

## Effect of Antituberculosis Drugs on Levels of Serum Proteins in Pulmonary Tuberculosis Patients

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### Abstract

Serum proteins fraction analysed by the microtech 672 PC, in sera of pulmonary tuberculosis patients. Samples were collected zero day, 15 days, 1 month, 2 months, 3 months, 4 months and 6 months post treatment. Significant increase has been observed in gamma globulin and significant decrease in albumin / Alfa-2 ratio and Beta globulin. Non significant decreases in A/G Ratio seen. A non significant increase have been opbserved in total protein level with respect ot zero days. An attempt was made to correlate changes in the Albumin / Alpha-2 levels in serum of T.B. patients on drug therapy. It was observed that before start of tuberculosis therapy the albumin / Alpha -2 Globulin ratio were low and the gamma globulin levels were high. After post treatment for 1 month, 2 months, 3 months and 4 months significant gradual increase in Albumin/Alpha-2 ratio was observed. After 6 month albumin / Alpha-2 value decrease as compared to control. A gradual significant decreased in gamma globulin level was observed after one month treatment of drug. Which reaches to control level on six months post treatment as improvement occurred. This study is a valuable guide in deciding upon the duration of therapy necessary for individual cases.

**Keywords:** Antituberculosis Drugs, Serum electrophoresis, Serum Protein, gamma globuline, albumin / alpha -2

### Introduction:

Human plasma contains 10 grams of solids in deciliter in soluble form of with approximately 76% are proteins. Serum protein consists of albumin and globulins and its level in normal subjects vary from 6.3 – 8.9 g/dl (1). Normal range of albumin is 3.8 – 4.8 g/dl,  $\alpha$ -1 0.24 – 0.5 g/dl,  $\alpha$ -2 is 0.5 – 0.75 g/dl,  $\beta$  is 0.58 – 1.10 g/dl and  $\gamma$ -globulin vary from 0.9 – 1.15 g/dl (2). Serum proteins perform many functions in the body. Due to their various kinds of functions, metabolism and site of origin, serum albumin and globulin are subjected to different influences and hence their concentration varies which is independent of one another. Alternations have been described in hepatic, renal, acute and chronic infectious diseases like tuberculosis (3).

Tuberculosis is a communicable disease which is found world wide and is caused by *Mycobacterium tuberculosis*, which usually affect the lungs but may also cause lesions in other organs or tissues of the human body. Tuberculosis of lungs is the commonest of all forms of tuberculosis as the

lungs are more commonly affected by tuberculosis than any other organ, partly because inhalation is the commonest mode of infection and partly because lung tissue provides a favorable environment for the growth of the organism. The specialists and the general practitioners usually give a progress of the disease mainly based on the level of ESR, which depends only on fibrinogen level of the blood. In many cases, this value is normal initially thus giving a false diagnosis (4). It is well established that changes in levels of serum protein occur in response to both acute and chronic infections. In air infection like mycobacterium tuberculosis, it is to be expected that changes in plasma protein levels will occur in patients. However, the change in level of each protein at any particular time should reflect the net effects of both the rate of synthesis and rate of catabolism as a result of host microbe interactions (5). In chronic infectious diseases like tuberculosis, the albumin content of serum proteins shows a decrease while the globulin content shows an increases leading to low albumin to globulin (A/G)

and albumin to Alpha2 Globulin (A/ Alpha2) ratios (6,7). Ewerbeck (8) and Knfichel and Kinele (9) studies showed that as the disease progressed, there were increase in the alpha and gamma globulins with corresponding decrease in the albumin, and it was suggested that increase in gamma globulin was due to antibody formation. Baldwin and Hand (10) in 1953 found patients with pulmonary Tuberculosis to have elevated Alpha globulins as well as gamma globuline. While the albumin fraction was reduced. The gamma globulin proved to decreases towards normal in the course of treatment. In the present study we have attempted to find out the changes in serum protein fraction in anti-tuberculosis drug treated patient, in comparison with non treated, and healthy controls.

