SHORT COMMUNICATION

C-REACTIVE PROTEIN LEVELS IN PATIENTS WITH PULMONARY TUBERCULOSIS

KANNAPIRAN, M, CHANDRA IMMANUEL, KRISHNAMURTHY, PV, AND RAGHUPATI SARMA, G

Tuberculosis Research Centre (Indian Council of Medical Research), Madras-600031

(Received original June 1988; revised November 1988)

ABSTRACT

Serum C-reactive protein (CRP) concentrations were determined in 67 patients with pulmonary tuberculosis, 12 patients with non-tuberculous respiratory disease and 12 each of healthy contacts of tuberculous patients and healthy volunteers. The mean serum CRP concentration was appreciably higher in the tuberculous patients than in the other three groups. Moderate or markedly elevated concentrations ($\geq$1 mg/dl) were observed in 58 (87%) of the 67 tuberculous patients whereas the concentrations were low in all the healthy volunteers and the healthy contacts, and in seven of the 12 patients with non-tuberculous respiratory disease. The correlation between CRP concentrations and viable counts of tubercle bacilli isolated from sputum of patients with pulmonary tuberculosis, though statistically significant, was weak ($r = 0.41$, $p < 0.01$).

Introduction

Human serum C-reactive protein (CRP) concentrations rise rapidly and attain high levels following infection or tissue injury resulting from causes such as inflammatory diseases, whereas the concentrations in healthy individuals are negligible. It has been suggested that the magnitude of increase of serum CRP concentrations over the normal levels reflects the extent of tissue injury and may predict the course of the ensuing illness(1). Determination of serum CRP concentrations has been employed as an aid to diagnosis and for management, particularly in rheumatoid arthritis and acute bacterial diseases such as pneumonia, pyelonephritis and pyogenic meningitis(2). Apart from the study of Grange and team in Indonesian subjects, there is very little information on serum CRP concentrations in chronic diseases such as tuberculosis(3). An investigation was therefore undertaken at our Centre to determine the serum concentrations of CRP in patients with pulmonary tuberculosis and to compare these with levels obtained in patients with non-tuberculous respiratory disease, healthy contacts of tuberculous patients, and healthy volunteers; this report presents the findings of this investigation.

Material and Methods

Subjects: Patients with sputum smear-positive pulmonary tuberculosis reporting at our Centre were included in this study. Patients with non-tuberculous respiratory disease were those attending the Institute of Thoracic Medicine, Chetput, Madras in whom tuberculous disease was ruled out by radiological and sputum smear examination. The healthy contacts were relatives of index cases admitted to controlled clinical trials of the treatment of pulmonary tuberculosis at our Centre, and healthy volunteers were laboratory staff working at this Centre. The subjects were not examined for any other concurrent illness.

Determination of serum CRP concentrations: Serum CRP concentrations were determined by the Radial Immuno-diffusion technique(4). In brief, a 1.5% solution of agar in citrate buffer (pH 7.0) was prepared by boiling the contents. After cooling the solution to 50°C in a water bath, antiserum to human CRP was added in a dilution of 1 in 100 and mixed well. Thirteen ml of this solution was poured into each of 'Steriware' petridishes (diameter: 9 cm). After allowing the agar to set, 19 wells of 2 mm diameter with a capacity of 10 μl each were punched in each plate. An aliquot of 10 μl of the samples (both serum and standards) was carefully pipetted into the wells, the plates covered and placed in a horizontal position in a humid chamber at room temperature. The diameter of the precipitin rings were measured after 72 hours using a graduated magnifying lens.

CRP standards and anti-serum were purchased from Behringwerke AG, West Germany and 'Steriware' plates from Steriware Private Limited, Faridabad, India.

