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Serum protein profile in patients with pulmonary tuberculosis

S. Kailasam, K. Jayasankar, M. Kannapiran, M.S. Krishnamurthy
P.V. Krishnamurthy & G. Raghupati Sarma

Tuberculosis Research Centre (ICMR), Madras

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A detailed study of the alterations in the serum protein fractions following commencement of effective anti-tuberculosis treatment was undertaken in 511 patients with pulmonary tuberculosis. The concentration of serum albumin was 20-30 per cent higher (P<0.001) and that of α_1, α_2 and γ -globulins significantly lower at the end of treatment than on admission (P <0.01), the magnitude of decrease being 7-17 per cent for γ -globulin, 19-30 per cent for α_1– globulin and 35-38 per cent for α_2– globulin. In all, 481 patients had a favourable response to treatment, 46 of whom had a bacteriological relapse within 18 months of stopping treatment. The likelihood of a relapse was found to be greater with higher α_2 -globulin concentrations at the end of treatment, the proportions of patients who relapsed being 3, 9, 13 and 15 per cent with α_2 -globulin concentrations of < 0.40, 0.40-0.59, 0.60-0.79 and ≥ 0.80 g/dl (P=0.02).

It has been well established that in chronic infectious diseases like tuberculosis, the albumin content of serum proteins shows a decrease while the globulin content shows an increase leading to low albumin to globulin (A/G) and albumin to α_2 -globulin (A/ α_2) ratios[1,2]. An investigation was undertaken to study the alterations in serum protein profile following commencement of effective anti-tuberculosis treatment, and to examine whether these changes are associated with immediate response to treatment and a subsequent likelihood of relapse.

Material & Methods

The investigations were undertaken in 511 patients with sputum-positive pulmonary tuberculosis; of these, 422 had organisms initially sensitive to isoniazid and streptomycin, and 89 resistant either to one or both the drugs. These patients were allocated at random to one of the following regimens :

R3: Rifampicin (12 mg/kg body weight) plus streptomycin (0.75 g) plus isoniazid (400 mg) plus pyrazinamide (35 mg/kg) daily for 3 months.

R5: The same as R3 plus a twice-weekly phase of 2 months with streptomycin (0.75 g), isoniazid (15 mg/kg) and pyrazinamide (70 mg/kg).

Z5: The same as R5, but without rifampicin.

Blood samples were obtained at 0 and 3 months from patients admitted to the

R3 regimen, and at 0, 3 and 5 months from those admitted to the R5 and 25 regimens and also from 18 healthy volunteers. The total serum protein content was determined by the Biuret method[3]. The different serum protein fractions were separated electrophoretically[4] at a pH of 8.6 (0.06M barbitone-sodium barbitone buffer, μ=0.05) using Whatman No. 3 filter paper at a constant voltage of 210 V for 5) h. The proteins were stained with methanolic bromophenol blue, and the different fractions were eluted with 0.01 M sodium hydroxide. The extinction was recorded at 540 nm, and the concentrations of the individual fractions were calculated on the basis of these values and the total protein content of each sample.

Counts of viable tubercle bacilli isolated from sputa of patients were set up as described earlier[5], and the classification of the radiographic extent of tuberculous disease was undertaken as detailed earlier[6].

A patient was deemed to have had an unfavourable bacteriological response if he had one or more of 3 cultures positive for *Mycobacterium tuberculosis* at each of the last 2 months of treatment, irrespective of the amount of growth. The response was also considered unfavourable in patients who had chemotherapy continued beyond the prescribed duration on account of clinical or radiographic deterioration, and in those who died of tuberculosis. A bacteriological relapse has been defined as the occurrence of 2 or more positive cultures for *M. tuberculosis* (of a total of 6 cultures) at different months in any 3 consecutive monthly examinations, irrespective of the amount of growth. Treatment was started only if at least one of the cultures had a growth of 20 colonies or more in the presence of at least one positive smear, and also in those who intermittently produced positive cultures (of any grade of growth) over several months.

Results

The mean values of the serum protein fractions and the A/G and A/ α_2 ratios in patients with initially drug-sensitive and drug-resistant organisms before start of treatment, and in healthy volunteers are presented in Table I. The serum albumin content was lower (about 40%), while the concentrations of all the globulin fractions were higher, ranging from about 20 per cent for β -globulin to about 200 per cent for α_1 -globulin, in the tuberculous patients than in the healthy volunteers leading to appreciably lower (about 60-65%) A/G and A/ α_2 ratios in the former. The mean values of all the protein fractions and the ratios were similar in patients with initially drug-sensitive and drug-resistant organisms.

