



Case Series

Evaluation of electrophoretic pattern in renal disease: A retrospective study

Dyna Ann Roby¹, Sukanya Shetty^{1,*}, Roopa Bhandary¹

¹Dept. of Biochemistry, KS Hegde Medical Academy, Karnataka, India



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α_2 - β =alpha2-beta

β_2 -M=beta 2 microglobulin

MM=multiple myeloma

HTN=hypertension

DM=diabetes mellitus

CKD=chronic kidney disease

ABSTRACT

Introduction: Protein electrophoresis is a technique that is well established and used for separating proteins based on their net shape, size and charge. Plasma proteins are separated into bands or zones according to their electric charge in an electrical field. Protein electrophoresis of the serum and urine along with other investigations are used for the evaluation of patients with chronic kidney diseases. The varied patterns of electrophoretographs observed in chronic kidney diseases brought to our attention to analyse the electrophoretic patterns in chronic kidney diseases retrospectively in the clinical laboratory.

Materials and Methods: The present study evaluated the electrophoretic pattern in renal disease for the period of 3 years. A total of 250 samples diagnosed for renal diseases were received by the laboratory for electrophoretic analysis.

Results: Out of the 250 samples, 78(51.2%) had low albumin levels and 50(20%) samples showed alpha2-beta bridging, 60 (24%) had high gamma globulins levels. Among the 50 samples who showed alpha2-beta bridging, 37 were diagnosed for multiple myeloma, 9 for Chronic kidney disease, 2 for acute kidney injury, 2 with systemic hypertension and type II diabetes mellitus and 2 with pancytopenia.

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1. Introduction

Electrophoresis is the separation or migration of charged solute particles under the influence of electric field in a liquid medium. Serum electrophoresis separates the various components of blood proteins into zones or bands under the influence of electric current.

The different bands have different zones with characteristic presence of different proteins (1) Albumin fraction → Albumin (2) alpha 1 fraction → alpha 1- lipoprotein, high density lipoprotein, alpha 1-antitrypsin (3) alpha 2 fraction → alpha 2 macroglobulin, haptoglobulin, beta-lipoprotein (4) beta fraction → transferrin, complement 3 (4) gamma fraction → fibrinogen, IgA, IgM, IgG

Serum proteins separate into 5 different electrophoresis zones

Albumin, and Prealbumin. Albumin is formed in the liver and is 60% of total proteins.

1. Alpha 1-globulin.

* Corresponding author.

E-mail address: dynaroby91@gmail.com (S. Shetty).

2. Alpha 2-globulin.

3. Beta-globulin.

4. Gamma-globulin.

A gradual loss of renal function occurs over a period of months to years, in Chronic kidney disease (CKD).

However there is a sudden damage, within few hours or few days in Acute kidney injury (AKI).

The diagnostic criteria for both these conditions include the study of serum protein, renal function tests and serum protein electrophoresis

The objective of the study was to evaluate α_2 - β bridging in electrophoretic pattern and to establish the association of these patterns with renal disorders.

To our knowledge this is the first report that reveals such a distribution.

2. Materials and Methods

Retrospective analysis was conducted in the clinical biochemistry laboratory, KS Hegde Medical College and Hospital, Deralakatte Mangalore over a period of 3 years

i.e Jan 2015-Jan 2018.

This study retrospectively collected total of 250 samples that was sent for electrophoresis to the clinical laboratory.

Electrophoresis was performed with a semi automated analyser on cellulose acetate plate at 80volt supplied by Helena Co. Electrophoretogram was quantitated on densitometer using Platinum Ver 4.0 Software supported by Helena Co:

Samples collected in plain vacutainers were centrifuged at 3000 rpm for 10 minutes to obtain serum.

All the data that was analysed was expressed in the form of percentage

Caution was taken to keep the identity of patients confidential

3. Observation and Results

Out of the 250 samples,

78(51.2%) - low albumin levels

50(20%) - alpha2-beta bridging

60 (24%) - high gamma globulins levels

62(24.8%) – normal

The samples with alpha2-beta bridging was selected and evaluated further.

