DIAGNOSTIC SIGNIFICANCE OF SERUM PROTEIN ELECTROPHORESIS

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Serum protein electrophoresis is a simple, reliable and specific method of separating different protein fractions. A study was carried out in 1556 symptomatic cases reported to Department of Chemical Pathology, Army Medical College for serum protein electrophoresis. Aims and objectives were to see the diagnostic significance of protein electrophoresis especially in comparison to other related investigations like serum protein estimation and albumin: globulin (A:G) ratio etc. Deluxe electrophoresis chamber and sample applicator were used. Gelman DCD-16 digital computing densitometer was used for quantification. Results revealed that protein electrophoresis was essential for diseases like paraproteinaemia, immunodeficiencies and α_1-antitrypsin deficiency. It is helpful along with other investigations in liver disease, nephrotic syndrome, collagen disease and malignancy. Three hundred and sixty five cases had subacute/ chronic infection, 340 had normal electrophoretic pattern, 250 cases were of congestive cardiac failure, 152 cases of nephrotic syndrome, 90 cases of hepatic cirrhosis, 53 cases of chronic renal failure and 25 cases of paraproteinaemia were identified. Its sensitivity and specificity were more than serum protein estimation by dye methods. It is recommended that full use of this diagnostic technique should be made for better diagnostic sensitivity and specificity.

INTRODUCTION

Electrophoresis is a technique for separation of different charged particles. It is based on movement of charged particles through a solution when subjected to an electrical field[1]. Since proteins are negatively charged at pH 8.6, subjecting them to electrical field will push protein particles towards anode. Weight and charge on different protein fractions separates them out into albumin (molecular wt 69000), α_1 and α_2-globulin (molecular wt 140,000), β and γ-globulin[2]. Electrophoresis initially started as moving boundary electrophoresis in which boundaries of medium used to move to separate protein fractions. Later on it was improved in the form of zone electrophoresis in which electrophoretic support medium was static and after application of serum sample, different protein particles used to migrate on it[3]. Various support media are used in electrophoresis depending upon the type of electrophoresis e.g. serum proteins, enzymes, lipoproteins etc[4]. It includes paper, cellulose acetate, starch gel, agarose gel and polyacrylamide gel. Factors affecting mobility include size and shape of particles, ionic strength of solution, viscosity and temperature of the medium[5].

Separation of serum protein fractions is very important for the diagnosis of different diseases like paraproteinaemias, haemoglobinopathies, immune deficiency and genetic abnormalities. It is also helpful along with other investigations in

chronic liver disease, malignancies and collagen disease. In multiple myeloma it is also helpful in monitoring the treatment[6].

The purpose of this study is to assess the diagnostic significance of serum protein electrophoresis. The study is also aimed at comparing protein electrophoresis with other serum protein investigations like total and differential protein estimation spectrophotometrically.

SUBJECTS AND METHODS

One thousand five hundred and fifty six cases reporting to chemical pathology laboratory of Army Medical College were selected for the study. Symptomatic cases without a prior definite laboratory diagnosis of their disease were included in the study. Cases on intravenous infusion of aminoacids were excluded from the study. Their detailed history was taken and clinical examination was carried out. Blood was drawn in plain tubes. Sera were separated immediately and stored. Electrophoresis was run in batches on Deluxe electrophoresis chamber. Sera were applied with the help of wire loop applicator. Cellulose acetate membrane was used as a support medium. Barbitone buffer was set at pH 8.6. It was prepared by adding sodium barbitone 25.4 g, barbitone 4.6 g and diethyl barbituric acid 4.6 g in 2.5 L of distilled water. Buffer pH was checked with the help of a pH meter before use in electrophoresis. Line of application was directed towards cathode, and 15 amp current was applied at 240 Volts for 30 minutes. Different protein fractions moved depending upon charge, molecular weight and size. Fixation was performed by methyl alcohol 85% and glacial acetic acid 15% for 5 min. Staining was done with the help of Ponceau-S stain 0.3% in 3% acetic acid for10 min, whereas clearing was done with 3% acetic acid for 20 min. Strips were dried in hot air oven and densitometric scanning was done for quantification of different protein fractions[7].