### Material and Methods:

The study was conducted on out-door patients in one of the Semi Govt. Hospital in Mumbai. 50 Patients were examined in respiratory Medicine Unit. Patients of either sex between age group 15 to 60 year with pulmonary tuberculosis were studied for serum protein pattern electrophoretically with respect to duration of treatment. Diagnosis of Tuberculosis (TB) was based on detection of Acid Fast Bacilli (AFB) and direct smear examination by Ziehl Nelson staining and also on culture for AFB (Warke and Khan, 2004) (11). Serum Protein fractions were analyzed by the Microtech 672 PC made in Italy, in Tata memorial Hospital Mumbai Patients were treated with the following antituberculosis drugs. All the drugs were given thrice a week through out for 6 months under the direct supervision of clinical staff.

- 1) Cap. Rifampicin (RIF) on empty stomach 1 Cap. (450 mg).
- 2) Tab. Isoniazid (INH) 2 Tab. (300 mg).
- 3) Tab. Ethambutol (ETH) 2 Tab. (600 mg).
- 4) Tab. Pyrazinamide (PZA) 2 Tab. (750 mg).

All drugs were given for two months. After two months tablets ethambutol and tablet Pyrazinamide were cease but treatment continued with Cap. Rifampicin and tab. Isoniazid for a period of further four months. Blood samples of all patients were collected on zero day (before start of treatment), 15 days, 1 month, 2 month, 3 month, 4 month and 6 month post treatment period. Approval from Institutional Ethics committee for collection of samples for research work was obtained.

### Sample Collection :

Venous blood samples were collected in plain bulb and serum was separated.

### Patients Selection Criteria :

#### Inclusion Criteria

- 1) Patients of either sex in the age group 15-60 years.
- 2) New cases of Pulmonary T.B. as diagnosed by using Revised National Tuberculosis Control Programme (RNTCP) diagnostic algorithm.

#### Exclusion Criteria

- 1) Retreatment cases of Pulmonary T.B. relapse, failure, treatment after defaulter with Category II.
- 2) Sputum smear negative for pulmonary T.B.
- 3) Patients suffering from active liver disease.
- 4) Patients suffering from severe renal cardiac disease admitted in ICU of the Hospital.
- 5) Patients suffering from other associated pulmonary diseases.
- 6) Pregnant and lactating female.
- 7) Patients suffering from HIV

#### Selection of Control

- 1) Healthy 5 volunteers of both sex in the age group of 15-60 years.
- 2) On the basis of following Lab investigations within the normal range;
  - a) Hb, CBC, ESR
  - b) LFT, AST, ALT.S. Bilirubin
  - c) BUN and Serum Creatinin

Protein fraction analysed by the microtech 672 pc Including sample application. Migration staining, destaining, clearing drying, scanning and densitometer in a fully automated process. Manufactures Interlab model microtech 672 pc made in Italy.

**Support medium:** Cellulose Acetate on mylar.

**Reagents:** Protein Electrophoresis kit REF SRE 147 K. was used. All reagents were ready to use

#### Procedure:

- 1) Sample plate was clean and dry.
- 2) Reagent level in Tanks
- 3) Blotting paper positions – migration chamber, sample holder, tank no-19 Next to migration chamber.
- 4) Migration buffer level in migration chamber.
- 5) Sample Application was in proper position.

#### Analysis:

Add 30 micro liter serum samples to the sample plate. Add 2 ml of Distilled water in each row of the sample plate. Keep the sample plate in the slot.

### Strip Placement:

The strip should be placed in the corresponding rows as sample. If samples are added in "Row" 1 then the strip should be placed in the "holder" No.1 in the "Row -1 of reagent tank position facing front side.

The strip surface with "lot no" is the upper side.

Starting Run :- ( Microtech 672 pc operation procedure) (12)

After the initialization machine is ready for Analysed Information Entry :- Total protein gm/dl to enter T.P value. The total serum protein content was determined by the Biuret Method (13). Date, graph, reading, band appears

### Results and Discussion:-

Table No. 1 show the effect of antituberculosis drugs on serum, total protein, Albumin, Alfa-1, Alfa-2. Beeta-1, Beeta-2, Betta, Gamma globulins, Albumin/Alfa-2 globulin ratio, Albumin/Globulin ratio. These are the markers for TB disease.

