

ACUTE PHASE PROTEINS IN TUBERCULOUS PATIENTS

Chandra Immanuel, G.S. Acharyulu, M. Kannapiran, R Segaran
and G. Raghupati Sarma

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

The serum concentrations of some acute phase proteins were determined on admission, during treatment, at the end of treatment and at 12 months after stopping treatment in 20 patients with pulmonary tuberculosis. Measurements were also made, on admission and at the end of treatment, in 19 patients with abdominal tuberculosis, and 11 children with tuberculous meningitis. All 20 patients with pulmonary TB had quiescent disease by the end of treatment and none had a bacteriological relapse during the follow-up period of 12 months. The response to treatment was considered favorable in 18 of the 19 patients with abdominal TB, and the CSF findings had returned to normal in 9 of 11 patients with TB meningitis. There was a significant decrease with treatment in the concentrations of C-reactive protein, ceruloplasmin, haptoglobin and α_1 -acid glycoprotein in all 3 groups of patients. While there was an increase in the concentrations of transferrin in patients with pulmonary and abdominal TB, there was a significant decrease in those with TB meningitis, α_2 -macroglobulin did not appear to function as an acute phase reactant in any of the 3 groups. Amalgamating the findings in all 3 groups of tuberculous patients, the proportions of patients with abnormal values on admission and at the end of treatment were 62% and 14% for C-reactive protein, 78% and 50% for ceruloplasmin, 86% and 26% for haptoglobin and 92% and 6% for α_1 -acid glycoprotein, respectively.

The immediate local response to tissue injury or infection is acute inflammation resulting in a number of systemic, metabolic and physiologic alterations, the event being referred to as the acute phase response. The acute phase reaction is accompanied by

Correspondence: Dr G. Raghupati Sarma, Deputy Director, Tuberculosis Research Centre, Chetput, Madras-600 031.

changes in the concentrations of several plasma proteins mainly of hepatic origin, referred to as acute phase proteins. A marked increase ranging from about 50% as with ceruloplasmin to several hundred-fold as with C-reactive protein and serum amyloid-A-protein is observed with some proteins, in contrast, the concentrations of some proteins such as albumin and transferrin have been found to decrease during the acute phase response¹. While the concentrations of some of these proteins have been reported in patients with pulmonary tuberculosis and tuberculous pleural effusion^{2,3}, little or no information is available in those with extra-pulmonary forms of the disease.

The serum concentrations of some of these proteins, notably C-reactive protein, have been used to assess the severity of several acute bacterial diseases, myocardial infarction and rheumatoid arthritis, and also to monitor the progress of these patients during treatment^{4,5}. The elevation in the erythrocyte sedimentation rate and the serum concentration of the α_2 - globulin fraction, both measures of acute phase response involving a rise in the concentrations of several plasma proteins, have been used without much success to assess the activity of disease and also to predict a bacteriological relapse which occurs in a proportion of patients with pulmonary tuberculosis after stopping treatment.^{6,7} It is possible that concentrations of some individual proteins might be better indicators of the activity of the disease. Further, identification of a protein whose serum concentration at the end of treatment can predict a bacteriological relapse would be of immense value. A preliminary investigation was, therefore, undertaken to compare the serum concentrations of some acute phase proteins in patients with pulmonary, and abdominal tuberculosis and tuberculous meningitis with those in healthy volunteers, and also to study the changes in the concentrations of these proteins during treatment with anti-tuberculosis drugs, to identify the proteins that could subsequently be studied in greater detail for assessment and predictive purposes.

Material and Methods

The acute phase proteins investigated were C-reactive protein, ceruloplasmin, haptoglobin, α_1 -acid glycoprotein, transferrin and α_2 -macroglobulin.

These investigations were undertaken in South Indian patients admitted to controlled clinical trials of pulmonary tuberculosis, abdominal tuberculosis and tuberculous meningitis conducted at this Centre. Blood samples were collected from these patients at different time-points, and serum separated and stored at -20°C till the concentrations of the acute phase proteins were determined.

Subjects

Pulmonary tuberculosis. Twenty consecutive patients admitted to a trial of the treatment of pulmonary tuberculosis with fully intermittent short-course regimens containing rifampicin, isoniazid, streptomycin and pyrazinamide were included in the present study. All the patients were aged 12 years or more and the disease was diagnosed on the basis of

the results of direct smear and culture examination of sputum. Blood samples were collected on admission (0 month), during treatment (2 months), at the end of treatment (6 months) and also at 18 months i.e., 12 months after stopping treatment). All these patients had attained bacteriological quiescence at the end of treatment and none had a bacteriological relapse during a follow-up period of 12 months after stopping chemotherapy.

