DOI: 10.7860/JCDR/2023/63001.18012

Case Report

Biochemistry Section

Monoclonal Gammopathy of Undetermined Significance with Three Bands on Serum Protein Electrophoresis: An Atypical Presentation

RASHMI RASI DATTA[1], ASHUTOSH AWASTHI[2]

ABSTRACT

Monoclonal Gammopathy (MGM) is a group of B-cell disorders characterised by secretion of abnormal monoclonal Immunoglobulin (Ig) that can be detected by presence of M-protein in serum and urine electrophoresis. Monoclonal protein (M-protein) can be best identified by high resolution agarose gel electrophoresis where, it is usually perceived as a discrete band in the γ or β globulin region. Sometimes atypical banding patterns are seen in serum capillary electrophoresis that pose diagnostic dilemmas and may require repeat testing and further confirmation with a diagnostic test. In the present case, a 60-year-old female patient was advised assessment for gammopathies owing to the presence of skeletal lesions and hyperproteinaemia clinically. Serum protein capillary electrophoresis revealed an unusual pattern with three prominent bands in the beta/early gamma region, indicative of triclonal gammopathy, although, it was monoclonal in nature which was depicted during confirmatory diagnostic testing. The authors further discuss the multiple aspects that may be attributed as a cause for such peculiar presentations which should be considered to avoid misdiagnosis.

Keywords: Heavy chains, Immunofixation electrophoresis, Immunoglobulins, Light chains

CASE REPORT

A 60-year-old female patient presented to the Department of Medicine of an Army Base Hospital with a complaint of dull aching pain in the lower back, often radiating to the abdomen since past two years. She also complaints that, the pain aggravated on physical movement. On examination, no abnormality was detected in the neck and abdominal region. However, her left breast was found to be scarred consequential to her childhood burn history. Her physical examination was unremarkable, except for slight tenderness on the lower back. The patient was advised ultrasound whole abdomen and the findings revealed the presence of a bulky uterus with no other abnormality detected. Subsequently, she was advised Magnetic Resonance Imaging (MRI) scan wherein, multiple non hypermetabolic to hypermetabolic lytic osseous lesions scattered throughout the axial skeleton were detected, which was suggestive of malignant pathology. Also, her endometrial thickness was found to be high (9.5 mm).

Based on the above findings, the patient was assumed to be a case of endometrial carcinoma and the bone lesions were attributed to metastasis. The patient was advised further laboratory investigations for correlation of MRI findings with the blood biochemical tests. The patient's sample was received in the reference laboratory of a diagnostic laboratory chain for biochemical investigations, which were performed on ROCHE Cobas Pro system based on Electro Chemiluminescence (ECLIA) technology. Based on the biochemical investigations, no relevant clinical data indicating involvement of specific organ system was found, except for deranged levels of total serum protein and uric acid [Table/Fig-1]. Blood levels of cancer antigens (CA125, Carcinoembryonic Antigen (CEA)) were also determined, which were found to be within normal ranges, CA 125=15.6 U/mL (normal range <35 U/mL) and CEA=0.547 ng/mL (normal range=<3.8 for non smokers and <5.5 for smokers). Considering the presence of skeletal lesions and hyperproteinaemia with accompanying hyperglobulinaemia, the patient was adviced further evaluation for gammopathies.

S. No.	Parameters	Result	Reference range*
1	Calcium	12.4 mg/dL	8.8-10.2 mg/dL
2.	Creatinine	0.79 mg/dL	0.5-0.9 mg/dL
3.	BUN	9.41 mg/dL	6-20 mg/dL
4.	Uric acid	6.41 mg/dL	2.4-5.7 mg/dL
5.	Total protein	10.1 g/dL	6-8 g/dL

[Table/Fig-1]: Routine biochemical parameters.
*Reference ranges as per manufacturer. Transferability of the expected values in patient population has been verified by the laboratory; BUN: Blood urea nitrogen

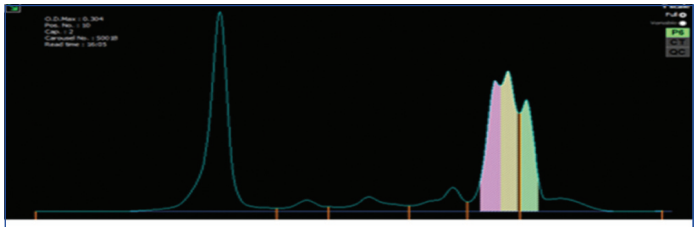
As a part of gammopathy evaluation, the patient's serum sample was received in the laboratory and was subjected to capillary protein electrophoresis in a Minicap electrophoresis Flex Piercing (FP) analyser (sebia platform). The proteins were separated by electrophoresis on alkaline-buffered agarose gels. Individual antisera against IgG, IgA and IgM heavy chains, and kappa and lambda light chains were applied [Table/Fig-2].

