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Case Report

Biochemistry Section

Diagnosing a Challenging Case of Primary Amyloidosis using Capillary Electrophoresis

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ABSTRACT

Amyloid Light chain (AL) amyloidosis is characterised by deposition of intact free light chains or their fragments in extracellular space. Here, authors describe the journey of a diagnostically challenging patient who presented with features of nephrotic syndrome and was finally diagnosed with AL amyloidosis. A 45-year-old female presented to the Outpatient Department (OPD) with gradually progressive generalised body swelling. On examination, hepatomegaly, cardiomegaly and macroglossia were observed. Renal biopsy, capillary serum protein electrophoresis, immunotyping and serum free light chain assay were performed along with routine blood and urine investigations to detect the presence of monoclonal protein. Bone marrow biopsy was conducted for confirmation of diagnosis. Proteinuria with hypoalbuminaemia 1.8 g/dL was detected during routine investigations. Renal biopsy showed presence of amyloid deposits in glomerular mesangium and walls of medium sized blood vessels which tested positive for Immunoglobulin (Ig) G, IgA, kappa and lambda chains on immune florescence. Serum protein capillary electrophoresis findings demonstrated increase in beta 2 fraction and distortion in gamma region. Immuno typing showed presence of monoclonal IgA heavy chains and lambda light chains. Bone marrow biopsy confirmed presence of plasma cell dyscrasia. Based on these findings authors concluded that capillary gel electrophoresis is more sensitive method than agarose gel electrophoresis in detecting beta migrating monoclonal proteins.

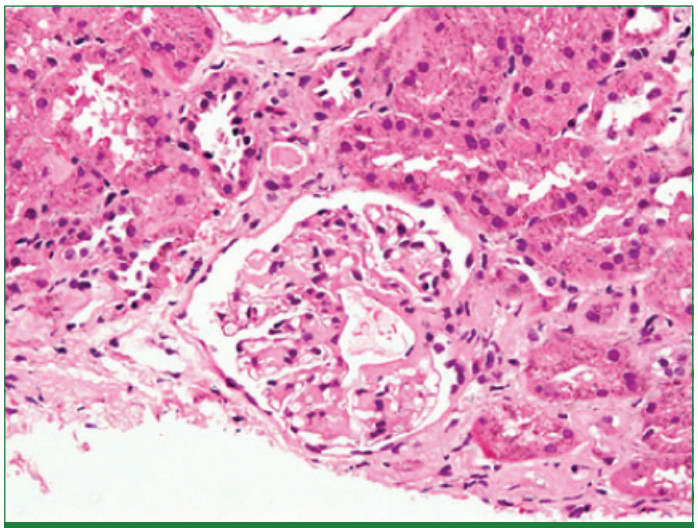
Keywords: Immunoglobulin light chain amyloidosis, Paraproteinaemia, Proteinuria

CASE REPORT

A 45-year-old female patient presented with gradually progressive generalised body swelling since two and a half months with no other complaints. Patient's past history was unremarkable except for having suffered from pulmonary Koch's 10 years ago which was adequately treated. Clinical examination revealed vital parameters in the normal range. The patient organomegaly was in the form of hepatomegaly, cardiomegaly and macroglossia.

Routine haematological (haemogram) and biochemical examination (Liver function and Renal Function tests) revealed no significant findings. There was sub nephrotic range proteinuria with 24 hour urine protein 2.8 g/day (<150 mg/day) and serum proteins were 5.2 g/dL (5.5-8 g/dL) with hypoalbuminemia (albumin 3.5-5 g/dL). Urine examination revealed inactive sediment with significant proteinuria; and absence of Bence Jones proteins. In the autoimmune workup, serum Rheumatoid Factor (RA), Antinuclear Antibody Test (ANA), double stranded Deoxyribose Nucleic Acid (dsDNA), Antineutrophil Cytoplasmic Antibody (ANCA) and Coomb's test all were negative. Serum complement 3 (C3) level was normal. Viral causes of Hepatitis and Human Immunodeficiency Virus (HIV) infection were ruled out.