Abdominal tuberculosis. Nineteen patients were included in this study. Of these, 10 were randomly allocated to a short-course regimen of rifampicin, isoniazid and streptomycin for 6 months and the remaining 9 to a standard regimen of streptomycin, isoniazid and ethambutol for a period of 12 months. All the patients were aged 12 years or more and the disease was diagnosed on the basis of histopathological, radiological or bacteriological findings. Blood samples from these patients were collected at the time of admission and at the end of chemotherapy (6/12 months). Of the 19 patients admitted, 18 were considered to have had a favourable response to treatment at the end of treatment.

Tuberculous meningitis. Eleven consecutive patients (1 in Stage I and 10 in Stage II), treated with short-course regimens of rifampicin, isoniazid, streptomycin, ethambutol and pyrazinamide of 9 months duration, were included in this study. The patients were aged 1-12 years and the diagnosis of tuberculous meningitis in these patients was based on clinical and CSF findings; blood samples were collected on admission and at the end of chemotherapy (9 months). At the end of treatment, the CSF findings were normal in 9 of the 11 patients; the recovery was complete in 4 patients while the rest had residual neurological sequelae (mild in 3, moderate in 3 and severe in 1).

Healthy volunteers were from laboratory staff working at this Centre and were aged 20 years or more.

The standards, antisera and the control specimens of the acute phase proteins were purchased from Behringwerke AG, West Germany.

Assay procedure. The concentrations of the acute phase proteins in the serum samples were estimated using the radial immunodiffusion technique employing the specific antisera and a minimum of 4 standards⁸. The assays were undertaken in batches; each batch had samples in duplicate from the different groups of subjects in addition to the standards. All assays were undertaken after randomising the samples and standards.

Results

Preliminary investigations (data not tabulated) had shown that the regression of the square of the diameters of the precipitin rings on the standard protein concentrations was linear; the correlation co-efficients ranged from 0.989 to 0.999 for different proteins. Variation between replicates within the plate was not significant ($p > 0.2$), and the mean co-efficient of variation for estimates obtained on two different occasions ranged from 2.1% with C-reactive protein to 10.4% with ceruloplasmin. While the differences between

the mean concentrations on two separate occasions were not significant for C-reactive protein, haptoglobin α_2 -acid glycoprotein and α_2 -macroglobulin ($p > 0.1$), those for transferrin ($p < 0.05$) and ceruloplasmin ($p < 0.03$) were statistically significant.

The mean serum concentrations on admission in patients with pulmonary and abdominal tuberculosis and tuberculous meningitis and rose in healthy volunteers together with the normal range⁹ are presented in table 1. In the 11 healthy subjects, the values were within the normal range⁹ in all for ceruloplasmin, in 10 for C-reactive protein, in 9 each for α_1 -acid glycoprotein and α_2 -macroglobulin, in 8 for haptoglobin and in 7 for transferrin.

Table 1. Serum acute phase protein concentrations in tuberculous patients and healthy volunteers

Acute phase protein	Mean and standard deviation of serum concentrations (mg/dl)				
	Pulmonary TB	Abdominal TB	Meningeal TB	Healthy volunteers	Normal range
C-reactive protein	7.2 ± 6.2	2.1 ± 2.3	1.1 ± 1.1	0.15 ± 0.27	0.01-0.8
Ceruloplasmin	76 ± 23	63 ± 29	78 ± 65	25 ± 5	15-45
Haptoglobin	512 ± 163	309 ± 162	509 ± 163	146 ± 77	40-180
α_1 -acid glycoprotein	269 ± 87	246 ± 90	245 ± 107	61 ± 23	35-140
Transferrin	228 ± 55	229 ± 78	410 ± 125	339 ± 68	200-340
α_2 -macroglobulin	310 ± 93	296 ± 104	491 ± 141	275 ± 112	Adults : 150-350
No. of subjects	20	19	11	11	Children : > 400

* Quoted from reference⁹.

