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CASE REPORT

Atypical Migration in Serum Immunofixation of Immunoglobulin A with Masked kappa Light Chains: a Case Report and Review of the Literature

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SUMMARY

Background: **We report a case of a patient with immunoglobulin A multiple myeloma associated with a masked kappa light chain. Serum immunofixation showed a monoclonal band in the IgA heavy chain lane without correspondence with the light chain and a monoclonal band in total kappa light chain lane without correspondence with the heavy chain.**
Methods: **To distinguish between heavy chain disease and immunoglobulin with "masked" light chains, two tubes containing the patient's serum were incubated with a very high concentration of anti-total kappa and anti-total lambda antisera for 48 hours at 4°C in order to facilitate immunoprecipitation of the involved light chain. After centrifugation, the supernatant was analyzed by using the IFs method on the Hydrasys 2 Scan Focusing Sebia® without dilution. Then we applied the anti-IgA, anti-total kappa and anti-total lambda antisera.**
Results: **The serum immunofixation test of the sample treated with a high concentration of anti-total kappa showed the disappearance of the monoclonal bands corresponding to IgA heavy chain lane and kappa light chain lane, indicating that precipitation had occurred and that the IgA did have kappa light chains that could not be detected by the standard immunofixation protocol. The serum immunofixation test of the sample treated with anti-total lambda showed the disappearance of the polyclonal background in lambda light chain lane, confirming that the precipitation with lambda light chains according to the previously mentioned protocol has done well.**
Conclusions: **This case illustrates some of the difficulties encountered and the corrective actions that can be taken for the detection of immunoglobulins with masked light chains.**
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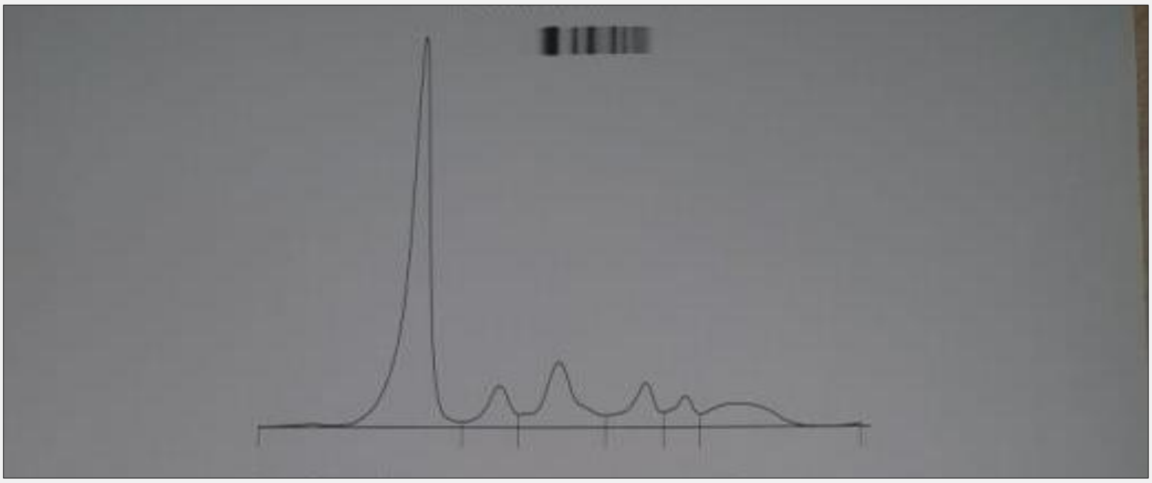
KEYWORDS

masked kappa light chain, multiple myeloma, IgA, immunoprecipitation

INTRODUCTION

Immunofixation (IF) is a technique where the proteins are immunoprecipitated on a gel, which is essential to confirm the homogeneity of monoclonal immunoglobulin (MI) and to characterize and type it. Its interpretation is generally easy; however, difficulties may be countered in daily practice. We report the case of a patient



Name	%	Standard values %	g/L	Standard values g/L
Albumin	57.7	55.8 - 66.1	38.1	40.2 - 47.6
Alpha 1	6.6	2.9 - 4.9	4.4	2.1 - 3.5
Alpha 2	14.5	7.1 - 11.8	9.6	5.1 - 8.5
Beta 1	7.6	4.7 - 7.2	5	3.4 - 5.2
Beta 2	4.3	3.2 - 6.5	2.8	2.3 - 4.7
Gamma	9.3	11.1 - 18.8	6.1	8.0 - 13.5

Figure 1. Serum protein electrophoresis performed on Capillarys Flex Piercing® showing moderated hypogammaglobulinemia associated with an inflammatory syndrome.

with IgA multiple myeloma with a masked kappa light chain.