The association between the concentrations of α_2 or γ -globulin, the A/G or A/ α_2 ratios and the disease status on admission, as measured by the radiographic extent of tuberculous disease and the log viable count of tubercle bacilli isolated from the sputa of the patients was examined (Table II). There was an increasing trend in the mean concentrations of α_2 and γ -globulins and a corresponding decreasing trend in the A/G and A/ α_2 ratios with an increase in the severity of disease as measured by either the radiographic extent of the disease or the count of viable tubercle bacilli ($P<0.01$).

Changes in the protein fractions during treatment are presented in Table III; the findings in patients with initially drug-

Table I. Serum protein concentrations in patients with pulmonary tuberculosis and in healthy volunteers

(Data are mean±SD)

Serum protein fraction (g/dl)	Drug-sensitive patients (422)	Drug-resistant patients (89)	Healthy volunteers (18)
Total protein	6.82 ± 0.59	6.83 ± 0.56	6.64 ± 0.44
Albumin	2.71 ± 0.56	2.78 ± 0.54	4.34 ± 0.35
α_1 -globulin	0.33 ± 0.11	0.32 ± 0.11	0.11 ± 0.04
α_2 -globulin	0.86 ± 0.20	0.87 ± 0.22	0.48 ± 0.10
β -globulin	0.67 ± 0.14	0.66 ± 0.14	0.55 ± 0.04
γ -globulin	2.24 ± 0.53	2.20 ± 0.53	1.16 ± 0.27
A/G ratio	0.69 ± 0.25	0.72 ± 0.26	1.93 ± 0.38
A/ α_2 ratio	3.52 ± 2.38	3.53 ± 1.75	9.49 ± 2.20

Figures in ‘parentheses are numbers studied

Table II. Association between disease status on admission and serum protein fractions and ratios

(Data are mean values)

Radio-graphic extent of disease	No. of patients*	α_2 (g/dl)	γ (g/dl)	A/G	A/α_2	Log viable count (ml^{-1})	No. of patients**	α_2 (g/dl)	γ (g/dl)	A/G	A/α_2
Slight	47	0.73	2.02	1.00	5.36	0	20	0.67	1.91	1.04	6.42
Limited	118	0.88	2.09	0.77	3.62	1-	53	0.80	2.19	0.78	3.99
Moderate	181	0.90	2.36	0.74	3.68	3-	162	0.84	2.21	0.73	3.58
Extensive	161	0.90	2.40	0.63	3.06	5-	217	0.91	2.28	0.67	3.11
Gross	4	0.89	2.39	0.60	2.83	7-	53	0.93	2.26	0.60	2.82

*Based on 511 patients: **Based on 505 patients only

sensitive and drug-resistant organisms have been amalgamated as these were similar. There was little change with treatment in the total protein content. However, there was a 20-30 per cent increase in the serum albumin content during treatment ($P<0.001$), and significant decreases ($P<0.01$) in all the globulin fractions excepting β -globulin in the 3 groups, the magnitude of decrease being 6-8 per cent for β -globulin, 7-17 percent for γ -globulin, 19-30 per cent for α_1 -globulin and 35-38 per cent for α_2 -globulin. Consequently, the A/G and A/ α_2 ratios were appreciably higher (41-66 and 87-114%, respectively) at the end of treatment in all the groups

Table III. Changes in the serum protein concentrations during treatment

(Data are mean ± SD)

Protein fraction (g/dl)	R3 (167)		R5 (179)			Z5 (145)		
	Months of treatment							
	0	3	0	3	5	0	3	5
Total protein	6.93 ± 0.52	6.91 ± 0.54	6.77 ± 0.62	6.78 ± 0.55	6.70 ± 0.52	6.78 ± 0.56	7.03 ± 0.52	6.84 ± 0.51
Albumin	2.79 ± 0.53	3.35 ± 0.46	2.74 ± 0.54	3.35 ± 0.51	3.51 ± 0.52	2.68 ± 0.58	3.48 ± 0.51	3.51 ± 0.55
α_1 -globulin	0.32 ± 0.10	0.26 ± 0.12	0.33 ± 0.12	0.27 ± 0.15	0.23 ± 0.11	0.34 ± 0.11	0.25 ± 0.11	0.26 ± 0.31
α_2 -globulin	0.86 ± 0.18	0.56 ± 0.14	0.86 ± 0.22	0.55 ± 0.15	0.53 ± 0.15	0.86 ± 0.21	0.58 ± 0.15	0.54 ± 0.13
β -globulin	0.67 ± 0.14	0.62 ± 0.16	0.66 ± 0.15	0.59 ± 0.15	0.61 ± 0.15	0.66 ± 0.13	0.63 ± 0.15	0.62 ± 0.15
γ -globulin	2.29 ± 0.53	2.12 ± 0.50	2.18 ± 0.52	2.01 ± 0.48	1.81 ± 0.46	2.24 ± 0.53	2.08 ± 0.50	1.91 ± 0.48
A/G ratio	0.70 ± 0.25	0.99 ± 0.29	0.72 ± 0.26	1.04 ± 0.34	1.17 ± 0.38	0.68 ± 0.26	1.04 ± 0.32	1.13 ± 0.37
A/ α_2 ratio	3.51 ± 1.57	6.57 ± 2.44	3.56 ± 2.16	6.68 ± 2.69	7.39 ± 2.85	3.34 ± 1.54	6.47 ± 2.35	7.16 ± 2.70