The mean concentrations of C-reactive protein, ceruloplasmin, haptoglobin and α_1 -acid glycoprotein were significantly higher in all 3 groups of tuberculous patients than in healthy volunteers ($p < 0.001$). The mean transferrin concentrations in patients with pulmonary and abdominal tuberculosis were significantly lower than in healthy volunteers ($p < 0.01$); in patients with tuberculous meningitis, however, these levels were higher than in the healthy subjects ($p < 0.01$). The mean concentrations of α_2 -macroglobulin were similar in pulmonary and abdominal tuberculous patients and healthy volunteers. The concentration of this protein in children is reported to be higher than in adults⁹, and 9 of the 11 children with tuberculous meningitis had values greater than 400 mg/dl. Comparing the values between the 3 groups of tuberculous patients, the mean value for C-reactive protein in patients with pulmonary tuberculosis was significantly higher than in patients with abdominal tuberculosis or tuberculous meningitis ($p < 0.01$ for both), while the mean value of haptoglobin in patients with abdominal tuberculosis was lower than those in the other two groups ($p < 0.01$ for both), the mean value for transferrin was higher in patients with

tuberculous meningitis than in the other two groups ($p < 0.01$). The differences between the mean concentrations in the other proteins were not significant with the exception of α_2 -macroglobulin; the mean concentration of this protein was significantly higher in children with tuberculous meningitis than in the other two groups ($p < 0.01$).

The mean concentrations of the different proteins at 0, 2, 6 and 18 months in patients with pulmonary tuberculosis are presented in Table 2. There was a sharp decrease in the mean concentrations of C-reactive protein, haptoglobin and α_1 -acid glycoprotein with treatment and the mean values both at 2 months and at the end of chemotherapy were appreciably lower than the values on admission ($p < 0.01$ for all). The mean values of these

Table 2. Serial serum acute phase protein concentrations in patients treated for pulmonary tuberculosis

Acute phase protein	Mean and standard deviation of serum concentrations (mg/dl)			
	0 month	2 months	6 months*	18 months**
C-reactive protein	7.2 ± 6.2	2.2 ± 2.4	0.8 ± 2.8	0.7 ± 1.4
Ceruloplasmin	76 ± 23	79 ± 48	60 ± 19	29 ± 8
Haptoglobin	512 ± 163	278 ± 159	152 ± 92	160 ± 94
α_1 -acid glycoprotein	269 ± 87	166 ± 76	86 ± 31	90 ± 45
Transferrin	228 ± 55	342 ± 133	332 ± 81	290 ± 51
α_2 -macroglobulin	310 ± 93	362 ± 149	346 ± 117	350 ± 102

* End of chemotherapy

** 12 month after stopping treatment

three proteins at 18 month (i.e., 12 months after stopping treatment) were similar to those at the end of treatment. The mean values of ceruloplasmin were similar at 0 and 2 months; there was a slight but significant fall by the end of chemotherapy ($p < 0.05$), and the mean value at 18 month was appreciably lower than that at the end of chemotherapy ($p < 0.01$). There was a significant increase in the mean concentration of transferrin with treatment ($p < 0.01$); however, there was a slight fall thereafter, and the mean value at 18 month was significantly lower than that at the end of chemotherapy ($p < 0.01$). The differences between the mean concentrations of α_2 -macroglobulin at the four different time-points were not significant ($p > 0.2$).

The mean concentrations of the acute phase proteins on admission and at the end of treatment in patients with abdominal tuberculosis and tuberculous meningitis are presented in Table 3 (The findings in patients with abdominal tuberculosis who received treatment for 6 or 12 months were similar and have therefore been amalgamated). In patients with abdominal tuberculosis there was a significant decrease in the concentrations of C-reactive protein, ceruloplasmin, haptoglobin and α_1 -acid glycoprotein ($p \leq 0.05$) and a significant increase in that of transferrin ($p < 0.01$) with treatment. The increase in the concentration of α_2 -macroglobulin was not significant ($p > 0.2$).

Table 3. Serum acute phase protein concentrations on admission and at the end of treatment in patients with abdominal tuberculosis and tuberculous meningitis

Acute phase protein	Mean and standard deviation of serum concentrations (mg/dl)			
	Abdominal TB		TB Meningitis	
	0 m	6/12 m	0 m	9 m
C-reactive protein	2.1 ± 2.3	0.8 ± 1.8	1.1 ± 1.1	0.2 ± 0.2
Ceruloplasmin	63 ± 29	40 ± 33	78 ± 65	41 ± 14
Haptoglobin	309 ± 162	119 ± 85	509 ± 163	154 ± 87
α_1 acid glycoprotein	246 ± 90	78 ± 42	245 ± 107	97 ± 28
Transferrin	229 ± 78	291 ± 68	410 ± 125	234 ± 56
α_2 macroglobulin	296 ± 104	346 ± 106	491 ± 141	502 ± 118

In patients with tuberculous meningitis, with the exception of α_2 -macroglobulin, there was a significant decrease in the levels of all acute phase proteins including that of transferrin with treatment ($p < 0.01$ for all).