CASE REPORT

We report a case of a 71-year-old woman hospitalized in the clinical hematology department of the Mohammed V Military Hospital in Rabat. She presented with a history of hypertension and hypothyroidism, associated with a profound asthenia, pain in her shoulders and hands for 9 months, and weight loss. The clinical examination found pain on palpation of two upper limbs, peripheral neuropathy grade 2, and motor deficit without tumor syndrome.

The radiological assessment was unremarkable. Spine and pelvis MRI (magnetic resonance imaging) scan showed myeloma infiltration of the axial bone without suspicious lesion.

Biological examination at the first appointment revealed non-regenerative normochromic normocytic anemia (Hb 8g/dL), renal failure with a Glomerular Filtration Rate (GFR) estimated by CKD-EPI equation of 10 mL/minute/1.73 m², a proteinuria of 1.22 g/L with a Proteinuria Creatininuria Ratio of 3,236 mg/g, hypocalcemia of 69 mg/L (80 - 105) with anormal albuminemia of 40 g/L (35 - 50), and beta-2-microglobulin of 13.34 mg/L (0.97 - 2.64). The myelogram showed a marrow plasmacytosis of 12%.

Serum protein electrophoresis performed on the Capillarys 2 Flex Piercing Sebia ® showed moderate hypogammaglobulinemia associated with an inflammatory syndrome (Figure 1).

The initial serum immunofixation (IFs) test performed on the Hydrasys 2 Scan Focusing Sebia® using anti IgG, IgA, IgM, total kappa and total lambda antisera, showed a monoclonal band in the IgA heavy chain lane which is unmatched in light chain lanes and the band in total kappa light chain lane is unmatched in heavy chain lane (Figure 2). This profile was completed by a second IFs using anti GAM, D, E, total kappa and free kappa antisera which revealed a monoclonal band in the IgA heavy chain lane that still remains without light chain correspondence while the band in the total kappa light chain lane corresponds to free kappa light chain (Figure 3).