Figures in parentheses indicate number studied. *Twenty patients (7 R3, 5 R5 and 8 Z5) have been excluded due to non-availability of data at all the specified time-points

($P<0.001$). No association was observed between the magnitude of change with treatment in the protein concentrations or the A/G or A/ α_2 ratio and the disease status on admission (data not presented).

Of the patients with initially drug sensitive organisms, all but one (R3) had a favourable response; however, a bacteriological relapse requiring treatment occurred in 21 (15%) of 136 R3, 4(3%) of 146 R5, and 15 (12%) of 129 Z5 patients within 18 months after stopping treatment. Among patients with initially drug-resistant organisms, response was unfavourable in 1 of 30 R3, 3 of 33 R5 and 5 of 16 Z5 patients ; of the remaining 70 patients, 6 (3 R3, 1 R5 and 2 Z5) had a bacteriological relapse within 18 months after stopping treatment. Since it appears that of all protein fractions examined, only the α_2 and the γ -globulin fractions are likely to be of any importance, further comparisons were made only for these two fractions and the A/G and the A/ α_2 ratios.

The mean α_2 and γ-globulin concentrations at the end of treatment in the 10 patients (9 of whom had initially drug-resistant organisms) who had an unfavourable response at the end of the scheduled period of treatment were 0.61 and 242 g/dl, values higher than in those who had a favourable response (0.54 and 1.94 g/dl, respectively). The mean A/G and A/ α_2 ratios in the former group (0.86 and 5.85, respectively) were lower than those in the latter group (1.10 and 7.07 respectively).

Comparison of the values in patients who had a bacteriological relapse with those who did not (Table IV), showed that those who relapsed on the 2 rifampicin regimens had higher α_2-globulin ($P<0.01$) and γ-globulin ($P > 0.2$) concentrations, and lower A/G ($P = 0.04$) and A/ α_2 ($P< 0.01$) ratios at the end of treatment than those who did not have a bacteriological relapse. For patients in the Z5 regimen, these values were similar in patients who relapsed and in those who did not. Amalgamating the results of patients from all regimens at the end of treatment, it was observed that the mean concentrations of both the globulin fractions (α_2 and γ) were higher, and the mean ratios (A/G and A/ α_2 were lower in patients who relapsed than in those who did not. Thus, the mean values for the α_2 and γ-globulin concentrations, and the A/G and A/ α_2 ratios in patients who had a bacteriological relapse were 0.58 and 2.02 g/d1 and 1.04 and 6.28 respectively; the corresponding values in patients who did not relapse, were 0.53 and 1.93 g/d1 and 1.11 and 7.15 respectively. The differences attained statistical significance ($P<0.04$) only with the α_2-globulin concentration and the A/ α_2 ratio. The mean at-globulin concentrations at the end of treatment in patients who relapsed and in those who did not (not tabulated) were 0.28 and 0.25 g/dl, respectively ; the difference was not significant ($P>0.2$).

The likelihood of a relapse was found to be greater with higher α_2-globulin concentrations at the end of treatment, the proportions of patients who relapsed

Table IV. Serum protein concentration at the end of chemotherapy in patients with bacteriological relapse

(Data are mean ± SD)

Regimen	Group	No. of patients	α_2 (g/dl)	γ (g/dl)	A/G	A / α_2
R3 + R5	Relapse	29	0.61 ± 0.12	2.03 ± 0.50	0.95 ± 0.35	5.71 ± 1.88
	No relapse	312	0.53 ± 0.15	1.94 ± 0.50	1.10 ± 0.35	7.13 ± 2.71
Z5	Relapse	17	0.53 ± 0.14	2.01 ± 0.54	1.18 ± 0.50	7.24 ± 2.78
	No relapse	123	0.54 ± 0.13	1.89 ± 0.46	1.14 ± 0.35	7.20 ± 2.71
All regimens	Relapse	46	0.58 ± 0.13	2.02 ± 0.51	1.04 ± 0.42	6.28 ± 2.35
	No relapse	435	0.53 ± 0..14	1.93 ± 0.49	1.11 ± 0.35	7.15 ± 2.71

being 3, 9, 13 and 15 per cent with α_2–globulin concentrations of <0.40, 0.40-0.59, 0.60-0.79 and ≥ 0.80 g/dl, respectively, a significant trend (P=0.02). Such a trend was not observed with the other globulin fractions.