Serum total protein, albumin and globulin estimation were carried out spectrophotometrically, based on dye binding principle[8]. Clinical correlation of the results of electrophoresis was carried out. Comparison of the results of total proteins and albumin by electrophoresis and spectrophotometric analysis was also performed.

RESULTS

Serum protein electrophoresis was run on sera of 1556 symptomatic cases. Out of these 1216 sera revealed some disease thus yielding a diagnostic sensitivity of 78.2%. Three hundred and forty sera (21.8%) of symptomatic patients were inconclusive for any disease. Major diseases diagnosed were subacute/ chronic infections (365 cases), cardiac lesions (250 cases), nephrotic syndrome (152 cases), malnutrition (142 cases), cirrhosis liver (90 cases), chronic renal failure (53 cases), acute infections (60 cases) and paraproteinaemias in 25 cases as shown in Fig-1. Miscellaneous cases revealed autoimmune haemolytic anaemia (09 cases), burns/eczema (11 cases), neoplasms (13 cases), immune deficiency (13 cases) and bisalbuminaemia in 9 cases as shown in Fig-2.

Results of serum total proteins and albumin estimation by spectrophotometric analysis revealed broad results of total proteins, albumin and total globulins. They revealed hypo-albuminaemia (310 cases) or hyperglobinaemia (119 cases). These results were less diagnostic as compared to serum protein electrophoresis. Hypoalbuminaemia was due to nephrotic syndrome, cirrhosis liver, malnutrition and acute chronic infections or malignancy etc. Hyperglobinaemia revealed by spectrophotometry was different from that revealed by electrophoresis. Some of the difficulties observed were on visual inspection of electrophoretic strips, included variations in staining, variations in sample volume, unsuitability of control samples and interpretation of dense albumin band. In nephrotic cases mean albumin was 19 g/L and mean α_2-globulin was 12.5 g/L.

In 60% of cases of monoclonal gammopathy other unexpected conditions brought them to the attention of medical personel. Mean concentration of γ-globulin region in myeloma cases was 28 g/L. Electrophoretic pattern emerging in some of the common diseases is shown in Fig-3. Electrophoretic pattern of myeloma diseases is shown in fig-4

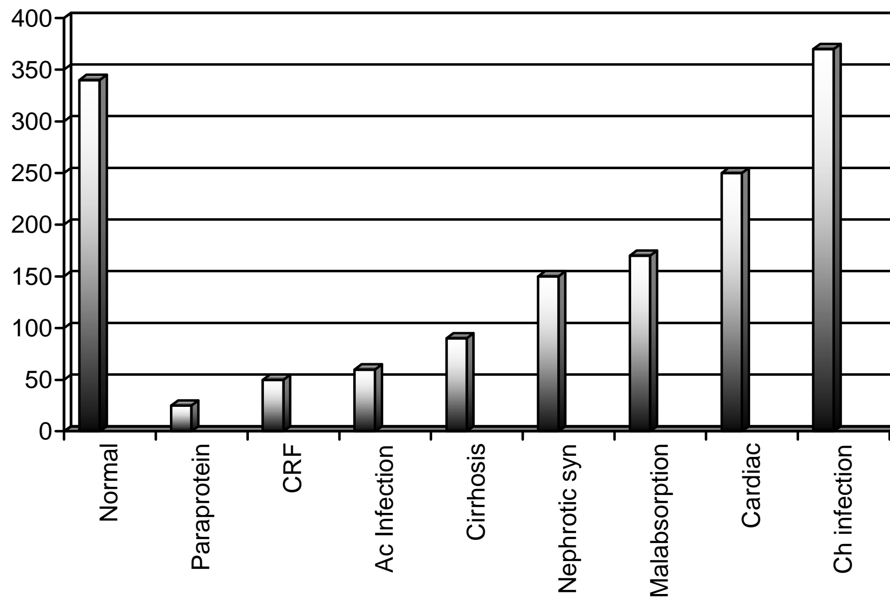

Fig 1: Major diseases diagnosed by serum protein electrophoresis (n=1503).