Diagrams

Table 1: Changes in the parameters with renal disorders

Parameters	Percentage and Number
Decreased albumin	51.2%(128)
α 2- β bridging	20%(50)
Increased globulin	24%(60)

Table 1 showed that 78(51.2%) had low albumin levels,50(20%) samples with alpha2-beta bridging and 60 (24%) had high gamma globulins levels.

Table 2: Renal diseases that presented with α 2- β bridging

Disease	Percentage and Number
MM with CKD	74%(37)
CKD	53.8% (7)
AKI	15 %(2)
DM & HTN with CKD	15%(2)
Pancytopenia with CKD	15%(2)

Table 2 showed that among the 50 samples with alpha2-beta bridging, 37 were diagnosed for multiple myeloma,9 for Chronic kidney disease, 2 for acute kidney injury, 2 with systemic hypertension and type 2 diabetes mellitus and 2 with pancytopenia

4. Discussion

Protein electrophoresis is a well established, inexpensive technique used for the separation of proteins based on their net charge, size and shape. It is routinely used in clinical

laboratories for the screening of protein abnormalities in various biological fluids(serum, urine, csf).

Generally electrophoresis of serum protein is considered as a laboratory investigation that is to be done for a patient with an increased serum globulin levels compared to albumin or when the patient shows any signs and symptoms which is suggestive of an underlying plasma cell disorder such as Waldenstrom’s macroglobulinemia, multiple myeloma, or primary amyloidosis.

Response to acute inflammation, necrosis, infarction, burns, malignancy, trauma, and chemical injury display reasonably predictable changes in plasma protein levels.

Interpretation of serum protein electrophoresis, mostly focuses on the gamma region, that composes predominantly of antibodies of the Immunoglobulin G.

To prevent progressive loss of renal function and further injury ealy diagnosis and detection is very important.

Patient evaluation with chronic kidney disease also typically includes serum and urine protein electrophoresis.

The mechanisms involved in renal proteinuria include abnormality in transglomerular passage of proteins due to increase in permeability of the glomerular filtration barrier, impairment in the reabsorption of proteins by the epithelial cells of proximal tubule, and release of enzymes/ proteins from damaged epithelial cells of tubule. Proteinuria can be considered a marker of renal risk and diagnostic tool in the general population and in patients with diabetes mellitus,chronic kidney disease etc prior to the treatment.

The study evaluated the electrophoretic pattern in renal disease for the period of 3 years. A total of 250 samples diagnosed for renal diseases were received by the laboratory for electrophoretic analysis.

Out of the 250 samples, 78(51.2%) had low albumin levels and 50(20%) samples showed alpha2-beta bridging, 60 (24%) had high gamma globulins levels. Among the 50 samples who showed alpha 2-beta bridging, 37 were diagnosed for multiple myeloma, 9 for Chronic kidney disease, 2 for acute kidney injury, 2 with systemic hypertension and type II diabetes mellitus and 2 with pancytopenia.

Different types of electrophoretic patterns were reported by Kowsalya et al, were in the band migrated in different regions in patients with renal disorders.¹

Studies^{2,3} have revealed that the diffused distribution of protein between alpha 2 and beta globulins is suggestive of either presence of an abnormal protein or production of an existing known protein.

Human α ₂ -macroglobulin (a2M), a glycoprotein weighing 720 kDa, mediates complex pathological and physiological activities through its functional role as carrier, targeting and binding protein.

Alpha-2 macroglubulin interacts with three major groups of peptides, cytokines and proteinases, which exerts important modulatory effects on inflammation, tissue repair