S. No.	Test	Result	Reference range (units)
1	Total IgA	40.50	0.52-4.68 g/L
2	Total IgG	5.85	6.5-16.4 g/L
3	Total IgM	0.43	0.39-3.38 g/L
4	Kappa free light chain	34.20	3.30-19.40 mg/L
5	Lambda free light chain	13.30	5.71-26.30 mg/L
6	Kappa lambda ratio	2.57	0.26-1.65 No units as it's a ratio
7	β2-microglobulin	3441.0	609-2366 ng/mL

[Table/Fig-2]: Gammopathy panel.

Serum Protein Electrophoresis (SPEP) exhibited an atypical banding pattern with three closely associated homogenous spikes/bands in the beta and early gamma region suggestive of triclonal gammopathy [Table/Fig-3]. Densitometry quantification was done and the concentration of each band was more than 1 g/dL (1.71, 1.80 and 1.35 g/dL, respectively) [Table/Fig-4]. The migration

pattern in the beta/gamma region on electrophoresis along with the concentration of the bands (more than 1 g/dL) was highly indicative of the presence of M-protein. Serum free light chain assay revealed elevated kappa free light chains (34.20 mg/L; normal 3.30-19.40 mg/L) with raised kappa lambda ratio (2.57, normal- 0.26-1.65). The complete findings confirmed the patient as a case of MGM, suggested non IgM MGM of Unknown Significance (MGUS). Serum Immunofixation Electrophoresis (IFE) was performed using the HYDRASYS agarose gel electrophoresis apparatus by Sebia (Norcross, GA, USA), according to the manufacturer's instructions, to determine the clonality (type) of M-proteins observed on capillary electrophoresis. The M-protein were identified following staining of the immunoprecipitates. IFE showed a single broad and discrete band in beta region corresponding with immunoglobulin A (IgA) heavy chain and another intense band in kappa light chain lane. Consequently, the three peaks observed on capillary protein electrophoresis with suspected triclonal involvement were established on IFE to be of IgA and kappa in origin [Table/Fig-5]. The clinician had to refer the patient to an oncology Institute for further management and the patient was lost to further follow-up.

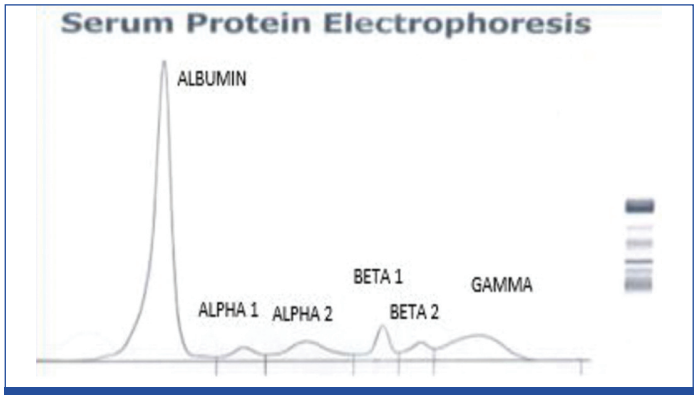


FRACTIONS	%	REFERENCE %	CONCENTRATION	REF.CONC.
Albumin	34.9 <	55.8-65.3	3.52	3.30-5.70
Alpha 1	2.6 <	3.0-4.8	0.26	0.10-0.40
Alpha 2	4.8<	7.0-11.6	0.48	0.30-0.90
Beta 1	5.7	4.7-7.1	0.58	0.30-0.70
Beta 2	35.5 >	3.1-6.4	3.59	0.10-0.50
Gamma	16.5	11.1-18.6	1.67	0.50-1.60

[Table/Fig-3]: Patient's serum capillary electrophoresis report.

Peaks	Percentage (%)	g/dL
Band 1	16.9	1.71
Band 2	17.8	1.80
Band 3	13.4	1.35

[Table/Fig-4]: Concentration of bands.



[Table/Fig-5]: Serum protein electrophoresis.

DISCUSSION

The MGM or plasma cell myeloma is a disorder characterised by proliferation of abnormal clone of plasma cells infiltrating the bone marrow. These cells secrete abnormal monoclonal Ig, the M-protein, a specific tumour marker, that can be appreciated as a discrete band in the serum and/or urine on SPEP [1-3]. It accounts for 10% of the haematological malignancies [2]. M-protein is best detected by high resolution agarose gel electrophoresis as a single discrete band, frequently in the γ or β globulin region. Rarely, it may also be perceived in the α2 globulin region [1,4]. The paraprotein identification can be done by immunofixation electrophoresis.

Serum Protein Electrophoresis (SPEP) is routinely used for the diagnosis of multiple myeloma and other haematological malignancies and it correlates well with biochemical, radiological and pathological findings. This conventional technique of serum electrophoresis still remains the gold standard for demonstration of M-protein in myeloma patients. The circulating M-protein can comprise of an intact Ig, the light chain only (either kappa or lambda), or the heavy chains (from one of the five Ig classes (G, A, M, D or E) [3].

