

# Serum Protein Electrophoretic Pattern as a Differential Diagnostic Tool

Dr. George Gborienemi Simeon (Corresponding Author)  
Department of Medical Laboratory, Niger Delta University, Bayelsa State, Nigeria.  
Tel: +234 (0) 8035478018 E-mail: ozunugborie@gmail.com

Anthony Rukari  
Department of Chemical Pathology, University of Port Harcourt Teaching Hospital, Port Harcourt, Nigeria.

## ABSTRACT

To ascertain the constituents of serum proteins in disordered organs, we evaluated these proteins by the zone electrophoresis method. The medium used was cellulose acetate paper with Barbitol (veronal) at pH 8.6 as the buffer. Disease conditions studied were cirrhosis, kwashiorkor, nephritic syndrome, post hepatic obstruction, acute glomerulonephritis, multiple myeloma and rheumatoid arthritis. Results reflect different patterns for each of the disease condition. There was generalized decrease in albumin concentration except for post hepatic obstruction while cirrhosis elucidated two potent markers, a unique Beta( $\beta$ ) - Gamma ( $\gamma$ ) fusion in one case and a high elevation of the gamma which spread to the Beta leaving a gap (juvenile cirrhosis). We quantitated the albumin by the bromocresyl green method and the total protein by the Biuret method to support the electropherogram obtained. Inclusion of serum protein electrophoresis will give integrity to differential diagnosis in organ derangement.

**Keywords:** serum protein, albumin, globulins, electrophoresis, diagnostic.

## INTRODUCTION

Serum proteins perform vagaries of function in the body on account of their variation in chemical constitution which is the amino acid constituents. They also exhibit differing physical properties such as specific gravity, solubility, immunological identity and electrical charge. These properties are exploited in separation methods for proteins. Electrophoresis is a separation method based on their physical properties. The rate of separation in this method depends on factors such as size and shape of the molecule, electrical field strength, net electrical charge, temperature and properties associated with the separating medium<sup>[1]</sup>. Serum protein electrophoretic patterns have been known to depend on fractions of two major types of proteins: Albumin and globulins. Albumin is produced by the liver under normal physiological conditions. The globulins ( $\alpha_1$  antitripsin, acid glycoprotein;  $\alpha_2$  microglobulin, haptoglobin;  $\beta$  hemopexin, transferrin, betalipoprotein complement C<sup>1</sup> 3;  $\gamma$  immunoglobulin A, M, G) have much smaller constituents and the subsets of these proteins and their relative quantity are the main concern in interpretation of protein electrophoresis<sup>[3,4]</sup>. Plasma protein are known to exhibit reasonably predictable changes as a response to acute condition such as burns, chemical injury, inflammation, malignancy and necrosis<sup>[10]</sup>.

The evaluation of serum proteins by method of zone electrophoresis came after the introduction of a separation method by Tiselius<sup>[5]</sup>. The method could not hold sway for long due to its complicated technical approach. The development of filter paper by Consden et al<sup>[6]</sup> possessing good chemical purity and inertness, regular porosity and homogenous thickness which were enduring properties as supporting medium acted as a stimulus in the application of electrophoresis. The use of cellulose acetate strips was introduced by Kohn and Fernberg<sup>[7]</sup>, the membrane being a modified form of paper to which acetyl group has been attached. The use of Barbitol (veronal) buffer at pH 8.6 was recommended by Kohn and Fernberg<sup>[7]</sup> which gave satisfactory separation. Many authors have offered several interesting observations on qualitative changes of migration of specific protein in pathological conditions<sup>[3]</sup>. In this study, the patterns of separation were studied in liver cirrhosis, nephritic syndrome, multiple myeloma, Rheumatoid Arthritis, hepatic obstruction and kwashiorkor.

## MATERIALS AND METHOD

Electrophoresis was carried out on cellulose acetate membranes (78x150mm cellogram 11) in a Shandon Southern Vokam Tank. Serum volume (1  $\mu$ l) was applied by means of applicator 7mm long and spaced 6mm apart on the cathode side of the centre line.

A constant voltage setting of 200-300v set at 10-30mA was applied for 1 hour. Strips of Whatman paper (No. 18) was used as wicks. The membranes were stained with Ponceau S dissolved in an aqueous solution containing trichloroacetic acid and sulphosalicylic acid.

## PROCEDURE

- i. Medium: Cellulose acetate

- ii. Buffer: Barbital Buffer, pH 8.6, prepared by dissolving 1.84g of Diethylbarbituric acid and 10.26g of sodium diethylbarturic acid in 20mls of distilled water and made up to a litre. To facilitate quick dissolution, gentle heat was used with magnetic stirring. Some crystals of thymol was applied as preservative.
- iii. Strip preparation: Strips were carefully floated on buffer allowing buffer to soak in from underside before wetting completely. It was then blotted dry with filter paper. Less shiny side of paper was used.
- iv. Application: Serum was applied on paper using Shandon applicator
- v. Run: Point of application nearest negative (-ve) electrode. Constant voltage setting was 200-300v at 10-30mA. It was run for 1 hour and air dried.
- vi. Staining: Ponceau S was used. 10g of Ponceau S was added to 37.5g of sulphosalicylic acid. This was made up to 500ml with distilled water, staining was done for 3mins.
- vii. Washing: Strips were washed 3 times in 0.9M acetic acid (5% acetic acid) and allowed to air dry.
- viii. Scanning: Strip was scanned and interpreted using a densitometric scanner.