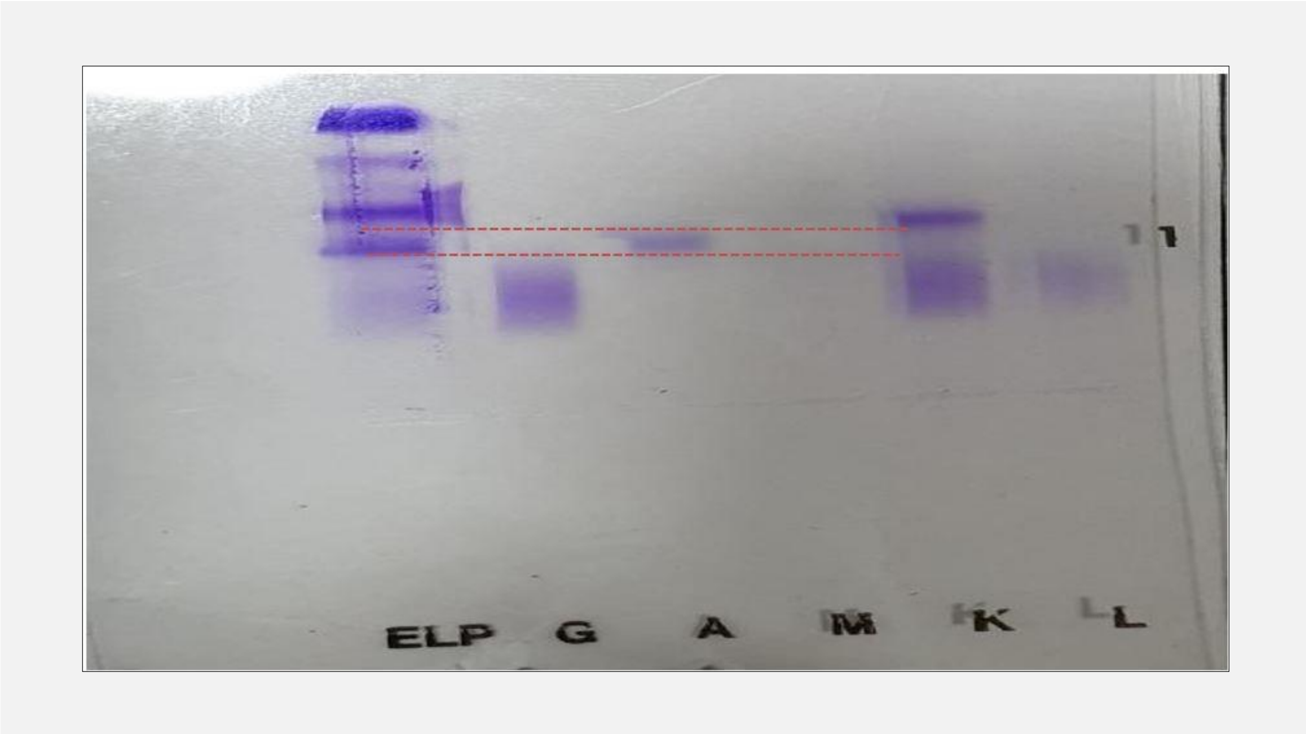


Figure 2. Serum immunofixation test showing a mismatch between the bands in the IgA heavy chain lane and kappa light chain lane.