The number of patients with abnormal values on admission and at the end of treatment are presented in Table 4 (The figures for α_2 -macroglobulin have not been presented as this protein did not appear to behave like an acute phase reactant in any of the 3 groups). Amalgamating the findings in all 3 groups of tuberculous patients, the values were abnormal on admission in 62% of the 50 patients for C-reactive protein, 78% for

Table 4. Number of tuberculous patients with abnormal values for some acute phase proteins on admission and at the end of treatment

Acute phase protein	Reference concentration for abnormal value (mg/dl)	Pulmonary TB		Abdominal TB		TB meningitis	
		0	6	0	6/12	0	9
		month	months	month	months	month	months
C-reactive protein	> 0.8	17	3	10	4	4	0
Ceruloplasmin	> 45	20	15	12	4	7	6
Haptoglobin	> 180	18	6	14	3	11	4
α_1 -acid glycoprotein	> 140	18	1	17	2	11	0
Transferrin	< 200	6	0	5	2	0	0
No. of patients		20		19		11	

ceruloplasmin, 86% for haptoglobin and 92% for α_1 -acid glycoprotein; the corresponding proportions at the end of treatment were 14%, 50%, 26% and 6% respectively.

Discussion

Following stimulus through infection or injury, the macrophage releases a monokine, interleukin 1, which stimulates the hepatocyte to secrete a number of acute phase proteins¹⁰. These acute phase reactants have been shown to serve a variety of functional roles during tissue repair of infection or inflammation and in several host immune-defence mechanisms C-reactive protein has been shown to cause bacterial capsular swelling, promotion of agglutination, complement fixation and enhancement of phagocytosis¹¹. Oxygen-derived free radicals and hydrogen peroxide produced by activated polymorphonuclear leucocytes, are believed to be the important agents causing the death of engulfed micro-organisms^{12,13}, and also to be the cause of inflammation and tissue injury¹⁴. Increased concentrations of ceruloplasmin, known to possess significant oxidase activity and capable of scavenging oxygen-derived free radicals^{14,15}, are probably responsible for limiting the damage caused by these radicals. Haptoglobin and transferrin have been shown to play important roles in restricting the availability of iron, an essential nutrient for the survival and proliferation of micro-organisms within the host¹⁶. Haptoglobin binds hemoglobin which is known to support bacterial growth^{16,17}, and transferrin binds free iron available within the cell¹⁸. The decrease in transferrin concentrations during the acute phase reaction is attributed to an excess of catabolism over synthesis¹⁶. While transferrin concentrations increased with treatment in patients with pulmonary, and abdominal tuberculosis, a decrease was observed in all patients with tuberculous meningitis. The significance of this finding needs to be evaluated further. The precise role of α_1 -acid glycoprotein in human immune-defence mechanisms is not clear, though it is believed to serve as a T-cell mediator¹⁹. α_2 -macroglobulin did not appear to function as an acute phase reactant in our patients, and this is in agreement with findings reported earlier²⁰.

A bacteriological relapse requiring treatment is known to occur in a proportion of patients with pulmonary tuberculosis who have quiescent disease at the end of treatment²¹. Investigations undertaken earlier at our Centre had shown that the mean serum concentration of the α_2 -globulin fraction at the end of treatment was significantly higher in patients who relapsed subsequently than in those who did not; it was however not possible to suggest the level of the concentration at which the outcome of a relapse could be predicted⁷. The erythrocyte sedimentation rate, which is also a measure of acute phase response, is not a suitable marker as it was found to be elevated in a substantial proportion of patients who had attained stable bacteriological quiescence after treatment⁶. It has been suggested that an elevated concentration of C-reactive protein is unequivocal evidence of an active tissue-damaging process^{1,22}, however, even this protein may not be suitable as its concentration at the end of treatment was found elevated in 3 of the 20 patients with pulmonary tuberculosis, none of whom relapsed during a follow-up period of 12 months. Ceruloplasmin and haptoglobin concentrations at the end of treatment were elevated in too

high a proportion of patients to merit their use for predictive purposes. Of all the proteins investigated, only α_1 -acid glycoprotein seemed to hold some promise as its concentrations were elevated in 90% of patients with pulmonary tuberculosis on admission, and only one of these had an abnormal value at the end of the scheduled period of chemotherapy. Whether elevated concentrations of this protein reflect the extent of tuberculous lesion before start of treatment, and whether its concentration at the end of treatment (either alone or in combination with other proteins) can predict a bacteriological relapse needs to be investigated in a much larger group of patients, and a prospective study is in progress at our Centre.

