Original Research Article

Plasma Protein Patterns in Pulmonary Tuberculosis Using Protein Electrophoresis

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Abstract: Serum protein was analyzed by electrophoresis and photometric method in sera of adults' patients with pulmonary tuberculosis and adults healthy individuals. Results were found to be as follows: A) The mean values of the different patterns in the patients group are as follows; total proteins (7.87g/dl, SD ± 1.18), albumin (3.49 g/dl, SD ± 0.80), α-1(0.63g/dl, SD ± 0.26), α-2 (1.84 g/dl, SD ± 0.564), β-1 (0.86 g/dl, SD ± 0.36), β-2 (0.70 g/dl, SD ± 0.47), and γ-globulins (0.97 g/dl, SD ± 0.43). B) The mean values of the different patterns in the healthy group are as follows; total proteins (7.290 g/dl, SD ± 0.61), albumin (4 g/dl, SD ± 0.54), α-1 (0.42 g/dl, SD ± 1.06), α-2 (1.26 g/dl, SD ± 0.46), β-1 (0.94 g/dl, SD ± 1.02), β-2 (0.57 g/dl, SD ± 0.18), and γ- globulins (0.84 g/dl, SD ± 0.48). It has been observed that there is a significant difference in total proteins, albumin, and α- 2 fractions. There are no changes in the rest of the patterns. In pulmonary tuberculosis, in spite of the lowered serum albumin, total protein level is maintained by a corresponding increase in the level of globulins resulting in the reversal of albumin/globulin ratio.

Keywords: electrophoresis, pulmonary tuberculosis.

INTRODUCTION

Tuberculosis is a contagious bacterial infection that is transmitted through the air, and can spread through the lymph nodes and bloodstream to any organ in the body. It is most often found in the lungs.

Because of the inactive living form in the body, the disease is symptomless in most TB patients. If the immune system weakens, (in HIV patients or elderly adults), TB bacteria can become active. Active TB bacteria are able to spread the disease to others. The disease cause death of tissue in the infected organs, and can be fatal if left untreated.

Plasma proteins

Albumin is a soluble, monomeric protein which comprises about one-half of the blood serum protein. It is synthesized in the liver at a capacity of approximately 120 mg/kg daily and has a half life of approximately 15 to 19 days. When serum albumin falls the synthetic rate can be almost doubled [1]**.**

Albumin is globular protein of molecular weight 65,000, having a roughly spherical structure. It functions primarily as a carrier for steroids, fatty acids, and thyroid hormones and plays a role in stabilizing extracellular fluid volume. Mutations in the ALB gene on chromosome 4 result in various anomalous proteins [2]. Disease can alter albumin by altering synthesis, increasing degradation, or by extravascular loss [3]**.**

The serum albumin level is considered a reliable marker for determining the severity of liver disease and their prognosis. It is also a good marker in follow-up of patients with liver disease to determine the effectiveness of therapy [4].

Over-production of albumin does not occur. A decreased amount of serum albumin may mean decreased liver production, or increased loss of this protein via hemorrhage, or via loss into the urinary or gastrointestinal tracts [5].

The alpha (α) and beta (β) globulin fractions are also predominantly produced in the liver. These fractions include proteins involved in the normal inflammatory response (acute phase proteins), proteins involved in lipids and iron transport. Significant increases in these fractions are seen primarily with inflammation, while decreases are mainly noted with liver disease (decreased production) [6].

Immunoglobulin (antibodies) produced in response to material or organisms that are foreign to the

body, are found in the γ globulin fraction [7]. Increases in (chronic infections, immune-mediated disease processes, some cancers and certain viral diseases), or as narrow-based increases that are supportive of underlying cancer of the immune system (lymphoma and multiple myeloma) as well as some infectious conditions [8].

Decreases in the γ globulin fraction are unusual and are typically seen in very young infants who may have not received sufficient colostrums (the first milk containing antibodies) from their mothers [9]. As a rule, these protein fractions do not change in isolation. For example, if there is inflammation of the liver, the albumin fraction may be decreased while the beta and γ globulin fractions are increased. Therefore it is important to evaluate the overall pattern of change and relate these findings to human symptoms and other laboratory data [10].

Components of Serum Protein Electrophoresis
Albumin
Albumin comprises from 55.8 % to 65% of the total serum protein concentration in human. The liver is the site of albumin synthesis [11].

The Globulins
Albumin – α 1- Interzone
Staining in this zone is due to α 1–lipoprotein (high density lipoprotein), A decrease may extend into the protein during puberty. Sharp band in α1– inter-zone may be a result of high levels of α –fetoprotein (AFP) from hepato-cellular carcinoma [12].

α1 –Zone
AFP, α 1- glycoprotein, thyroid-binding globulin, and transcortin are the constituents of α1-protein fraction. A decrease in this band is seen in deficiency states such as AFP deficiency, nephrotic syndrome and liver cirrhosis. Free light chains (Bence Jones protein), may bind to and retard the α1- band and thus lead to a decreased α 1- zone, whereas acute-phase reactants as in other malignancies or during inflammatory responses may increase the α 1– protein band [13].