In view of significant proteinuria, a further evaluation was planned using renal biopsy and serum protein electrophoresis after obtaining informed consent from the patient. The renal biopsy revealed enlarged glomeruli with an amorphous eosinophilic substance deposited in the mesangium which was negative on Silver Methenamine (SM) and Periodic Acid-Schiff (PAS) stain [Table/Fig-1]. Similar material was also demonstrated in the walls of medium sized blood vessels and tubules. The fluffy eosinophilic deposits showed a congo red positivity with apple green birefringence on polarised light. There was no evidence of a proliferative glomerulopathy in the glomeruli or basement membrane thickening as evident on SM stain. Immuno histochemical staining for Serum Amyloid A protein (SAA), amyloid was negative.

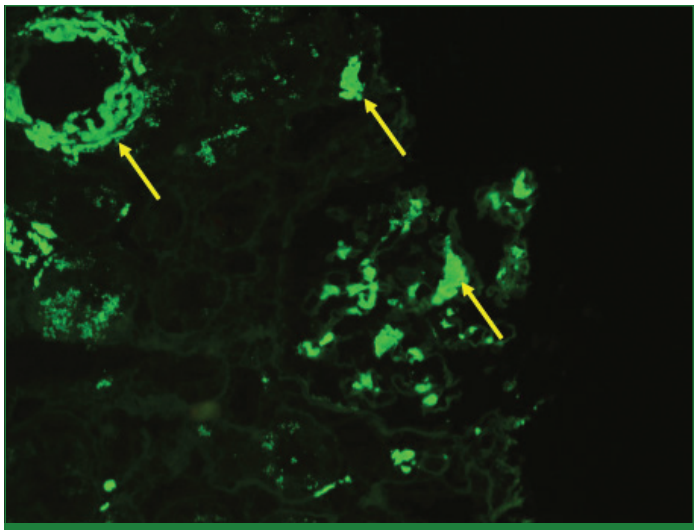


[Table/Fig-1]: Haematoxylin and Eosin (H&E) stain showing amyloid deposits (congo red positivity with apple green birefringence on polarised light) in the glomerular mesangium and tubules. 20X.

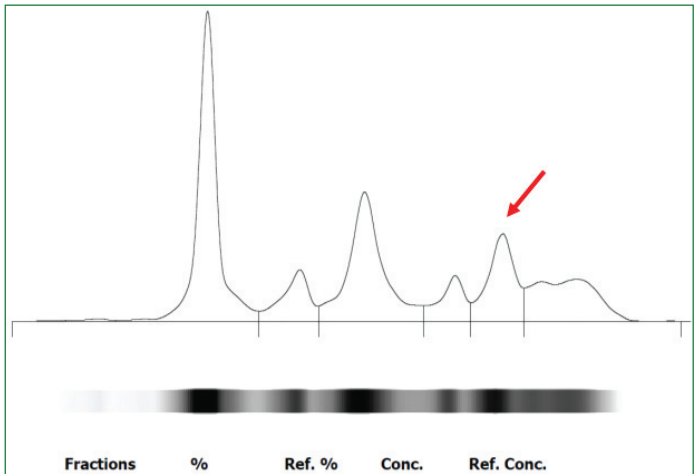
Immunofluorescence (IF) revealed coarse granular deposits of IgG and IgA in mesangium which showed an intensity of 2+ (on a subjective scale of 0 to 4+). The deposits were strongly positive for lambda (L) light chains as compared to kappa (K) light chains (L=4+; K=1+) [Table/Fig-2]. Fluffy deposits with similar staining characteristics were deposited in the blood vessels. IgM, complement 3 (C3) and complement component 1q (C1q) stains on IF were negative. Based on the nature of deposits on light microscopy and IF, a final diagnosis of AL amyloidosis was made. Also, following renal biopsy, serum electrophoresis was performed to look for evidence of a monoclonal immunoglobulin.

Serum protein electrophoresis showed the evidence of increase in alpha 2 and beta 2 globulin fractions and decrease in albumin fraction with slight deformation in gamma region [Table/Fig-3]. On immunotyping a sharp narrow peak was seen in IgA and lambda

region. Also, broad diffuse peaks were seen in IgG and Kappa region [Table/Fig-4].