Acknowledgement

We are grateful to the medical and nursing staff of the Centre for organizing the collection of blood specimens.

References

1. Kushner I, Broder ML, Karp D. Control of acute phase response. Serum C-reactive protein kinetics after acute myocardial infarction. *J Clin Invest* 1978; **61** : 235-242.
2. Sulochana G, Khalifulla PA, Padmanabhan L. Adenosine deaminase, α_1 antitrypsin, α_1 acid glycoprotein, ceruloplasmin and protein in the diagnosis of tuberculous pleural effusion. *Indian J Chest Dis Allied Sci* 1988; **30** : 15-18.
3. Grange JM, Kardjito T, Setiaabudi I. A study of acute phase reactant proteins in Indonesian patients with pulmonary tuberculosis. *Tubercle* 1984; **65** : 23-29.
4. Morley JJ, Kushner I. Serum C-reactive protein levels in diseases. *Ann NY Acad Sci* 1982; **389** : 406-418.
5. Whither JT, Dieppe PA. Acute phase proteins. *Clin Immunol Allergy* 1985; **5** : 425-446.
6. Tuberculosis Chemotherapy Centre, Madras. A concurrent comparison of isoniazid plus PAS with three regimens of isoniazid alone in the domiciliary treatment of pulmonary tuberculosis in South India. *Bull WHO* 1960; **23** : 535-585.
7. 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**: 551-557.
8. Vaerman TP. Single radial immuno-diffusion. *Methods Enzymol* 1981; **73** : 291-305.
9. Ritchie RP. Specific proteins. In Todd, Sanford and Davidson. *Clinical Diagnosis and Management by Laboratory Methods*. J.B. Henry (ed). Vol. 1; 16th edition W.B. Saunders Company, Philadelphia, London, Toronto; 1979; p. 228.
10. Kushner I. The phenomenon of acute phase response. *Ann NY Acad Sci* 1982; **389** : 39-48.
11. Pepys MB, Baltz ML. Acute phase proteins with special reference to C-reactive protein and related proteins (pentaxins) and serum amyloid A protein. *Adv Immunol* 1983; **34** : 141-212.

12. Jacketh PS, Andrew PW, Aber VR, Lowrie DB. Hydrogen peroxide and superoxide release by alveolar macrophages from normal and BCG-vaccinated guinea-pigs after intravenous challenge with *Mycobacterium tuberculosis*. *Br J Exp Pathol* 1982; **62** : 419-428.
13. Lowrie DB. The macrophage and mycobacterial infections. *Trans R Soc Trop Med Hyg* 1983; **77** : 646-655.
14. Goldstein IM, Kaplan HB, Edelson HS, Weissmann G. Ceruloplasmin : An acute phase reactant that scavenges oxygen-derived free radicals. *Ann NY Acad Sci* 1982; **389** : 368-379.
15. Goldstein IM, Kaplan HB, Edelson HS, Weissmann G. Ceruloplasmin : A scavenger of superoxide anion radical. *J Biol Chem* 1979; **254** : 4040-4045.
16. Ogata RT. Factors determining bacterial pathogenicity. *Clin Physiol Biochem* 1983; **1** : 145-159.
17. Eaton JW, Brandt P, Mahoney JR. Haptoglobin : A natural bacteriostat. *Science* 1982; **215** : 691-693.
18. Gordon AH. The acute phase plasma proteins. In : *Plasma Protein Turnover*. R. Bianchi, G. Mariani and A.S. McFarlane (Eds). Baltimore, University Park-Press, 1976; p. 381.
19. Downton SB, Cohen HR. Acute phase reactants in inflammation and infection. *Semin Hematol* 1988; **25** : 84-90.
20. Metcalfe J, Tavill AS. A proposed role of alpha-1-acute phase globulin synthesis by the perfused rat liver. *Br J Exp Pathol* 1975; **56** : 570-571.
21. Fox W. Whither short-course chemotherapy ? *Br J Dis Chest* 1981; **75** : 331-357.
22. Pepys MB. C-reactive protein fifty years on. *Lancet* 1981; **II** : 653-657.