Monoclonal Light Chains with alpha 2 mobility on Serum Protein Electrophoresis

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Abstract

Multiple myeloma (MM) is a neoplasm characterized by malignant proliferation of plasma cells that produce excessive quantities of a single type of immunoglobulin (Ig) called as monoclonal immunoglobulin or M-protein or paraprotein. M-protein produced can be either an intact antibody with both heavy and light-chain components or only light chains or rarely only heavy chains. Presence of M-protein in serum protein electrophoresis (PEP) is useful in the diagnosis, prognosis, and treatment of MM and other plasma cell dyscrasias. These M-proteins are identified commonly in beta and gamma regions and very rarely in alpha 2 region, appearing as a narrow band in agarose electrophoresis or as a sharp symmetric spike (M-spike) or peak in capillary zone electrophoresis. Here, we present an unusual case of monoclonal light chains producing two M- spikes in the alpha 2 globulin region in capillary zone electrophoresis.

Introduction

Multiple myeloma (MM) is a neoplasm of B cell lineage which is characterized by excessive proliferation of abnormal plasma cells. These abnormal plasma cells secrete a specific immunoglobulin type referred to as monoclonal immunoglobulin (Ig) that leads to a condition called monoclonal gammopathy. Serum protein electrophoresis is often used as a preliminary test in suspected cases of multiple myeloma to detect the presence of M-protein, which when present produces a narrow band in agarose electrophoresis or a sharp symmetric spike (M-spike) or peak in capillary zone electrophoresis (1). Serum protein electrophoresis is a technique used to separate serum proteins into different fractions depending on their size and the charge they carry in a buffer medium under the influence of an electric field (2). Zone electrophoresis refers to the migration of charged molecules of proteins in a porous support medium like agarose, cellulose acetate, capillaries, etc., such that each protein zone is sharply separated from the neighbouring zone by a protein free area (3).

Capillary zone electrophoresis (CZE) is a technique in which the buffer filled capillary tube is used as the support medium. By the application of high voltage, serum proteins are separated into 6 fractions viz. albumin, alpha 1(α-1), alpha 2(α-2), beta 1(β-1), beta 2 (β-2), and gamma (γ) globulin regions. The paraproteins (M-proteins), when present, produce a sharp spike in any of the globulin regions in CZE (2, 4). M-protein spike is generally observed in gamma or

beta region and very rarely seen in alpha 2 globulin region (5). Here, we present a case of monoclonal light chain producing M-spikes in alpha 2 globulin region on the protein electrophoresis performed using CZE technique.

Case details

A 64 year-old male presented to the orthopedic out-patient department with complaints of lower backache. He was evaluated at home town where the lumbosacral spine X-ray showed lumbar spondylosis. MRI-lumbosacral spine showed at L4-L5 and L5 level a broad posterior disc osteophyte complex causing canal stenosis with bilateral facetal hypertrophy contributing to lateral recess stenosis and moderate compression of bilateral traversing nerve roots. Abnormal marrow signal intensity in D10 vertebra with bulging of the posterior cortex with epidural soft tissue thickening causing canal stenosis and significant compression of the traversing nerve roots was also noticed. Based on the MRI findings a serum protein electrophoresis was ordered. Serum protein electrophoresis by capillary zone electrophoresis performed in minicap (Sebia) automated analyzer showed, hypogammaglobinemia with two small peaks in alpha 2 region (Figure 1). The laboratory, based on the findings of the protein electrophoresis, suggested to perform serum free light chain assay and serum immunotyping to confirm that the spikes in alpha 2 region in PEP were due to paraprotein. The results of the investigations done are as follows (serum free light chains were performed using Binding site- free lite kit in Optilite analyzer):

- Serum free kappa : 17,052 mg/L (reference interval : 3.3 – 19.4 mg/L)
- Serum free lambda : 11.69 mg/L (reference interval : 5.7 – 26.3 mg/L)
- kappa/lambda ratio : 1458.7 (reference interval : 0.26 -1.65)