Of the 46 patients who relapsed within 18 months after stopping treatment, 32 had an early relapse (within 6 months), and the remaining had a late relapse (7-18 months). The mean α_2 -globulin concentrations in the early and late relapses were 0.61 and 0.53 g/dl, respectively (P=0.06); the mean A/ α_2 ratios in the 2 groups were 5.99 and 6.93, respectively (P>0.2). The mean r-globulin concentrations and A/G ratios (results not presented) were similar in the 2 groups.

Discussion

The study of serum protein profile before and during treatment with short course regimens was undertaken primarily to identify factors of prognostic significance in patients with pulmonary tuberculosis. Weak associations, though statistically significant, were observed between the extent of tuberculous disease on admission and the concentrations of α_2 and γ -globulins and the A/G and A/ α_2 ratios. During treatment, the mean decrease in the concentration of α_2 -globulin was more pronounced than that of the other globulins, and the increase in the A/ α_2 ratio was substantially higher than that of the A/G ratio. Similar observations were made by Gilliland and others[1] who suggested that the A/ α_2 ratio could be employed to assess the activity of the disease and to monitor the progress of the patient during chemotherapy.

Almost all the patients with initially drug-sensitive organisms, and a substantial proportion of those with organisms initially resistant to streptomycin or isoniazid or both had a bacteriologically favourable response at the end of the scheduled periods of chemotherapy with effective short-course regimens containing rifampicin and pyrazinamide in addition to isoniazid and streptomycin; however, bacteriological relapses requiring treatment occurred in varying proportions of patients with the 3 regimens investigated after stopping treatment. Results reported in this paper suggest that of all the protein fractions examined, the α_2 -globulin fraction is probably of greater prognostic significance than the other fractions. The classification of the proportions of relapses according to the α_2 -globulin concentrations at the end of treatment showed an increase with increasing concentrations; it was, however, not possible to determine the level of the concentration at which the outcome of a relapse could be predicted. The concentration of this fraction is known to be elevated in conditions where tissue necrotic changes are known to occur. The observation that the α_2 -globulin concentration was higher in patients who relapsed than in those who did not suggests that active tissue destruction is possibly still in progress in the former. The mean concentration of this protein fraction was also slightly higher in patients who relapsed early (within 6 months after stopping treatment) than in those who had a later relapse (7-18 months) suggesting a greater degree of active tissue destruction in the early relapses.

The α_2 -globulin fraction consists of a number of proteins with similar electro-

phoretic mobilities, some of which like ceruloplasmin are acute phase proteins. The concentrations of these proteins are known to be increased in response to inflammatory reactions involving tissue damage and infection[7] The elevated concentrations of the α_2 -fraction in tuberculous patients before start of treatment, and the slightly higher concentrations in patients who had an unfavourable response or in those who had a bacteriological relapse after stopping treatment might be due to the higher concentrations of these acute phase proteins. However, no firm conclusions on the possible significance of the findings reported here can be drawn until data are available on the α_2 -globulin and the constituent acute phase protein concentrations at time-points between the end of treatment and the occurrence of relapse.

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References

1. Gilliland, I.C., Johnston, R.N., Stradling, P. and Abdel-Wahab, E.M. Serum proteins in pulmonary tuberculosis. *Br Med J* **1** (1956) 1460.
2. Gilliland, I.C., Stradling, P. and Abdel-Wahab, E.M. Serum protein changes following BCG vaccination. *Br Med J* **1** (1958) 87.
3. White, W.L. and Frankel, S. *Seiverd's chemistry for medical technologists,* 2nd ed. (The C.V. Mosby Co., Saint Louis) 1965 p 147.
4. Block, R.J., Durrum, E.L. and Zweig, G. *A manual of paper chromatography and paper electrophoresis,* 2nd ed. (Academic Press, Inc., New York) 1974 p 554.
5. Tuberculosis Chemotherapy Centre, Madras. A controlled comparison of two fully supervised once-weekly regimens in the treatment of newly diagnosed pulmonary tuberculosis. *Tubercle* **54** (1973) 23.
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 **23** (1960) 535.
7. Kushner, I. The phenomenon of acute phase response. *Ann NY Acad Sci* **389** (1982) 39.

Reprint requests : The Director, Tuberculosis Research Centre, Spur Tank Road Chetput, Madras 600031