In the index case, the patient was advised assessment for gammopathies owing to the presence of skeletal lesions and hyperproteinaemia clinically. Serum protein capillary electrophoresis revealed an unusual pattern with three prominent bands in the beta/gamma region, indicative of triclonal gammopathy. IFE was done subsequently by the study laboratory (test request not made by the clinician) to determine clonality of the heavy/light chains involved. This exhibited a single broad and discrete band in beta region corresponding with IgA heavy chain and another intense band in kappa light chain lane. IgA monoclonal Ig produces a broad band near beta and early gamma region due to higher molecular weight. IgA monoclonal Ig produces a wide band near beta and early gamma region owing to higher molecular weight. Also, IgA monoclonal Ig molecules have the tendency to self-aggregate and form multimers, which hence, yields a broad band [5,6]. Monomeric IgA molecules have a faster mobility as compared to the slow moving multimeric (dimers, trimers) molecules that yields a broader band [7]. The difference in mobility of IgA monomers and dimers may produce two or more distinct bands on SPEP raising a suspicion of involvement of multiple clones.

For unusual banding patterns arising as a result of polymerised paraproteins, it is advised to repeat the test after pretreating the sample with beta-mercaptoethanol which results in their dissociation [8,9]. This however, was not done in the present case by the study laboratory. Additional aspects that need to be well-thought-out as a source for such observations in SPEP are sample condition, technical and procedural variability, observer inconsistency, differences in sensitivity of detection and technology used. Further, the location of IgA band in SPEP is close enough to that of fibrinogen band which occasionally can lead to misinterpretation. This is observed in case of inadequately clotted blood samples owing to improper collection or presence of anticoagulant in specimen vial. This requires repeating the electrophoresis with a fresh sample or pretreating the sample with ethanol to precipitate out fibrinogen. Such conditions can be resolved on IFE as fibrinogen does not show band in heavy chain lanes and kappa light chain lane on IFE but, occasionally may show a thin band in lambda light chain lane [7].

CONCLUSION(S)

The IgA gammopathy has myriad of presentations on protein electrophoresis which, if unaware can lead to misdiagnosis. From the diagnostic perspective for gammopathy evaluation, it is advisable that, the clinician should always request for the complete panel of tests comprising Survey Planning and Coordination Element (SPCE), IFE, and light chain estimation with their ratio and Ig levels to ensure conclusive diagnosis.

Acknowledgement

The authors would like to thank the technical team involved in sample processing.

REFERENCES

[1] Hussain A, Almenfi H, Almehdewi AM. Laboratory features of newly diagnosed multiple myeloma patients. Cureus. 2019;11(5):e4716. Doi: 10.7759/cureus.4716.
[2] Rajkumar SV. Multiple myeloma: 2022 update on diagnosis, risk stratification, and management. Am J Hematol. 2022;97(8):1086-107. Doi: 10.1002/ajh.26590. Epub 2022 May 23. PMID: 35560063; PMCID: PMC9387011.
[3] Dash NR, Mohanty B. Multiple myeloma: A case of atypical presentation on protein electrophoresis. Indian J Clin Biochem. 2012;27(1):100-02. Doi: 10.1007/s12291-011-0178-3. Epub 2011 Nov 18. PMID: 23277721; PMCID: PMC3286584.

[4] Kyle AR, Rajkumar SV, Lust AJ. Monoclonal gammopathy of undetermined significance and smoldering multiple myeloma. Wintrobe's clinical hematology. Chapter 97. 11th ed. Philadelphia: Lippincott Williams & Wilkins; 2004. Pp. 2566-67.
[5] Keren D. Protein Electrophoresis in Clinical Diagnosis. Great Britain: Hodder Arnold; 2003.
[6] Bansal F, Bhagat P, Srinivasan VK. Immunoglobulin A gammopathy on serum electrophoresis: A diagnostic conundrum. Indian J Pathol Microbiol. 2016;59:134-36.
[7] Keren D. High-Resolution Electrophoresis and Immunofixation: Techniques and Interpretation. 2nd ed. Stoneham, MA: Butterworth Publishers; 1994.
[8] Keren DF, Gulbranson R, Carey JL. 2-mercaptoethanol treatment improves measurement of an IgMκ M-protein by capillary electrophoresis. Clin Chem. 2001;47:1326-27.
[9] Valentine H, Dawnay A. The effect of paraprotein polymerisation on quantitation by capillary zone electrophoresis and Hevylite®. Ann Clin Biochem. 2021;58(6):586-92. Doi: 10.1177/00045632211029327. Epub 2021 Jul 4. PMID: 34159795.

PARTICULARS OF CONTRIBUTORS:
1. Consultant Biochemist and Head, Department of Biochemistry, SRL Reference Laboratory, Gurugram, Haryana, India.
2. Pathologist, Department of Pathology, SRL Reference Laboratory, Gurugram, Haryana, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Rashmi Rasi Datta,
A-206, Sector-10, Plot-7, Pragjyotispur Apartments, Dwarka, New Delhi-110075, India.
E-mail: rashmi.datta@srl.in

PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Jan 24, 2023
- Manual Googling: Mar 10, 2023
- iThenticate Software: Apr 17, 2023 (17%)

ETYMOLOGY: Author Origin

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: **Jan 20, 2023**
Date of Peer Review: **Feb 24, 2023**
Date of Acceptance: **Apr 24, 2023**
Date of Publishing: **Jun 01, 2023**