α 1– α 2– Interzone
Two faint bands may be seen representing α 1–antichymotrypsin (an acute-phase protein) and vitamin D binding protein. These bands fuse and intensify in early inflammation due to an increase in α 1 –anti-chymotrypsin [14].

α 2 –Zone
Ceruloplasmin, α 2 –macroglobulin, and haptoglobin contribute to α 2 –protein band. The 2 –zone is decreased in hemolytic anemia when haptoglobin binds with free hemoglobin and rapidly removed by phagocytes. As part of the acute-phase response haptoglobin may also be elevated, especially during inflammation [15].

Higher levels of α 2 –macroglobulin are shown in children, elderly people and may present a sharp front to the α 2- band. The α 2 –band may be raised in liver cirrhosis, diabetes mellitus, and malignant tumors **[16].**

α 2 – Interzone
High levels of pre- α 2- lipoprotein are found in Frederickson type II hyper-cholesterolemia and in nephritic syndrome (large size).

β –Fraction
Higher resolution techniques separate the β–fraction into a β 1 –band and a β 2 –band. Transferrin comprises the β 1- band. An increase of β 1 –proteins is seen in iron-deficiency anemia due to elevated levels of free transferrin, pregnancy and estrogen therapy. Complement protein 3 (C3) and β– lipoprotein form the β 2– band. IgA, IgM, and sometimes IgG also can be identified in the β2- fraction.

γ- Fraction
Most of the γ –band is made up by the various immunoglobulin of the classes (IgG, IgA, IgM, IgD and IgE), but they can also be found in the β – γ – and β – globulin area.

The γ- zone is decreased in agamma globulinemia and hypo-gammaglobulinemia syndromes (e.g. IgA deficiency, newborns).

γ –globulins is increased in malignant lymphoma (multiple myeloma (IgA and IgG paraproteinemias), Waldenström's macroglobulinemia, Hodgkin's disease, chronic lymphocytic leukemia (usually IgM), chronic infections, liver cirrhosis, amyloidosis, and rheumatological, granulomatous and connective tissue disorders (e.g. rheumatoid arthritis, systemic lupus erythematosus). Conditions that cause an increase in the γ –region, with a homogenous spike-like peak in the focal region of the γ – globulin zone are of special interest. These are called monoclonal gammopathies [17]**.** In Waldenström's macroglobulinemia, only IgM para proteins can be found [18].

Rationale
Electrophoresis is important in evaluating the overall pattern of change in one single test. Relatively little is known about the procedure and interpretation of results of electrophoresis in Sudan. Training in electrophoresis will indeed extend our experience in this field, allowing most institutions to carry on the same procedure. Performing electrophoresis locally

will decrease the cost of investigation compared to the high cost when doing it outside the Sudan.

General objective
To study the serum protein patterns in Sudanese adults with endemic diseases (pulmonary tuberculosis), using high resolution electrophoresis.

Specific objectives
1- To introduce the high resolution protein electrophoresis technique in Sudan. This will significantly improve the chance of resolving a complex mixture of proteins into individual constituents, and so improve diagnosis.
1. To provide new information (our own data) on the plasma protein patterns in Sudan, and to compare the results with those obtained from some countries.
2. To determine the impact of socio-economic status and some diseases (mainly endemic) on the plasma protein concentration and fractions.
3. To evaluate the performance of the test regarding diagnostic and prognostic parameters in plasma protein abnormalities (paraproteins).

MATERIALS AND METHODS
Study Design
Retrospective, cross- sectional, case control study.

Study Area
This study had been conducted in Sudan in two different areas including; Khartoum, and New Halfa.

Study Duration
From July 2004 up to 2010

The Study Groups
A. Patients group (Pulmonary tuberculosis)
Sample size is 31 adults with pulmonary tuberculosis attending Khartoum North Hospital in Khartoum and New Halfa. Selection of patients was based on the microscopy for acid fast bacillus in two sputum samples.

B. The control group (Healthy individuals)
Sample size is 41 healthy individuals. We excluded individuals with diseases, or taking drugs known to affect serum protein levels or electrophoretic patterns. Among drugs known to affect serum protein levels or electrophoretic patterns are; Phenytoin (Dilantin), procainamide, oral contraceptives, methadone, therapeutic gamma globulin, aspirin, bicarbonates, chlorpromazine, corticosteroids, and neomycin.

Laboratory analysis
Five milliliters of blood samples were collected from each individual, sera were separated and analysed immediately. Serum protein electrophoresis (SPE) for the participants and reference sera was undertaken using the Biotec Fischer Protein kit on the Filipo system (Biotec Fischer W. Germany).

Serum Total Protein
Serum total protein concentrations were measured calorimetrically using the biuret reaction, on BA Semi- Auto chemistry analyzer results were compared with the published reference range.

Statistical analysis
The data were presented as mean $\pm$ SD. Statistical analysis was performed by the statistical software SPSS 13 using ANOVA test. $P < 0.05$ was considered statistically significant

RESULTS
Patients
The mean values of the different patterns in this group are as follows; total proteins(7.87g/dl, SD $\pm$ 1.18), albumin(3.49 g/dl, SD $\pm$ 0.80), α-1(0.63g/dl, SD $\pm$ 0.26), α-2(1.84 g/dl, SD $\pm$ 0.564), β-1(0.86 g/dl, SD $\pm$ 0.36), β-2(0.70 g/dl, SD $\pm$ 0.47), and γ- globulins(0.97 g/dl, SD $\pm$ 0.43).