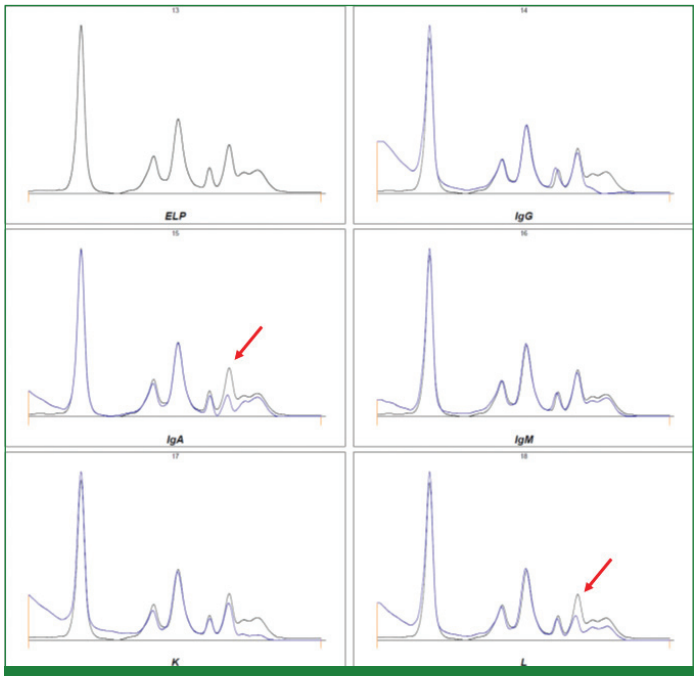


[Table/Fig-2]: Monoconal lambda light chain deposits in glomerulus and tubules as seen in immuno fluorescence (yellow arrows).



Fractions	%		Ref. %	Conc.	Ref. Conc.
Albumin	34.1	<	55.8 - 66.1	1.8	4.0 - 4.8
Alpha 1	7.7	>	2.9 - 4.9	0.4	0.2 - 0.4
Alpha 2	24.1	>	7.1 - 11.8	1.3	0.5 - 0.9
Beta 1	6.1		4.7 - 7.2	0.3	0.3 - 0.5
Beta 2	13.0	>	3.2 - 6.5	0.7	0.2 - 0.5
Gamma	15.0		11.1 - 18.8	0.8	0.8 - 1.4

[Table/Fig-3]: Serum Protein electrophoresis (on Minicap-Flex Piercing).



[Table/Fig-4]: Immunotyping by immunosubstraction (Minicap-Flex Piercing).

A 2-Dimensional (2D) echocardiography was suggestive of restrictive cardiomyopathy with pericardial effusion. Non Contrast Enhanced CT (CECT) scan of Kidney, Ureter, and Bladder (KUB) was normal and therefore contrast Computed Tomography (CT) scan was not performed. No lytic lesions were identified on bone scan. Bone marrow biopsy report described presence of a plasma cell dyscrasia with increase in plasma cells (17%). Free light chain assay showed markedly increased free lambda levels i.e., 118 mg/L (8.3-27 mg/L) and normal free kappa levels 22.1 mg/L (6.7-22.4 mg/L) [Table/Fig-5]. Kappa:lambda ratio (κ:λ ratio) was calculated and found to be 0.19 (0.26-1.65).

Test parameter	Concentration	Reference interval
IgG	1150 mg/dL	700-1600 mg/dL
IgA	585 mg/dL	70-400 mg/dL
IgM	125 mg/dL	40-230 mg/dL
Free kappa	22.10 mg/L	6.7-22.4 mg/L
Free lambda	118 mg/L	8.3-27 mg/L

[Table/Fig-5]: Estimation of immunoglobulin levels by nephelometry.
Ig: Immunoglubulin; mg: Milligrams; dL: Decilitre

DISCUSSION

Amyloidosis is a condition associated with a number of inherited and inflammatory disorders in which extracellular deposits of fibrillar proteins are responsible for tissue damage and functional compromise [1]. AL chain amyloidosis (primary amyloidosis), a subset of systemic amyloidosis, is associated with plasma cell dyscrasias. The amyloid fibril protein of the AL type is produced from free Ig light chains secreted by a monoclonal population of plasma cells [1]. Plasma cell dyscrasias represent a spectrum of monoclonal gammopathies in which a clone or multiple clones of premalignant or malignant plasma cells overproduce and secrete an abnormal monoclonal antibody or a part of it into the bloodstream. Monoclonal gammopathy of undetermined significance, the least severe form, is present at one end of this spectrum while multiple myeloma is present at the other end. However, many patients with AL amyloidosis do not have classical multiple myeloma or any other overt B cell neoplasm but have monoclonal immunoglobulins or free light chains, or both, in the blood and/or urine [1-3]. Serum protein electrophoresis plays a crucial role in diagnosis, monitoring disease progression and prognosis of plasma cell dyscrasias and amyloidoses [2].