#### Total protein :-

In non-treated T.B. patients was elevated marginally as compared to control group (6% high) and 1 month post-treatment periods gradually (7% and 14%). However it start slow decline from 2 month through 3, 4 and upto 6 month post treatment period. The value comes altogether equal to control on 6 month post treatment period (10%, 10%, 7%).

#### Albumin :-

In control group of subject mean value of albumin was found to be 4.24 gm% which significantly decrease in pretreated (zero days) T.B. patients to  $3.58 \pm 0.572$ . Thus mean decrease in albumin in T.B. patients with respect to control in 16%. The values slightly increases on treatment with T.B. drugs and it reaches to  $3.82 \pm 0.39$  on 15<sup>th</sup> day post treatment, the magnitude of increase being 10% as compared to control.

Afterward the post treatment values significantly increases from 1 month, 2 months, 3 months and 4 months upto 6 months as  $4.55 \pm 0.75$ ,  $4.52 \pm 0.58$ ,  $4.32 \pm 0.72$ ,  $4.52 \pm 0.85$  and  $4.35 \pm 0.48$  respectively. The percent increase being 7.5%, 6.3% and 2.3% respectively with respect to control. On 6<sup>th</sup> month the value was only 2.3% high with respect to control.

#### $\alpha$ -1 Globulin :-

The mean value of  $\alpha$ -1 protein in control group was found to be 0.38 gm/dl which decreases to  $0.24 \pm 0.078$  in pulmonary tuberculosis patients (Pretreated group). After post treatment, values decreases from 15 days, 1 month. 2 months and 3 months as  $0.22 \pm 0.048$ ,  $0.14 \pm 0.053$ ,  $0.21 \pm 0.069$  and  $0.15 \pm 0.07$  corresponding to 47.8%, 44.8%, 45.2% and 61.4% respectively.

After post treatment values further increases on 4 month and 6 month as  $0.61 \pm 1.10$  and  $0.62 \pm 0.29$  respectively. The value of 6 month post treatment period in significantly higher as compared to control group.

#### $\alpha$ -2 Globulin :-

The healthy subjects of control group showed mean value of  $\alpha$ -2 globulin as 0.62 gm/dl which slightly increases in pretreated pulmonary T.B. group. The value being  $0.64 \pm 0.65$ .

15 days after treatment of anti T.B. drugs, the value further increases to  $0.78 \pm 0.33$  which is higher as compared to control group of patients. After treating for 1 month and 2 month, the value in patients become  $0.75 \pm 0.16$  and  $0.70 \pm 0.21$  respectively. The lowest value was observed on 3 month of  $0.57 \pm 0.28$  which significantly increases on 4 and 6 month as  $0.67 \pm 0.32$  and  $0.87 \pm 0.13$  respectively.

#### $\beta$ -1 and $\beta$ -2 Globulins :-

Similar pattern has been observed for  $\beta$ -1 and  $\beta$ -2 globulins. In control group of healthy subjects, the mean value for  $\beta$ -1 globulin was 0.28 g/dl and for  $\beta$ -2 0.68 g/dl. In pulmonary T.B. patients before treatment,  $\beta$ -1 value increase to  $0.60 \pm 0.55$  whereas  $\beta$ -1 was slightly decreases in pulmonary T.B., the value being  $0.40 \pm 0.41$ .

After post treatment of 15 days, 1 month and 2 month  $\beta$ -1 value decreases to  $0.11 \pm 0.30$ ,  $0.14 \pm 0.29$  and  $0.13 \pm 0.22$  as compared to control group, while  $\beta$ -2 globulin decreases slightly after 15 days upto six month as compared to control group.

Increase in  $\beta$ -1 value has been observed in 3 month post treatment, the magnitude being  $0.78 \pm 1.77$ , while on 4 month and 6 month post treated period it decreases and come towards normal.

#### $\beta$ - Globulins :-

In control group of healthy subjects mean value was 0.76 g/dl which was greater as compared to pretreated T.B. patients, value being  $0.24 \pm 0.43$  g/dl.