Investigation I: An investigation was undertaken to assess replicate, plate-to-plate and between-reader variation, and also to get an idea of the concentrations that are likely to be encountered in patients with pulmonary tuberculosis. Serum samples from eight patients with pulmonary tuberculosis [five with severe disease (viable count of tubercle bacilli in sputum $\geq 10^6$/ml) and three with minimal or moderate disease (viable count $< 10^6$/ml], three healthy contacts of index cases and three patients with non-tuberculous respiratory disease were employed. These samples were set up neat (undiluted) and in 1 in 2 dilution, in duplicate. In addition, standard CRP concentrations of 0.64, 1.27, 2.54, 5.07 and 7.60 mg/dl were set up in quadruplicate (one replicate in each plate). The wells containing the standards and the serum samples were randomised and coded and the diameters of the precipitin rings were read independently by two readers. The concentrations in the samples were estimated by reference to the diameters of the precipitin rings of the standards.

Investigation II: Concentrations of CRP were determined in serum samples (kept stored at—20°C) from 67 patients with pulmonary tuberculosis, 12 with non-tuberculous respiratory disease, and 12 each of healthy contacts of index cases of pulmonary tuberculosis and healthy volunteers as in investigation I after randomisation of the wells containing the samples and the standards.

Counts of viable tubercle bacilli isolated from sputa of patients with pulmonary tuberculosis were set up as described in an earlier report(5).

Results

Investigation I: The diameters of the precipitin rings ranged from 4.8 to 11.3 mm for standard CRP concentrations ranging from 0.64 to 7.60 mg/dl (A preliminary experiment had shown that the sensitivity of the method was 0.64 mg/dl). There was a linear relationship between the standard CRP concentrations and the square of the diameter of the precipitin rings and the correlation was almost perfect ($r = 0.999$). The co-efficient of variation for the different standards set up in quadruplicate ranged from 1.2 to 2.1%. The differences in the estimated concentrations between duplicates, the within-plate and plate-to-plate differences, and the difference in the recorded diameters of the precipitin rings between the readers were not significant ($p > 0.1$). The estimated CRP concentrations ranged from less than 0.64 to 3.3 mg/dl in the three patients with non-tuberculous respiratory disease and from less than 0.64 to 15.7 mg/dl in the eight patients with pulmonary tuberculosis; the precipitin ring was not discernable (i.e. the values were negative) for the three healthy contacts.

Investigation II: The distribution and the mean serum concentrations of CRP are presented in the Table [Indeterminate concentrations (<0.64 mg/dl)

TABLE

DISTRIBUTION OF SERUM CRP CONCENTRATIONS IN PATIENTS WITH PULMONARY TUBERCULOSIS, NON-TUBERCULOUS RESPIRATORY DISEASE, HEALTHY CONTACTS OF TUBERCULOUS PATIENTS AND HEALTHY VOLUNTEERS

Group	Total No. of subjects	No. of subjects according to serum CRP concentrations (mg/dl)			
		Negative (<0.64)	0.64–0.99	1.0–9.9	≥10.0
Pulmonary tuberculosis	67	5	4	45	13
Non-tuberculous respiratory disease	12	7	0	5	0
Healthy contacts of tuberculous patients	12	10	1	1	0
Healthy volunteers	12	12	0	0	0

have been treated as negative]. The CRP concentrations in patients with pulmonary tuberculosis were appreciably higher than in the other three groups of subjects, and 58 (87%) of the 67 patients had either moderately or markedly elevated concentrations ($\geq$1 mg/dl). The mean concentration for the 67 patients was 5.6 mg/dl, with the highest value observed being 12.9 mg/dl. CRP concentrations were low (<1 mg/dl) in all the healthy volunteers, in all but one of the healthy contacts of tuberculous patients, and in seven of twelve patients with non-tuberculous respiratory disease.

The correlation between serum CRP concentrations and viable counts of tubercle bacilli isolated from sputa of patients with pulmonary tuberculosis though statistically significant, was weak ($r=0.41$, $p<0.01$). There was no correlation between CRP concentrations and the age, sex or the total White Blood Cell Count of the subjects investigated. (The erythrocyte sedimentation rate [ESR] was not determined).