Table 1: The mean values of the different protein patterns of patients

Prot fractions	No	Mini (g/dl)	Max (g/dl)	Mean (g/dl)	SD
TP	31	6.0	10.8	7.87	1.18
Alb	31	2.2	5.3	3.49	0.80
α1	31	0.1	1.3	0.63	0.26
α2	30	0.3	3.0	1.84	0.56
β1	31	0.3	1.9	0.86	0.36
β2	4	0.4	1.4	0.70	0.47
γ	31	0.2	2.0	0.97	0.43

Control
The mean values of the different patterns in this group are as follows; total proteins(7.290 g/dl, SD $\pm$ 0.61), albumin(4 g/dl, SD $\pm$ 0.54), α-1(0.42 g/dl, SD $\pm$ 1.06), α-2(1.26 g/dl, SD $\pm$ 0.46), β-1(0.94 g/dl, SD $\pm$ 1.02), β-2(0.57 g/dl, SD $\pm$ 0.18), and γ- globulins(0.84 g/dl, SD $\pm$ 0.48).

Table 2: The mean values of the different protein patterns of the control group

Protein fractions	No	Mini (g/dl)	Max (g/dl)	Mean (g/dl)	SD
TP	41	5.9	8.3	7.290	0.61
Alb	41	3.1	5.2	4.01	0.54
α1	41	0.1	0.7	0.42	1.06
α2	41	0.4	2.1	1.26	0.46
β1	41	0.2	1.6	0.94	1.02
β2	7	0.3	0.8	0.57	0.18
γ	41	0.2	1.8	0.84	0.48

It has been observed that there is a significant difference in total proteins, albumin, and α- 2 fractions. There are no changes in the rest of the patterns.

DISSCUSION

The reduction in serum albumin levels in this study is in agreement with a study in India by Ramakrishnan *et al* [20] who found that, serum albumin levels were significantly reduced in patients with PTB. The possible causes for the low serum albumin and zinc in PTB patients were considered to be nutritional factors, enteropathy and acute phase reactant proteins (APRP).

The hepatic synthesis of APRP is induced by cytokines such as IL-6 and TNF-α [19], which inhibit the production of serum albumin and cause dramatic shifts in the plasma concentration of certain essential micronutrients and albumin.

Since alpha-1 acid glycoprotein (AGP) is increased in pulmonary tuberculosis [21], the significant increase in alpha-1 zone in this study may be due to increase in AGP.

AGP has a high affinity, low capacity binding for basic drugs positively charged at physiological PH. As an acute phase protein its level is increased in various disease states in a manner that is likely to influence the free plasma level of a drug, the ability to attain minimum effective concentration and overall in vivo effectiveness. AGP is a glycoprotein known to display disease specific changes in glycosylation and although this secondary modification is not directly involved in drug binding, it may influence the conformation of the binding site.

Previous studies revealed that α-1-acid glycoprotein binds mainly to the tuberculosis drugs: rifampicin, isoniazid, pyrazinamide, p-amino salicylic acid, capreomycin, ethionamide, levofloxacin and ofloxacin out with the therapeutic plasma range tested.

In spite of lowered serum albumin in this group, total protein level is maintained by a corresponding increase in the level of globulins resulting in the reversal of albumin/globulin ratio.

Albumin/alpha-2 globulin ratio is 1.9 in this study. It is significantly lower than that of the control ratio (mean = 3.2). This finding is in agreement with that of K. L. Agrawal *et al* [22] their studies showed that, the clinical status is better correlated with albumin/alpha-2 globulin ratio and it was considered to be a better criterion of the severity of the disease process.

It was shown by Freigang [23] that, in 85% of the patients with pulmonary tuberculosis and in 100% of those with non-pulmonary tuberculosis, direct correlation was established between clinical progress and the albumin/alpha-2 globulin ratios. Also they found that, an increase in the albumin/alpha-2 globulin ratios indicated a favorable prognosis, whereas a fall in this ratio pointed to a poor outcome, and the E.S.R. value cannot be correlated with the protein fractions and the severity of the disease.

CONCLUSION AND RECOMMENDATIONS
Conclusion

In pulmonary tuberculosis, in spite of the lowered serum albumin, total protein level is maintained by a corresponding increase in the level of globulins resulting in the reversal of albumin/globulin ratio.

Recommendations

Serum protein electrophoresis is inexpensive and widely available in hospitals and clinics outside the Sudan.

In Sudan electrophoresis is commonly used to monitor patients with multiple myeloma.

Serum protein electrophoresis is the accepted method for monitoring the clinical course of multiple myeloma, and other diseases caused by the abnormalities in one or more of the different patterns of the serum protein.

Serum protein electrophoresis is not often used in the screening of diseases other than multiple myeloma in Sudan, with understanding and knowledge how to interpret results; this will change in the future.

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