cor pulmonale which was caused by pulmonary emphysema, but in these patients the IgG level was significantly higher than that in the group of normal subjects and of emphysema patients.

The concentration of serum IgG could be influenced by several factors, such as aging, race and sex.¹² In addition, most patients with chronic obstructive pulmonary disease have a tendency to suffer from acute and chronic broncho-pulmonary infections. In our series the average age of cor pulmonale patients was 59.6 years (range 48 to 80), while that of emphysema patients was 62.0 years (range 43 to 74). Between these two groups there were no statistical significance in mean age. And in these two groups of patients acute or chronic broncho-pulmonary infections were not present. All these patients were clinically free from cardiac failure

at the time of study.

The cause of the elevated serum IgG in patients with cor pulmonale is not clear, but the most likely explanation is that chronic cor pulmonale might lead to tiny patchy myocardial necrosis, which may release a myocardial tissue component. This released heart tissue might be changed to antigenic substance by unknown mechanism, to which the host may respond, producing humoral antibodies, probably in IgG class. It was found by our previous studies⁴⁻⁸ that there is a good correlation between the serum IgG levels and the titer of circulating anti-heart antibodies in patients with chronic cor pulmonale. Furthermore, the mean value of serum IgG levels in the positive group for anti-heart antibodies was significantly higher than that of the negative group.

Since the discovery of a new Ig class, IgE, in which reaginic antibodies reside, many investigators^{13,14,15} have measured the serum level of this Ig in various allergic disorders. Results of the present study found that the mean value of the serum IgE in the group of asthma was higher than in normal subjects. While in 10 of 15 sera from allergic asthma the IgE level exceeded 300 U/ml, that in one of 7 sera from non-allergic asthma group was over 300 U/ml. An elevated level (over 300 U/ml) was found in 4 of 9 sera of patients with pulmonary emphysema accompanied by chronic cor pulmonale and in 3 of 12 patients without definite cor pulmonale. On the basis of these findings determinations of IgE in the serum might be helpful in the diagnosis of different types of bronchial asthma or chronic obstructive pulmonary disease. An elevated IgE level constitutes to indication of the presence of an atopic disease. Furthermore, the elevated IgE levels in patients with chronic cor pulmonale would suggest the possibility that an atopic disease may be one of the causative factors for chronic cor pulmonale.

SUMMARY

In patients with chronic obstructive pulmonary disease, quantitative analysis of serum IgG, IgA and IgM were carried out by use of the radial immunodiffusion method. The concentration of IgE in sera was also determined by the radioactive radial immunodiffusion method.

The mean value of serum IgG level in the group of cor pulmonale was higher than that in groups of emphysema, asthma and normal subjects. The mean value of serum IgA level was

higher in two groups of cor pulmonale and emphysema than in the group of asthma and of normal subjects. There was no difference of serum IgM levels between these four groups. The mean value of serum IgE in the group of allergic asthma was higher than that in the group of non-allergic asthma or normal subjects and an elevated serum IgE level was also found in patients with cor pulmonale.

Findings of this study suggested that the raised level of serum IgG in patients with chronic cor pulmonale might reflect production of anti-heart antibodies against cardiac tissue.

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* unofficial translation by the current author.