From 15 days to 1 month, 2 months, 3 months and 4 months post treatment period a significant gradual decrease in  $\beta$ -globulin was observed. On sixth month post treatment period  $\beta$ -globulin value was high as compared to control group.

#### $\gamma$ -Globulin :-

In control group of healthy subjects mean value of  $\gamma$ -globulin was found to be 1.06 mg/dl which significantly increases in pretreated T.B. patient to  $2.74 \pm 0.43$  which was 74% higher than control. The value decreases on treatment with T.B. drugs and it reaches to  $2.34 \pm 1.11$  on 15 days. The magnitude of increase being 20% higher as compared to control. After one month treatment of T.B. drugs till six months significantly gradual decrease in  $\gamma$ -globulin

observed which reaches to normal level on six month post treatment.

#### **A/G Ratio:-**

In control group of healthy subject mean value of A/G ratio was 1.23 which lower in pretreated pulmonary tuberculosis patients before start of treatment to  $0.758 \pm 0.184$  which was 39% lower as compared to control group.

After post treatments of 15 days, 1 month, 2 months and 3 months a gradual increases in A/G ratio that is  $0.81 \pm 0.145$ ,  $1.01 \pm 0.254$ ,  $1.065 \pm 0.245$  and  $1.108 \pm 0.597$  post treatment. After 4 month and six month A/G ratio value reaches to normal level.

#### **Albumin / $\alpha$ -2 ratio :-**

In control group of healthy subject mean value of ratio was 6.126. In pretreated pulmonary tuberculosis, value was  $5.58 \pm 1.244$  which was lowered as compared to control group.

After post treatment 15 days, 1 month, 2 months, 3 months and 4 months gradual increases in Albumin / $\alpha$ -2 ratio that is  $6.20 \pm 1.651$ ,  $6.13 \pm 1.651$ ,  $6.46 \pm 1.652$ ,  $7.57 \pm 2.63$ ,  $6.735 \pm 1.652$  was observed. After six month Albumin /  $\alpha$ -2 value decreases to  $5.00 \pm 1.242$  as compared to control.

#### **Discussion:-**

Albumin and Globulin are the two main serum proteins both of which carry a negative charge and therefore, migrate towards the anode during electrophoresis. Albumin has the highest rate of migration and gamma globulin the lowest. The other serum proteins Alpha-1, Alpha-2 and Beta-Globulin are located between albumin and gamma globulin according to their relative mobilities. Variations in the fractions occur in some Pathological condition.

From our results it is evident that Albumin, Alpha-1, Beta-2, Beta and Albumin/G and Albumin/ Alpha-2 in a healthy population are higher, as compared to tuberculosis patients while total protein alpha-2, Beta-1, gamma globulin are lower as compared to Tuberculosis patients.

Result show a decrease in albumin concentration, an increase in the levels of total protein, and some of fractions of globulin in pulmonary tuberculosis

patients, which is similar to the observations of other researchers (4, 14, 15). Total serum proteins have been variously reported to increased (4, 6, 16, 17).

Electrophoretic pattern revealed that the increase in total protein was due to the increase in globulins. The decrease in serum albumin of tuberculosis

patients may be attributed to several factors. The loss of appetite in tubercus patients due to endotoxins produced by bacteria and lack of exercise disturb the metabolic process of the patients, which results in low intake of proper diet thus leading to malnutrition. Another possible mechanism which may be at work in infectious disease, such as tuberculosis is that the globulin formation increases in the body as a result of increased immune response where antibodies are produced.

Decrease in the A/G ratio and A/Alpha-2 Ratio (Table 1) found in case of pulmonary tuberculosis. The clinical status is better correlated with A/G ratio and A/Alpha-2 ratio and it is considered to be better criterion of the severity of the disease process. We have also observed A/Alpha-2 globulin ration and A/G fall in tuberculosis. This alteration statistically significant observation is in conformity. Thus, A/G and A/Alpha-2 globulin ratio is better criterion of the severity of the disease process as observed by other workers.

Albumin / Alpha-2 ratio provides a useful objective index for assessing activity of disease in patients with tuberculosis (L.C. Gilliland and R. N. Johnston, 1956) (6).