## RESULTS

We investigated six disease conditions: cirrhosis, nephrotic syndrome, multiple myeloma, rheumatoid arthritis, hepatic obstruction and kwashiorkor. The clinicopathological features of these diseases are presented as interpreted from the electropherogram. Each disease sample was run with a control (normal) sample. Figures (1-6) show typical pattern for each of the disease condition.

1. Liver Cirrhosis: We observed an increased in both  $\beta$  and  $\gamma$  globulin with the increase more marked in the gamma region. In two cases there where  $\beta$ - $\gamma$  fusion while in the other, the fusion is missing. Albumin was decreased in both cases.

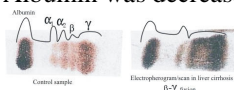


Figure 1 and figure 1a. Electropherogram of Pattern shown in liver Cirrhosis

2. Nephrotic Syndrome: Marked decreased in albumin observed,  $\alpha_1$  and  $\alpha_2$  globulins were also decrease along with  $\gamma$ -globulin.



Figure 2. Electropherogram of pattern shown in nephrotic syndrome

3. Multiple Myeloma: A characteristic finding here is a clone of myeloma protein associated with  $\beta$ -globulin. Albumin was reduced.

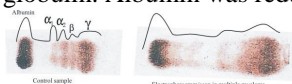


Figure 3. Electropherogram of pattern in Multiple myeloma

4. Acute glomerulonephritis: Decrease in albumin was well marked. The globulin were also affected as they show moderate decrease.

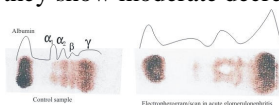


Figure 4. Electropherogram of Acute Glomerulonephritis

5. Rheumatoid Arthritis: Here there was a small to moderate increase in  $\gamma$ -globulin. The increase in  $\alpha_2$ -globulin is marked.

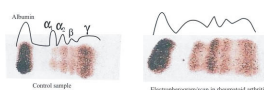


Figure 5: Electropherogram of Rheumatoid Arthritis

6. Kwashiorkor: Here there was uniform decrease in all fractions when compared to the control reform sample.

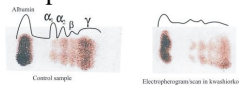


Figure 6. Electropherogram of Kwashiorkor

**Table 1: Values of Albumin and total protein in the different disease conditions**

	Control n=10	Cirrhosis (n=10)	Nephrotic syndrome (n=10)	Multiple myeloma (n=10)	Acute glomerulo- nephritis (n=10)	Rheumatoid arthritis (n=10)	Kwashiorkor (n=10)
Albumin (g/l)	45±5	31±5	32±5	30±6	29±6	30±4	25±8
Total protein (g/l)	65±10	95±10	60±5	100±5	58±6	94±5	50±5

Values are mean ± SD

## DISCUSSION

Serum protein electrophoresis has gained a secure place in terms of valuable diagnostic aid for many diseases. In this study we have examined six (6) disease conditions. Emphasis was placed on the pattern of the serum protein changes rather than alternations in concentration of the individual fractions and this has provided diagnostic usefulness. We observed a decrease in the concentration of serum albumin in 90% of all the cases investigated. This finding is in tandem with the works of [8,9].

The mobility of the charged particles is a function of the charge which in turn varies with pH. Since we are dealing with ampholytes (protein), at the isoelectric point ( $p^i$ ) mobility was zero. At pH below  $p^i$ , the ampholytes has a net positive charge and moves towards the cathode; above the ampholyte has a net negative charge and migrate to the anode. As pH increases, the total negative charge increases and the total positive charge decrease. At pH of 8.6, which the separation of the protein was performed, all but the gamma globulins carry a net negative charge and migrate towards the anode.

Cirrhosis is a chronic progressive disease of the liver essentially inflamatory characterized by proliferation of connective tissues resulting in the degeneration and death of the parenchyma cells. We can easily account for the fall in albumin in this case since the liver is the site for synthesis of albumin any disease affecting this organ will definitely impair its synthesis. The samples for cirrhosis show a unique fusion of the  $\beta$ - $\gamma$  suggestive of cirrhosis other than billiary, see figure 1. Another lacked the  $\beta$ - $\gamma$  fusion see figure 1a suggestive of juvenile cirrhosis. These findings are in agreement with previous works of [10]. It becomes clear from the above that the increase in concentration of serum proteins comes about as a result of increase in total globulin and in most cases the  $\gamma$ -globulin. We recognize the fact that although the serum albumin decrease in cirrhosis that of globulin was high.