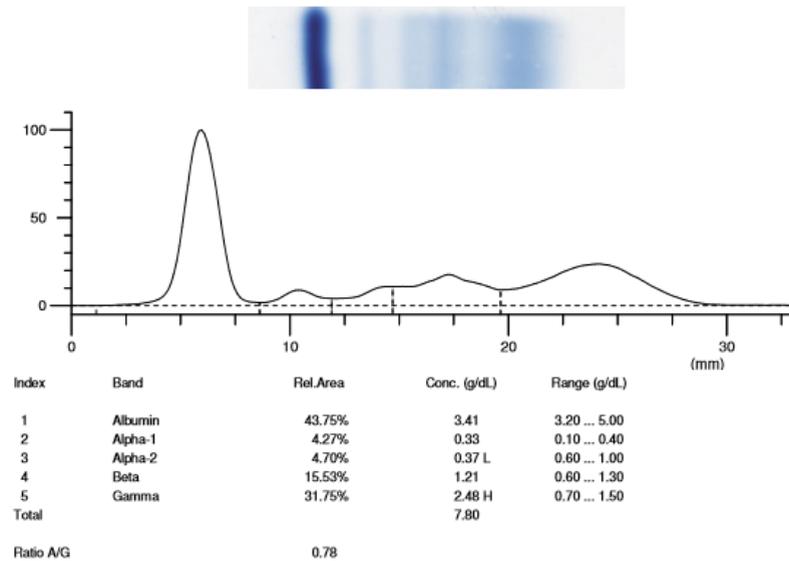


Fig. 1: Electrophoretic patterns showing α_2 - β bridging

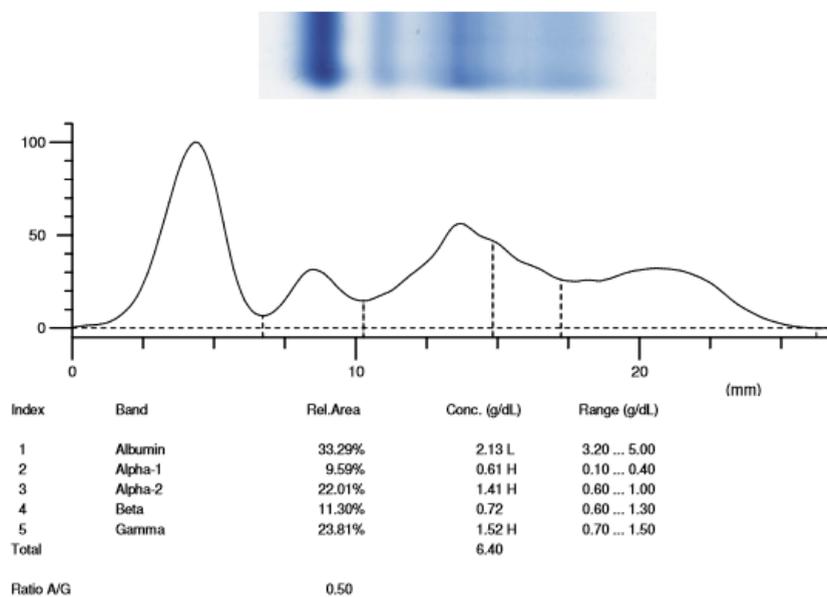


Fig. 2: Electrophoretic patterns showing α_2 - β bridging

and immunity.

The pathogenic role of alpha-2 macroglobulin in renal disease has not been studied extensively. As alpha-2 macroglobulin is involved in the, regulation of matrix-degrading enzymes, transport of growth factors and modulation of fibrinolysis factors, all of which are pathogenic mechanisms of glomerular injury, it is logical to consider the possibility of a relatively close connection between alpha-2 macroglobulin and its response of glomerular tissue to injury.

As a result there can be an increase in alpha 2 globulins leading to deposition and hence bridging which has also been reported by Yang Ah et al.²

Various studies have indicated the deposition of light chains in renal disorders.

Colina et al reported that the rise in the concentrations of polyclonal free light chains may result in similar progressive renal pathologies that could be associated frequently with monoclonal free light chains. In addition, elevated polyclonal free light chains indicates that the known systemic toxicity of monoclonal free light chains