#### **Conclusion:**

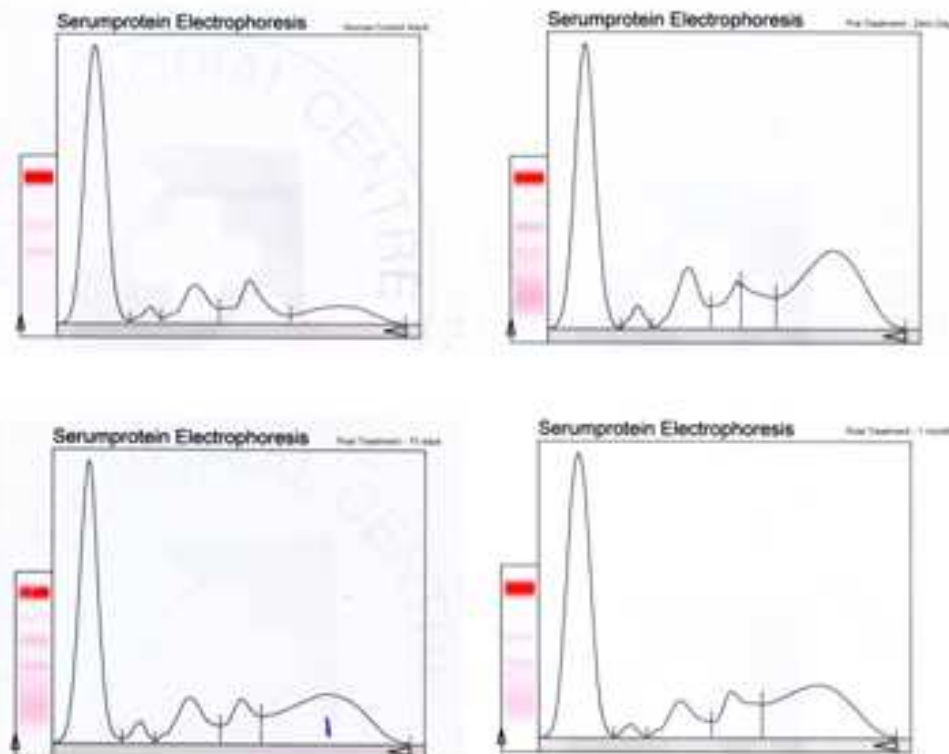
It was observed that before start of tuberculosis therapy the Albumin/ Alpha-2 globulin ratio were low and Gamma globulin level were high.

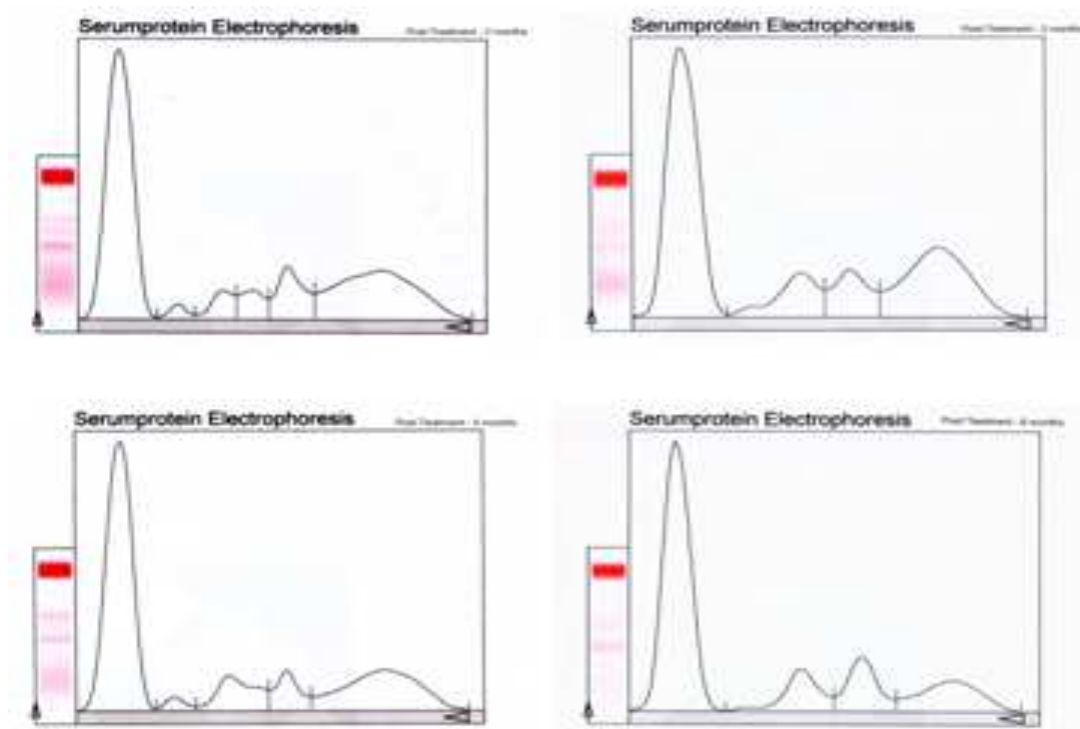
Serum protein changes occurred because of antituberculosis drugs. It was indicated that as the patients recovered there was a gradual significant decreased in Gamma globulin towards the normal value and also after antituberculosis treatment Albumin/Alpha-2 globulin gradually increases toward the normal levels on Six months Post treatment as improvement occurs. This study is valuable guide in deciding upon the duration of therapy necessary for individual case.