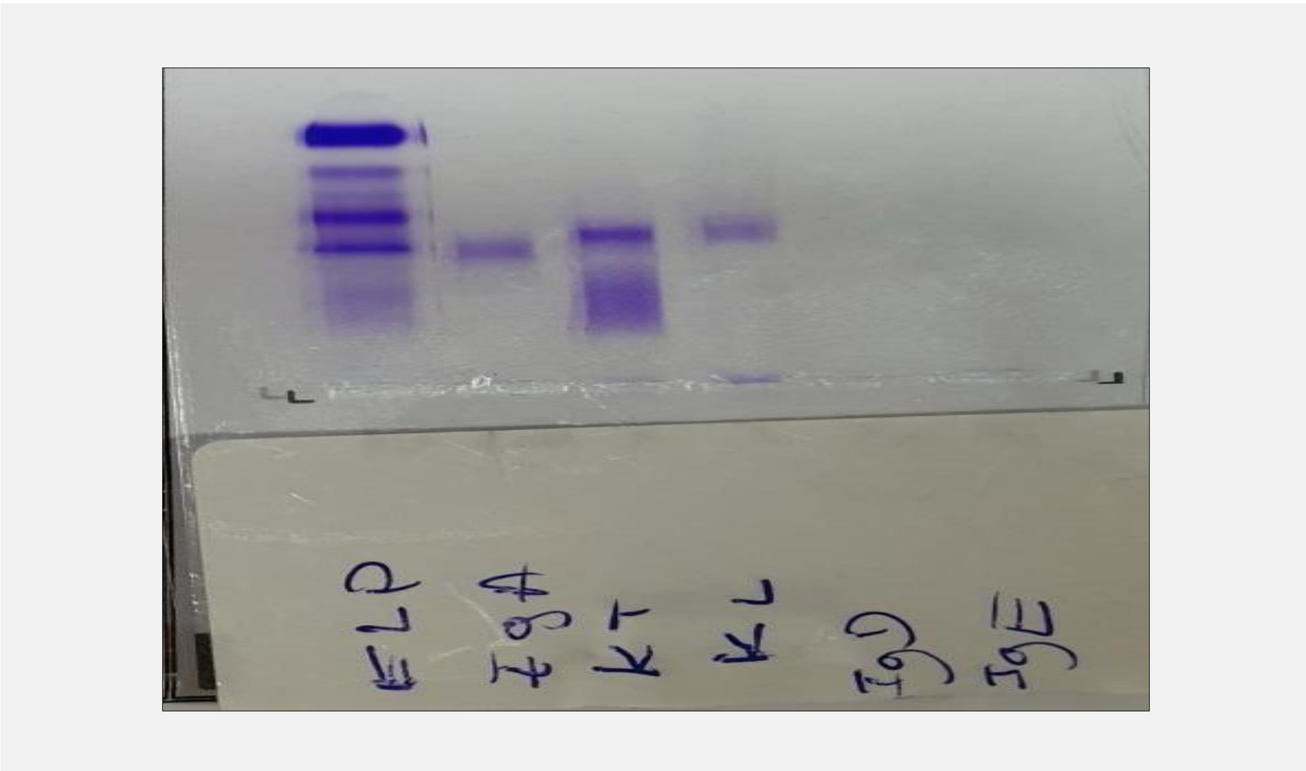


Figure 3. Serum immunofixation test using anti IgA, total kappa, free Kappa, IgD and IgE antisera: Total kappa and free kappa show the same level of migration.

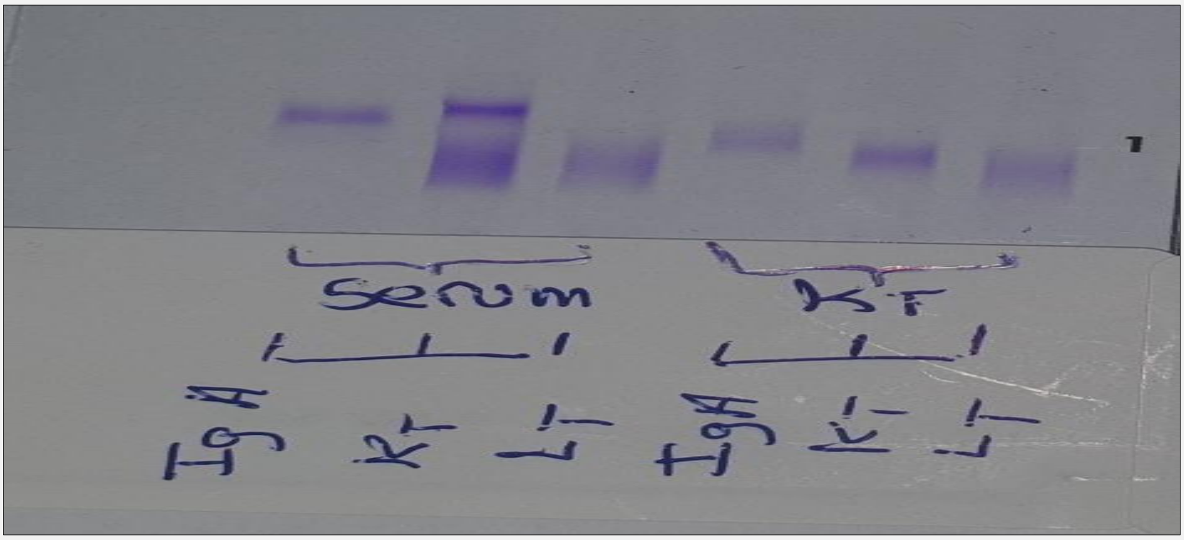


Figure 4A. The first 3 lanes correspond to serum immunofixation without incubation with anti-total kappa antiserum.

The last 3 lanes correspond to serum immunofixation after incubation with anti-total kappa antiserum and show the disappearance of monoclonal bands in the IgA heavy chain lane and total kappa light chain lane.