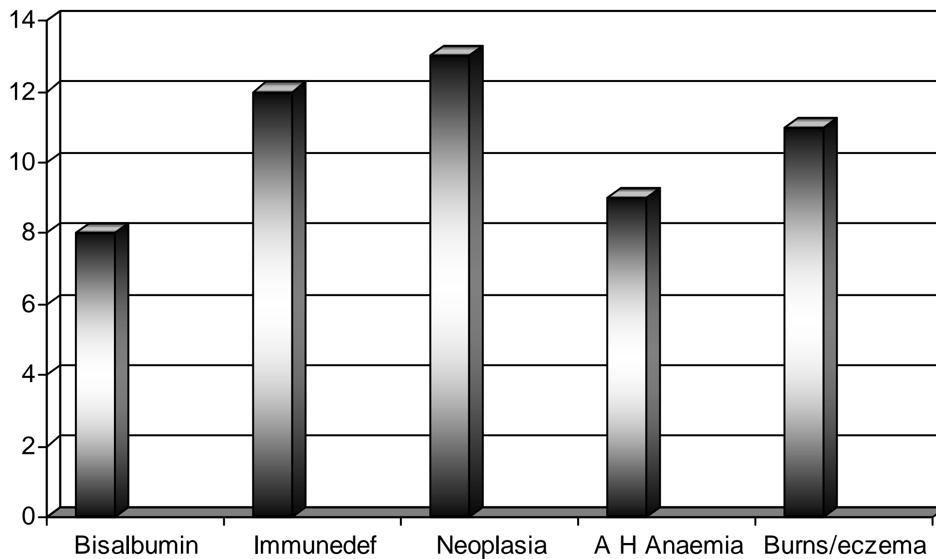

Fig 2: Miscellaneous diseases diagnosed by serum protein electrophoresis (n = 53).

albumin, α-1, α-2, β, γ-globulin

+ve **-ve**

Normal

Nephrotic syndrome

Cirrhosis liver

Multiple myeloma

Bisalbuminaemia

Fig 3: Electrophoretic pattern of different diseases.

albumin,α-1,α-2 ,β, γ-globulin

+ve **-ve**

Fig 4: Electrophoretic pattern of myeloma.

Key: Normal controls on the top, different types of myeloma pattern (M- band) as indicated by the arrow.

DISCUSSION

Advent of serum protein electrophoresis has greatly enhanced diagnostic accuracy in different disease processes. Initially started tests of total protein and albumin estimation by dye binding technique has just become a screening procedure. Electrophoresis has proved to be very useful in subfractioning different types of proteins. Although an absolute indication in gammopathies and haemoglobinopathies, it has turned out to be a very useful diagnostic tool. Its main significance is in the diagnosis and monitoring of monoclonal or polyclonal gammopathies. Acute and chronic infections could be very easily differentiated by raised gamma globulin without raised α_1 and α_2-globulins and usually low albumin in chronic infections[9]. In acute infections, there is an acute phase reaction, with raised α_2-globulins, without polyclonal increase in γ-globulins[10].

Electrophoresis proved very useful when routine investigations were inconclusive. Patients usually presented with fever, low back pain, generalised malaise, easy fatigability or pathological fractures. Raised erythrocyte sedimentation rate, hypercalcaemia, unexplained anaemia, proteinuria, renal insufficiency and recurrent infections also necessitate protein electrophoresis[11]. Clinical correlation of the results is essential as in our experience some of the condition like haptoglobulin - haemoglobin complexes, large transferrin band in iron

deficiency anaemia or fibrinogen bands were seen simulating M band of monoclonal gammopathy. Likewise a monoclonal gammopathy at times have appeared as somewhat broad band on cellulose acetate membrane, owing to complexing of monoclonal protein with other plasma components or aggregates of IgG, dimers of IgM or ploymers of IgA[12]. A monoclonal band should be confirmed by immunofixation technique. Urine electrophoresis along with serum electrophoresis is invaluable in cases of proteinuria. Rare cases of α_1-antitrypsin deficiency or bisalbuminaemia were unexpectedly diagnosed by electrophoresis[13]. It is very important to mark the line of application as it may give false appearance of some band in the γ-globulin region. Correct monitoring of buffer pH, voltage, equal quantity of test and control applied and skill of technologist is of prime importance in improving the quality of strips and proper interpretation of results[14].

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