**Table 1: Effect of antituberculosis drugs on serum, total protein, Albumin, Alfa-1, Alfa-2, Beeta-1, Beeta-2, Beta, Gamma globulins, Albumin/Alfa-2 globulin ratio, Albumin/Globulin ratio**

Duration Of Treatment	Total Protein	Albumin	$\alpha$ -1	$\alpha$ -2	$\beta$ -1	$\beta$ -2	$\beta$	$\gamma$	A/G Ratio	Albumin Alfa-2
Control	8.02	4.24	0.38	0.62	0.28	0.68	0.76	1.06	1.23	6.126
Zero days	8.557 ± 0.439	3.585 ± 0.572	0.242 ± 0.078	0.642 ± 0.650	0.60 ± 0.556	0.40 ± 0.416	0.242 ± 0.415	2.742 ± 0.431	0.758 ± 0.184	5.58 ± 1.244
P value	0.55	0.04	0.20	0.80	0.48	0.91	0.03	0.04	0.13	0.03
15 days	8.642 ± 0.427	3.828 ± 0.390	0.228 ± 0.048	0.785 ± 0.333	0.114 ± 0.302	0.342 ± 0.907	1.00 ± 0.660	2.342 ± 1.111	0.81 ± 0.145	6.20 ± 1.651
1 month	9.157 ± 0.745	4.557 ± 0.750	0.142 ± 0.053	0.742 ± 0.161	0.142 ± 0.299	0.242 ± 0.415	0.657 ± 0.461	2.671 ± 0.612	1.01 ± 0.254	6.13 ± 1.651
2 month	8.9 ± 0.860	4.528 ± 0.587	0.214 ± 0.069	0.70 ± 0.216	0.13 ± 0.220	0.328 ± 0.427	0.485 ± 0.459	2.5 ± 0.616	1.065 ± 0.245	6.46 ± 1.652
3 month	8.9 ± 1.89	4.328 ± 0.722	0.157 ± 0.078	0.571 ± 0.287	0.785 ± 1.774	0.371 ± 0.386	0.385 ± 0.484	1.942 ± 1.370	1.108 ± 0.597	7.57 ± 2.63
4 month	8.628 ± 1.025	4.528 ± 0.853	0.614 ± 1.100	0.671 ± 0.325	0.057 ± 0.151	0.114 ± 0.302	0.585 ± 0.285	2.057 ± 0.756	1.235 ± 0.853	6.735 ± 1.652
6 month	8.114 ± 0.456	4.357 ± 0.482	0.628 ± 0.292	0.871 ± 0.138	0.157 ± 0.415	0.314 ± 0.831	1.371 ± 0.999	0.414 ± 0.744	1.251 ± 0.452	5.00 ± 1.242

**Fig.1: Serum Protein Electrophoretic Pattern in Anti T. B. drugs Therapy in T. B. Patients**





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