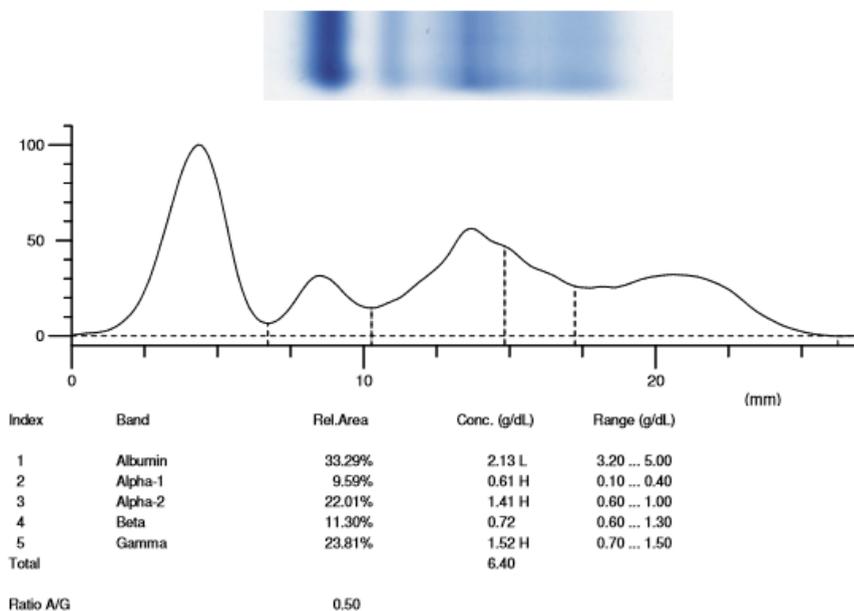


Fig. 3: Electrophoretic pattern showing M band in the α region

may have further biologic relevance in CKD.⁴

During synthesis of intact immunoglobulins by plasma cells, free light chains are produced in excess of heavy chains. These excess polyclonal free light chains are released into the circulation, from where they are rapidly removed by the kidneys. They have a half-life of 2 to 6 hours. In patients with CKD, as the glomerular filtration rate decreases, the renal clearance of polyclonal free light chains decreases and concentrations in serum rise.

Clonal process that is malignant or potentially malignant are associated with monoclonal gammopathies. On the contrary, polyclonal gammopathies are usually caused by any reactive or inflammatory process, and they are mostly in association with nonmalignant conditions.

Monoclonal gammopathy of renal significance (MGRS) regroups all renal disorders caused by a monoclonal light chain. MGRS is associated with high morbidity due to severity of renal and sometimes systemic lesions. The spectrum of renal disorders is wide in MGRS. Hence early diagnosis is very crucial.⁵

Our finding is suggestive of the presence of significant amount of these chains between the alpha2-beta region which needs further investigation.

Raymond et al also reported that renal disorders occur in association with abnormalities of light chain metabolism and demonstrates that significant renal failure and proteinuria occur in association with light chain deposition in the kidney.⁶

Various studies have found alpha 2 macroglobulin to be increased in diabetic nephropathy which correlates with our study as well.⁷⁻⁹

Multiple myeloma in the alpha region is a possibility. It is observed that in rare cases of MM a homogenous peak is observed in the alpha 2 region.^{10,11}

In the pathogenesis of amyloidosis the role of beta 2 microglobulin has been implicated in long-term dialysis patients. In patients with chronic renal failure, beta 2 microglobulin levels were in parallel to the increased levels in serum creatinine. beta 2 microglobulin correlated with the residual glomerular filtration rate, and its removal is membrane-dependent, thereby showing an increased concentration.¹²

Almost all chronic kidney diseases share similar pathogenic mechanisms that begins from the initial injury, leading to hyperfiltration of glomerulus, proteinuria, and finally progresses to renal scarring and functional loss. Consistent experimental data supports the major role of proteinuria in advancing kidney disease progression to end-stage renal disease through several pathways, including complement activation and induction of tubular chemokine expression. These events, in turn, lead to infiltration by inflammatory cells in the interstitium and sustained fibrogenesis. The extent of proteinuria is widely recognized as a severity marker of chronic kidney disease and as a predictor of decline in glomerular filtration rate in the future. A decrease in proteinuria invariably forecasts into a protection from renal function drop in patients.¹³

Many a times person to person variation while introducing troughs and peaks in an electrophoretogram also affects the judgement and hence the result.

5. Conclusion

The alpha 2 beta bridging observed in the present study could be due to increased production of alpha 2 macroglobulin, polyclonal free light chains and beta 2 microglobulins.

6. Source of funding

None.

7. Conflict of interest

None.

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Author biography

Dyna Ann Roby Post Graduate

Sukanya Shetty Professor and HOD

Roopa Bhandary Assistant Professor

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