Serum immunotyping, performed in minicap (Sebia) automated analyzer, showed both the peaks in alpha 2 region to be kappa free light chains (Figure 2) .The patient was further investigated with PET CT whole body, which showed multiple ill-defined lytic bony lesions in axial skeleton with few lesions showing low-grade FDG uptake. Bone marrow aspiration cytology showed diffuse plasmacytosis (23% plasma cells) composed of mature, few immature plasmablasts, binucleate, occasional trinucleate and quadrinucleate forms consistent with plasma cell myeloma. Bone marrow trephine biopsy revealed a hypercellular marrow with diffuse plasmacytosis consistent with myeloma marrow. The other relevant biochemistry and haematology investigations include, Serum beta-2 microglobulin : 8.54 mg/L (724.34 nmol/L), serum protein : 5.9 g/dL (59 g/L), serum albumin : 4 g/dL (40 g/L), globulin : 1.9 g/dL (19 g/L), serum calcium : 8.2 mg/dL (2.05mmol/L), serum creatinine: 1.1 mg/dL (97.24 micromole/L), serum urea: 43 mg/dL (7.16 mmol/L) and Hemoglobin: 6.5 gm/L. Patient was diagnosed to have multiple myeloma and was started on Chemotherapy using VRD regimen with bortezomib, lenalidamide and dexamethasone, planned for 6 cycles. Each cycle had bortezomib given intravenously on days 1, 8, 15 and 22, dexamethasone given intravenously on days 1, 8, 15, and 22. Lenalidamide was given orally once daily from day 1 to day 22. Follow up serum protein electrophoresis after initiation of 1st cycle of chemotherapy showed that the peak in the alpha 2 region had disappeared (Figure 3) with corresponding serum free kappa level decreasing to 2123 mg/L . Serum immunotyping was negative for kappa free light chains (Figure 4).

Discussion:

Multiple myeloma is the second-most common hematological malignancy and is characterized by excessive proliferation of abnormal plasma cells that secretes monoclonal immunoglobulins (M- protein) (6, 7). Serum protein electrophoresis (PEP) to identify the presence of M- protein and immunofixation electrophoresis (SIFE) or immunotyping to identify the type of monoclonal immunoglobulin that is involved in the disease process are commonly used as screening tests in the diagnosis of multiple myeloma. Other investigations that are usually done include quantification of serum free light chain, urine protein electrophoresis and urine immunofixation electrophoresis for Bence Jones protein. Bone marrow aspirate and trephine biopsy are obtained for carrying out cytogenetic, fluorescent in situ hybridization (FISH) and immunophenotyping studies (8).

Relative reduction or increase in M-protein concentration or their disappearance in serum protein electrophoresis, is one of the parameter of the Uniform Response Criteria (IURC) designed by the International Myeloma Working group (IMWG) to guide the management of patients with multiple myeloma (9). Therefore, identification of paraproteins in PEP is a critical diagnostic step to further guide the investigations and design the management in patients with MM. M-proteins produced in multiple myeloma are either an intact immunoglobulin with both heavy and light-chain components or only light chains or rarely only heavy chains. In the intact immunoglobulin type, the heavy chain is from one of the five immunoglobulin classes G, A, M, D or E, while the light chain is either κ (kappa) or λ (lambda)(10). The commonest immunoglobulin class involved in MM is IgG followed by IgA (as intact Immunoglobulins) and only light chains comprises 15% of patients with MM (7). In electrophoretogram, these M-proteins are commonly observed in gamma-globulin region. They are also observed in β-2 and β-1 regions in diminishing order of frequency and rarely seen in α-2 globulin region. Generally monoclonal intact immunoglobulins of type IgA, IgG and IgM are not observed on the α-2 region and are usually observed in γ, β-1, and β-2 regions (10). Normally the proteins that migrate in α-2 fractions include alpha-2 macroglobulin, ceruloplasmin and haptoglobulin. These are acute phase reactants that are raised in inflammatory conditions. Hence a tall peak in α-2 region in CZE may occur in inflammatory disorders and in nephrotic syndrome (due to increased alpha-2 macroglobulin) closely resembling a monoclonal peak. A radio contrast dye used for imaging studies also produces a spike or a split in this region in CZE, mimicking a paraprotein(11). In vitro hemolysis and haptoglobins of different phenotypes may induce a split in the α-2 globulin region raising

a suspicion for M-protein (12). Although the abnormal patterns observed in α-2 region are more common due to the above mentioned conditions yet, those produced by the paraprotein should not be missed. The case in discussion here had two small spikes in α-2 region and was present along with hypogammaglobinemia. This led to the suspicion of paraprotein which was confirmed to be due to monoclonal kappa light chains by serum immunotyping. Presence of two M-spikes in α-2 globulin region due to monoclonal light chains has not been reported so far. Literature search showed that the M- proteins migrating to α-2 region in electrophoresis are few in numbers and such migrations had IgA preponderance (13, 14). For the case in discussion, the two M spikes on the α-2 region were shown to be because of kappa free light chains (Fig : 2). Most of the time, in light chain myeloma, spikes are often not observed in serum protein electrophoresis, except for the presence of hypogammaglobinemia. In our case too, the peak produced by high concentration of monoclonal kappa (17,052 mg/L) in PEP disappeared and serum immunotyping showed absence of monoclonal gammopathy once the concentration of kappa free light chains decreased to 2123 mg/L. The reason for two M-spikes observed in our case can be attributed to polymerization of monoclonal light chains (kappa) that was present at high concentration (17,052 mg/L) in serum. Their alpha 2 mobility can be reasoned out to be due to their low molecular weight compared to intact immunoglobulins (15).