Unique pattern was also identified for nephrotic syndrome in which there was decrease in albumin. We adduce thus to the fact that in this case, the glomerular structures and nephrons have been compromised resulting in loss if urinary protein. Since albumin is a low molecular weight protein, it is the protein that is mostly lost reflecting hypoalbuminemia in the electropherogram, as shown in figure 2.

The pattern for glomerulonephritis is as shown in figure 4. We observed a marked decrease in albumin. Globulins were decrease with gamma globulin as an exception. In view of the acute nature,  $\alpha_1$ ,  $\alpha_2$  and  $\beta$ -globulins were elevated. Earlier workers have shown that there is usually an outburst of production of antibodies in such conditions [11].

We also reviewed the electropherogram for multiple myeloma. Multiple myeloma may be present in a variety of clinical patterns depending on the degree of organ involvement, pressure from plasma cell tumors, and effect of abnormal proteins and specific metabolic complications [12, 14, 15]. It is known that the condition arise due to a diffuse bone marrow or discrete tumor production which result in the appearance of solitary lytic lesson in the bone. This is followed by a primary hematogenous spread in which the peripheral blood is inundated with abnormal cells called plasma cells [13]. All these varieties nevertheless share adherent  $\beta$ -cell functions and produces immunoglobulins of varying types and amount. In all the cases of multiple myeloma investigated there was marked increase in total protein especially the  $\gamma$ -globulin. A unique finding here was a clone of myeloma protein associated with the  $\beta$ -region.

A notable finding in kwashiorkor is the decrease in all fractions as shown in figure 3. We observed a moderate increase in the albumin concentration in post hepatic obstruction.

We assessed the strip for rheumatoid arthritis. There was marked increase in the gamma globulin,  $\alpha_2$ , was also elevated but total protein was low. Here, increase in gamma globulin tends to lend credence to earlier submission<sup>[4]</sup> that rheumatoid arthritis is related to autoimmune reactions. It is believed that the antibodies formed starts a cycle of events leading to arthritis. The use of serum protein electrophoresis will increase specifically and sensitivity in diagnosis. Its inclusion as routine investigation is highly recommended.

#### ACKNOWLEDGEMENT

The authors wish to thank the Research Ethical Committee of the University of Port Harcourt Teaching Hospital, Nigeria for granting us approval for this study. We also thank the staff of the Laboratory Department were the study was conducted.

#### REFERENCES

- (1). Kaplan, A. and Savory, J. (1965). Evaluation of cellulose acetate Electrophoresis system for serum protein fractionation. *Clin Chem* 11:937
- (2). Sunderman, F.W. Jr. (1964). Studies of serum protein VI. Advances in Clinical Interpretation of Electrophoretic fractions. *J. Clin. Path.* 30. 112
- (3). Alexzner, R., Weber, D, Liu, F.I. (1999). Differential Diagnosis of monoclonal gamopathies. *Arch. Pathol Lab. Med.* 123:106-13.
- (4). Kyle, R.A. (1999). Sequence of testing for Monoclonal gammopathies. *Arch Pathol Lab. Med.* 123:114-8
- (5). Tiselus, A., (1937). Harvey Lecture 35, 57.
- (6). Consden, R., Gordon, A.H., Martin, A.J. (1946). Zone Electrophoresis. *Biochem J.* 40, 38.
- (7). Kohn, J. and Ferberg, J.G. (1965). Electrophoresis on cellulose acetate, Shandon Instrument. Applications. No. 11. The Shandon Scientific Co., London.
- (8). Schultze, H.E., Hereman, F.F. (1966a). *Molecular Biology of Human Proteins* Vol. 1 p. 430 Elsevier, Amsterdam 1.
- (9). Landers, J.P. (2000). Molecular diagnostics on Electrophoretic Microchips. *Anal Chem.* 75: 2919-27
- (10). Anderson, N.L., Anderson, N.G. (2002). The Human plasma proteome History, Character, and disgnostic prospects. *Mol cell pro* 1:845-67.
- (11). Molloy, M.P. (2000). Two dimensional electrophoresis of membrane proteins using immobilized pH gradients. *Anal Biochem* 280:1-10
- (12). Viskari, P., Landers, J.P. (2006). Unconventional defection methods for microfluidic dencer. *Electrophoresis* 27:1797-810
- (13). Boccadro, M., Pieri, A. (1997). Diagnosis, Prognosis and Stanlarge Treatment of Multiple Myeloma. *Hematol Oncol Clin North Am.* 11:111-31.
- (14). Dispenzeri, A., Gertz, M.A., Therneau, T.M., Kyle, .R.A. (2001). Retrospective cohort study of 148 patients with polyclonal gamopathy. *Mayo Clin Proc.* 76:476-87.
- (15). George, E.D., Sedovsky, R. (1999). Multiple Myeloma: recognition and management. *Am Fam, physician:* 59:1885-94.

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