Discussion

Following stimulus through infection or injury, the macrophage releases a monokine, interleukin-1, which stimulates the hepatocyte to secrete a number of proteins referred to as the acute phase proteins (acute phase reactants)(6). The role of these proteins, particularly that of CRP, in health and disease is under intensive investigation(7). The serum concentration of CRP is less than 1 mg/dl in healthy subjects(2,7), and it has been suggested that an elevated concentration of CRP is unequivocal evidence of an active tissue-damaging process(8). Results presented in this report show that the association between CRP concentrations and the viable count of tubercle bacilli isolated from sputa of the patients with pulmonary tuberculosis, (though significant statistically), was weak. Hence, the use of CRP concentrations to assess disease activity may be no better than the determination of the ESR or the concentration of the α_2-globulin fraction or the ratio of albumin to α_2-globulin (A/α_2) in serum. The concentration of α_2-globulin is known to be elevated and the A/α_2 ratio lower in conditions where tissue necrotic changes are known to occur(9).

The changes in serum CRP concentrations during treatment with short-course regimens containing rifampicin, isoniazid, streptomycin and pyrazinamide were studied in another investigation on 20 patients with sputum smear-positive pulmonary tuberculosis (unpublished findings). All patients were bacteriologically quiescent at the end of treatment. There was a sharp decrease in the CRP levels with treatment, the mean serum concentrations on admission and at the end of treatment (six months) being 7.2 and 0.8 mg/dl, respectively ($p<0.01$). Further investigations are in progress to study if CRP concentrations at the end of treatment can predict a bacteriological relapse that occurs in a small proportion of the patients after stopping treatment.

The only detailed study of acute phase proteins including that of CRP in pulmonary tuberculosis is that of Grange and others in Indonesian subjects(3). Employing a nephelometric technique, these authors reported concentrations of CRP substantially higher than those reported in this paper; thus, the mean concentrations in control subjects and patients with pulmonary tuberculosis were 6 mg/dl (range: 2-86 mg/dl) and 14 mg/dl (range: 2-116 mg/dl), respectively. The differences between the findings of the two investigations cannot be ascribed to the difference in the method of estimation alone; whether genetic factors could influence CRP concentrations in different racial populations needs to be investigated.

Acknowledgement

We thank the Director and the staff of the Institute of Thoracic Medicine, Madras for their enthusiastic co-operation. We are grateful to the medical and nursing staff of our Centre for organizing the blood collections and to Mr. V. Sundaram for assistance with statistical computations.

REFERENCES

1. Kushner I, Broder ML, Karp D. Control of acute phase response. Serum C-reactive protein kinetics after acute myocardial infraction. J. Clin. Invest. 1978; *61*: 235-42.
2. Morley JJ, Kushner I. Serum C-reactive protein levels in disease. Ann. N.Y. Acad. Sci. 1982; *389*:406-18.
3. Grange JM, Kardjito T, Setiabudi I. A study of acute-phase reactant proteins in Indonesian patients with pulmonary tuberculosis. Tubercle 1984; *65*:23-29.
4. Vaerman JP. Single Radial Immunodiffusion. Methods in Enzymology 1982; *73*:291-305.
5. Tuberculosis Chemotherapy Centre, Madras. A controlled comparison of two fully supervised once-weekly regimens in the treatment of newly diagnosed pulmonary tuberculosis. Tubercle 1973; *54*:23-45.
6. Kushner I. The phenomenon of the acute phase response. Ann. N.Y. Acad. Sci. 1982; *389*: 39-48.
7. Downton SB, Colten HR. Acute phase reactants in inflammation and infection. Semin. Hematol. 1988; *25*: 84-90.
8. Pepys MB. C-reactive protein, fifty years on. The Lancet 1981; I; 653-7.
9. Kailasam S, Jayasankar K, Kannapiran M, Krishnamurthy MS, Krishnamurthy PV, Raghupati Sarma G. Serum protein profile in patients with pulmonary tuberculosis. Indian J. Med. Res. 1985; *81*:551-7.

Request for reprint/correspondence: Dr. G. Ragupati Sarma, Tuberculosis Research Centre, Chetput, Madras-600031