Conclusion

M- protein with alpha 2 mobility though rare can still occur and produce a spike in alpha 2 region in serum protein electrophoresis. These paraprotein spikes in alpha 2 may be overlooked and mistaken for spikes that are usually produced by components due to various other causes. Paraprotein should be suspected and investigated further when abnormal pattern or small spike in alpha 2 region coexists with hypogammaglobinemia. Immunotyping is performed with specific antisera against IgG, IgA, IgM heavy chains and against free and bound Kappa and Lambda light chains. The superimposition of the antisera pattern allows for visualization of the disappearance and / or decrease of monoclonal fraction on the antisera pattern and to indicate a gammopathy.

Authors' contribution

Dr. Danalakshmi S: Conception of the idea, drafting the article, Critical revision and final approval of the version to be published

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Conflicts of interest

There are no conflicts of interest.

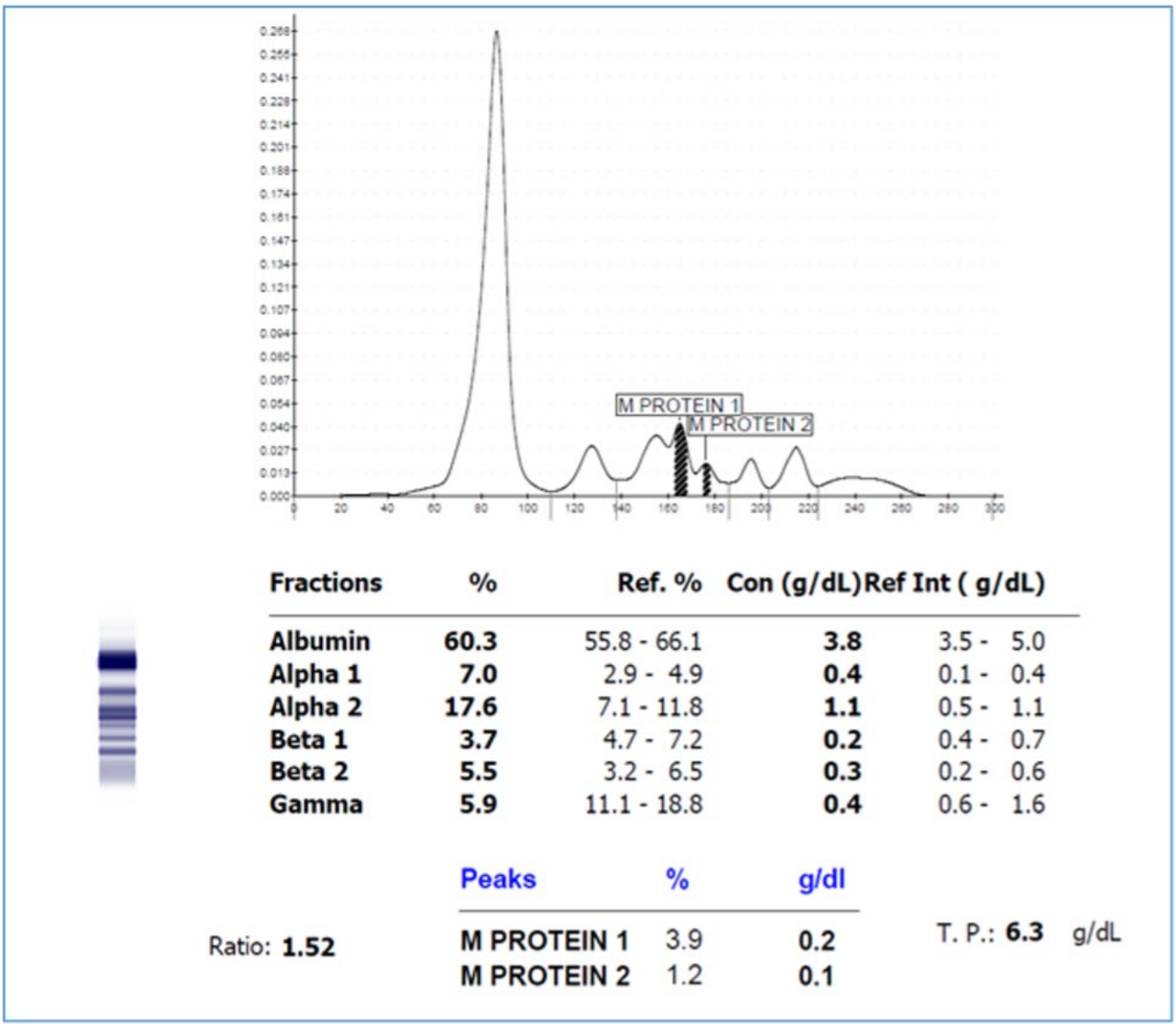

Figure 1: Electrophoretogram of capillary zone electrophoresis.
Serum protein electrophoresis, done using Sebia- Minicap, shows two M-protein spikes in alpha 2 region and hypogammaglobinemia

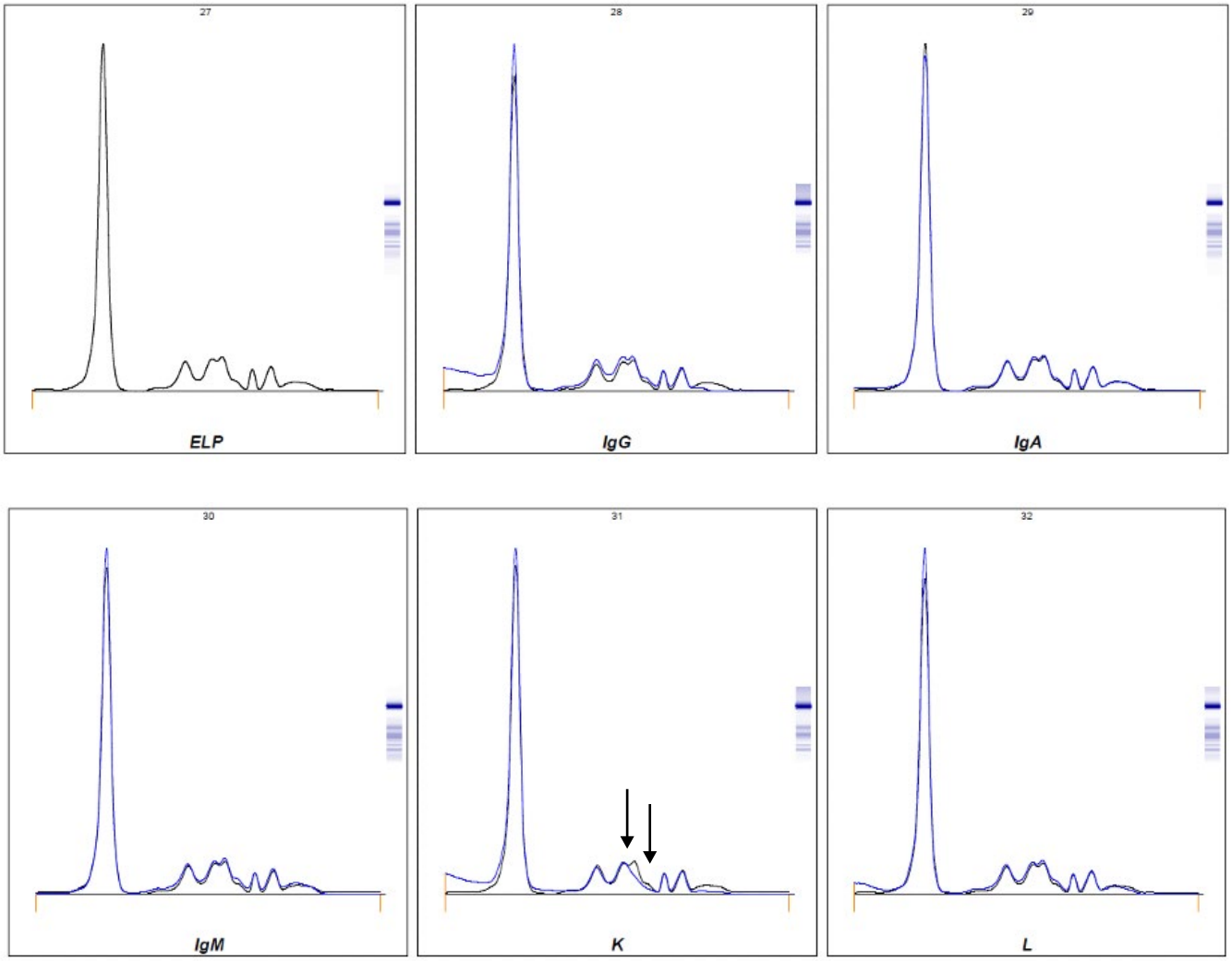

Figure 2: Serum protein immunotyping using Sebia minicap, shows immunosubtraction (two arrow marks) in kappa confirming the spikes to be free kappa light chains (monoclonal Kappa component presenting with disappearance of the kappa antisera pattern as compared to ELP pattern).