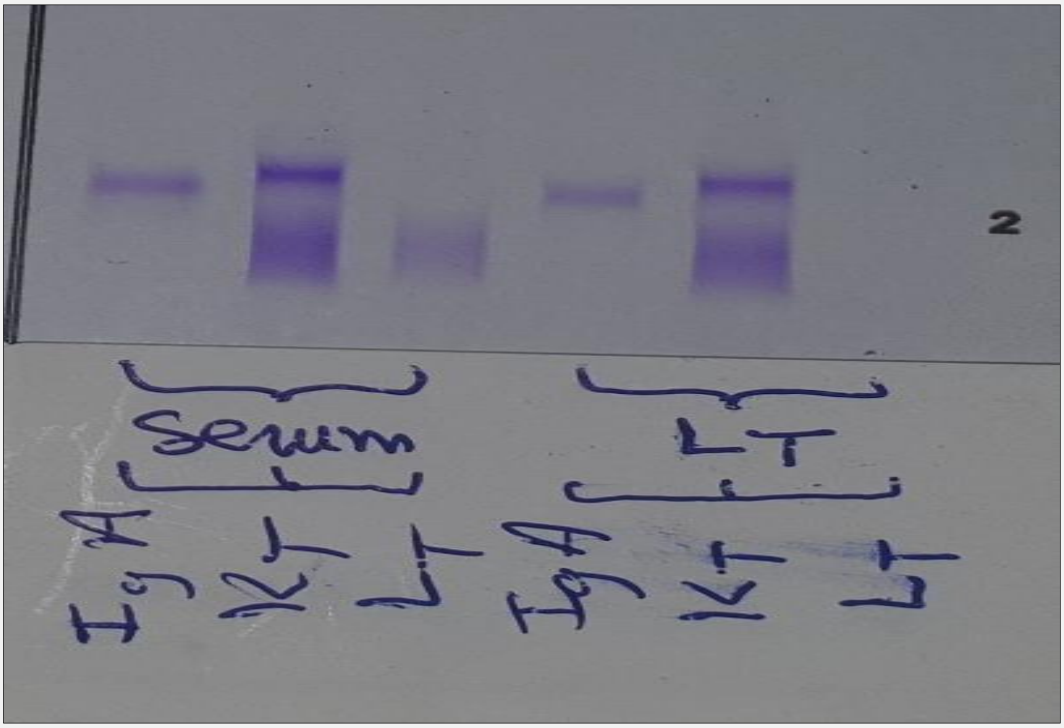


Figure 4B. The first 3 lanes correspond to serum immunofixation without incubation with anti-total Lambda antiserum.

The last 3 lanes correspond to serum immunofixation after incubation with anti-total Lambda antiserum and show the disappearance of the polyclonal back ground in Lambda.

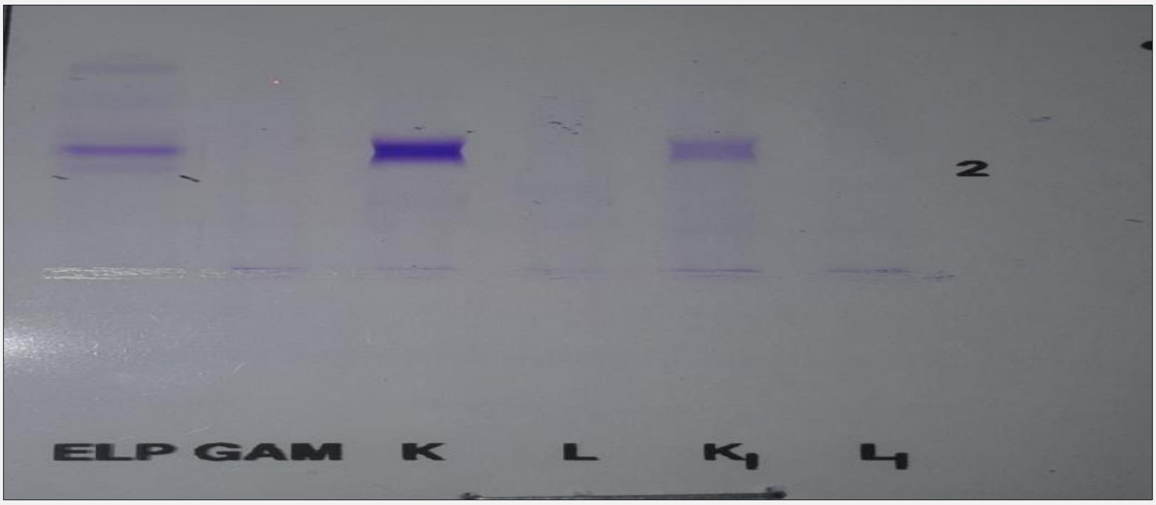


Figure 5. Urine immunofixation test showing the presence of a monoclonal band in the kappa free light chain lane.