Serum proteins can be separated by electrophoresis on the basis of their charge, molecular size and shape. Protein electrophoresis, an important lab investigation, is routinely used for screening abnormal proteins in serum and other body fluids like urine and CSF. Capillary electrophoresis is the latest technique which has been found to be more sensitive in detection of paraproteins than agarose gel electrophoresis [4, 5]. Here, in the present case, authors highlighted the advantage of using capillary electrophoresis over agarose gel electrophoresis.

In patients of unexplained proteinuria, cardiomyopathy, hepatomegaly or neuropathy a diagnosis of AL amyloidosis should be suspected [6, 7]. As in this patient a subnephrotic range proteinuria was detected, a kidney biopsy was conducted and the findings obtained were coherent with the findings seen in capillary protein electrophoresis and immunotyping.

On capillary electrophoresis decrease in albumin fraction and increase in alpha 1, alpha 2 and beta 2 fractions, with slight deformation in gamma region were detected. As it is already known that monoclonal proteins can migrate anywhere from alpha 2 to gamma region, rise in any protein fractions encountered on capillary electrophoresis should be interpreted with caution [8]. The increase in beta 2 region could be attributed to multiple factors like presence of C3 complement factors, fibrinogen or IgA immunoglobulin which may migrate in beta region owing to its higher molecular weight [9,

10]. Therefore, to confirm author's suspicion of primary amyloidosis and to rule out the possibility of any inflammatory response, immunotyping was performed. On immunotyping a sharp peak with significant immunosubtraction was observed in IgA and lambda region corresponding to the increase observed in beta 2 region and a broad peak was seen in IgG and Kappa region corresponding to the deformation observed in gamma region. This indicated the presence of monoclonal proteins (IgA and lambda) in the serum of this patient.

To further support the patient findings, quantitative levels of immunoglobulins along with free light chain assay (measured by nephelometry) was performed which revealed significantly raised levels of IgA and lambda. IgG and kappa levels were found to be within normal reference range. The kappa lambda ratio was calculated as 0.19 which strongly indicated presence of a population of plasma cells that are producing clonal lambda free chains. To establish the final diagnosis, bone marrow biopsy was done which showed clonal plasma cell burden of 17%. So based on all the blood, radiological and biopsy findings the patient was diagnosed with AL amyloidosis (primary amyloidosis).

Here, authors would like to highlight the fact that at the outset the serum protein electrophoretic pattern observed did not show the presence of any sharp peak in gamma region, classically seen in multiple myeloma and other plasma cell dyscrasias and it was only after immunotyping that authors could determine the reason for increase in beta 2 fraction. Immunotyping also helped to rule out secondary amyloidosis which would otherwise have caused a polyclonal hypergammaglobulinemia and no significant immunosubtraction in any of the heavy or light chains in gamma fraction.

It is already known that the sensitivity of capillary electrophoresis in detecting monoclonal proteins, especially those migrating in beta region, is more than agarose gel electrophoresis [5]. Further agarose gel electrophoresis based kits do not always differentiate between beta 1 and beta 2 regions. Studies have shown that capillary electrophoresis is more sensitive than agarose gel electrophoresis in detecting modest increase in beta region [11,12].

CONCLUSION(S)

In the present case, authors observed minimal but significant increase in beta 2 fraction on capillary electrophoresis that could have been missed if agarose gel electrophoresis was used, as the beta migrating monoclonal IgA proteins would be obscured by healthy beta migrating proteins. Since, some abnormal pattern was observed in capillary protein electrophoresis, immunotyping was performed. Usually in laboratories not having the facility of capillary electrophoresis, immunofixation is performed after protein gel electrophoresis only when some abnormality is observed in the electrophoretogram.

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