To distinguish between heavy chain disease and immunoglobulin with "masked" light chains, and according to a protocol defined by the Sebia® Company, two tubes containing the patient's serum were incubated with a very high concentration of anti-total kappa and anti-total lambda antisera for 48 hours at 4°C in order to facilitate immunoprecipitation of the involved light chain. After centrifugation, the supernatant was analyzed by using the IFs method on the Hydrasys 2 Scan Focusing Sebia® without dilution, and then we applied the anti-IgA, anti-total kappa and anti-total lambda antisera.
The serum immunofixation test of the sample treated with a high concentration of anti-total kappa showed the disappearance of the monoclonal bands corresponding to IgA heavy chain lane and kappa light chain lane, indicating that precipitation had occurred and that the IgA did have kappa light chains that could not be detected by the standard immunofixation protocol (Figure 4A). The serum immunofixation test of the sample treated with anti-total lambda showed the disappearance of the polyclonal background in lambda light chain lane, confirming that the precipitation with lambda light chains according to the previously mentioned protocol has done well (Figure 4B).
The urine immunofixation test revealed monoclonality for kappa free light chain (Figure 5). In conclusion, this patient has IgA kappa multiple myeloma with kappa free light chains.

DISCUSSION

The immunofixation test is generally easy to interpret; however, it fails to determine the type of light chain of some monoclonal immunoglobulins (MI) having a change in their molecular configuration, this is the case of "non-reactive" light chains that prevent the precipitation reaction with anti-light chain antisera. In rare cases, IMs can adopt a structural conformation in which the reaction with antiserum against light chains does not occur or occurs very weakly, and this is due to accessibility of the epitopes recognized by the antiserum [1]. We illustrate here the importance of serum incubation with a high concentration of anti-kappa and anti-lambda light chain antiserum in two tubes for 48 hours to facilitate immunoprecipitation of the involved light chain and to distinguish between heavy chain disease and immunoglobulin with "masked" light chains.
This simple, reproducible protocol can be used to characterize monoclonal immunoglobulins that do not precipitate using anti-light chain antisera [2].
Previously, the techniques used for the detection of masked light chains were immunoselection, separation, isolation of monoclonal protein from serum, reduction and alkylation technique. Immunoselection is a simple and reproducible method while the reduction and alkylation techniques are sensitive and reliable. All these techniques are time-consuming, expensive and cumber some [1-5].
The IgA myeloma with masked free light chains remains a very rare entity. We describe in this article the first Moroccan case of IgA multiple myeloma associated with a masked kappa light chain. To our knowl-

edge, 2 cases have been reported in the literature. The first case was described by Hashimoto N et al. in 1970, regarding a 64-year-old patient, in which the revelation of masked kappa light chains associated with IgA is carried out after reduction and alkylation of purified paraprotein. In the same study, the authors suggested that the inability to detect the specificity of the kappa light chain in IgA paraprotein is due to the paraprotein tertiary structure which differs slightly from normal IgA proteins compared to most monoclonal proteins and to the folding of the polypeptide chains. Therefore, kappa light chain determinants may be "hidden" by these structural alterations; these light chains become unavailable to react with a specific anti-kappa antiserum [2]. The second case is an IgA multiple myeloma with a masked lambda light chain, described by Netto et al. in 1981 [1]. The detection technique was immunoselection.
In addition, 5 cases of immunoglobulin D with masked light chains have been reported in the literature: By Cejka et al., Vladutiu AO, Netto et al., and Spira G et al. [1,3-5]. The most recent case was described in 2018 at the military hospital in Rabat. The demonstration of the masked light chain was performed by using immunoprecipitation technique following the protocol described in this article [6].

CONCLUSION

The serum immunofixation test is a simple and quick technique to perform; however, its result is not easy to interpret. This case illustrates some of the difficulties encountered and the corrective actions that can be taken for the detection of immunoglobulins with masked light chains.

Declaration of Interest:
The authors declare no conflict of interest.

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