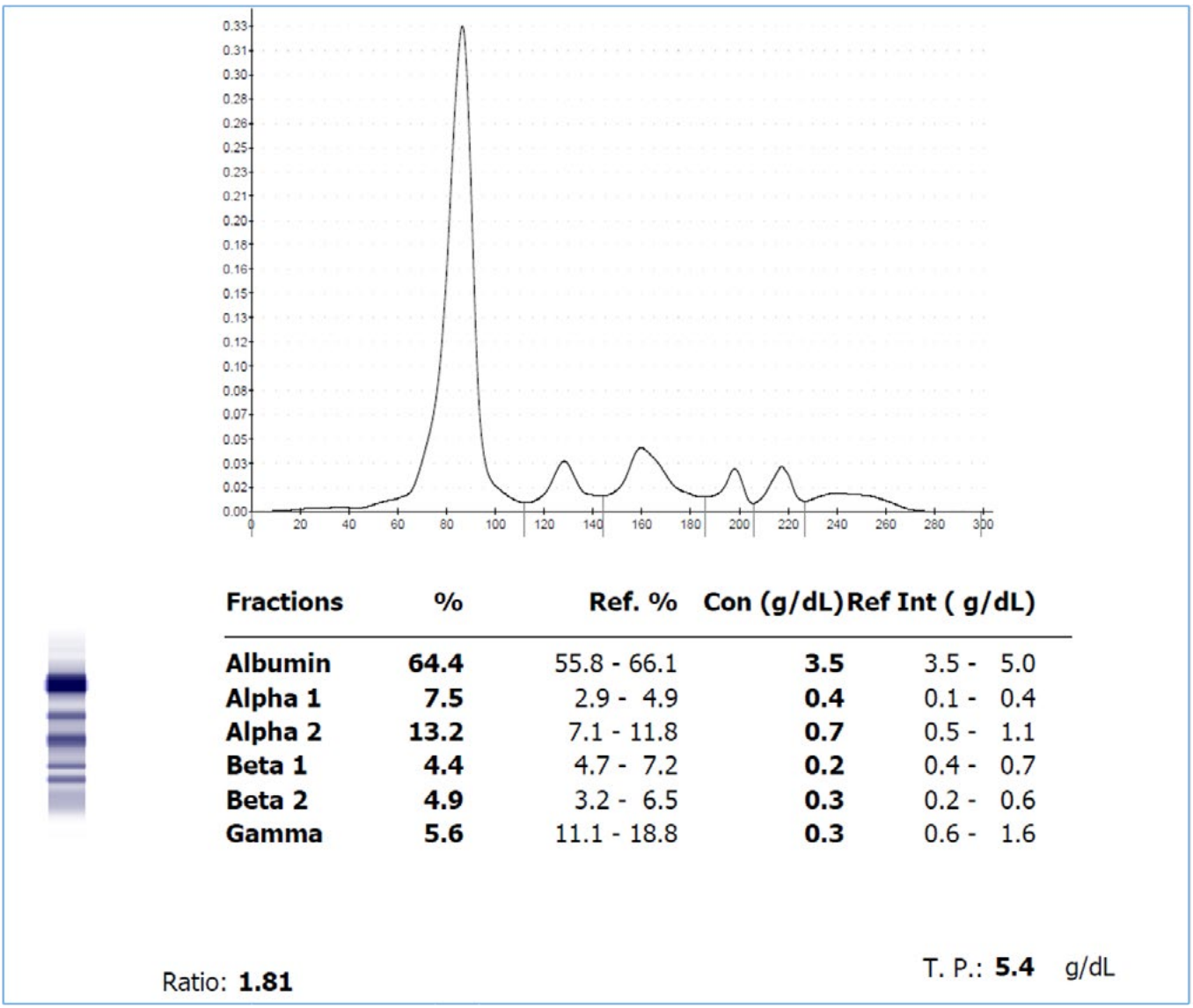

Figure 3: Serum protein electrophoresis (done using Sebia- Minicap, capillary electrophoresis) – post initiation of first cycle of chemotherapy, shows absence of M-spikes in alpha 2 globulin region.

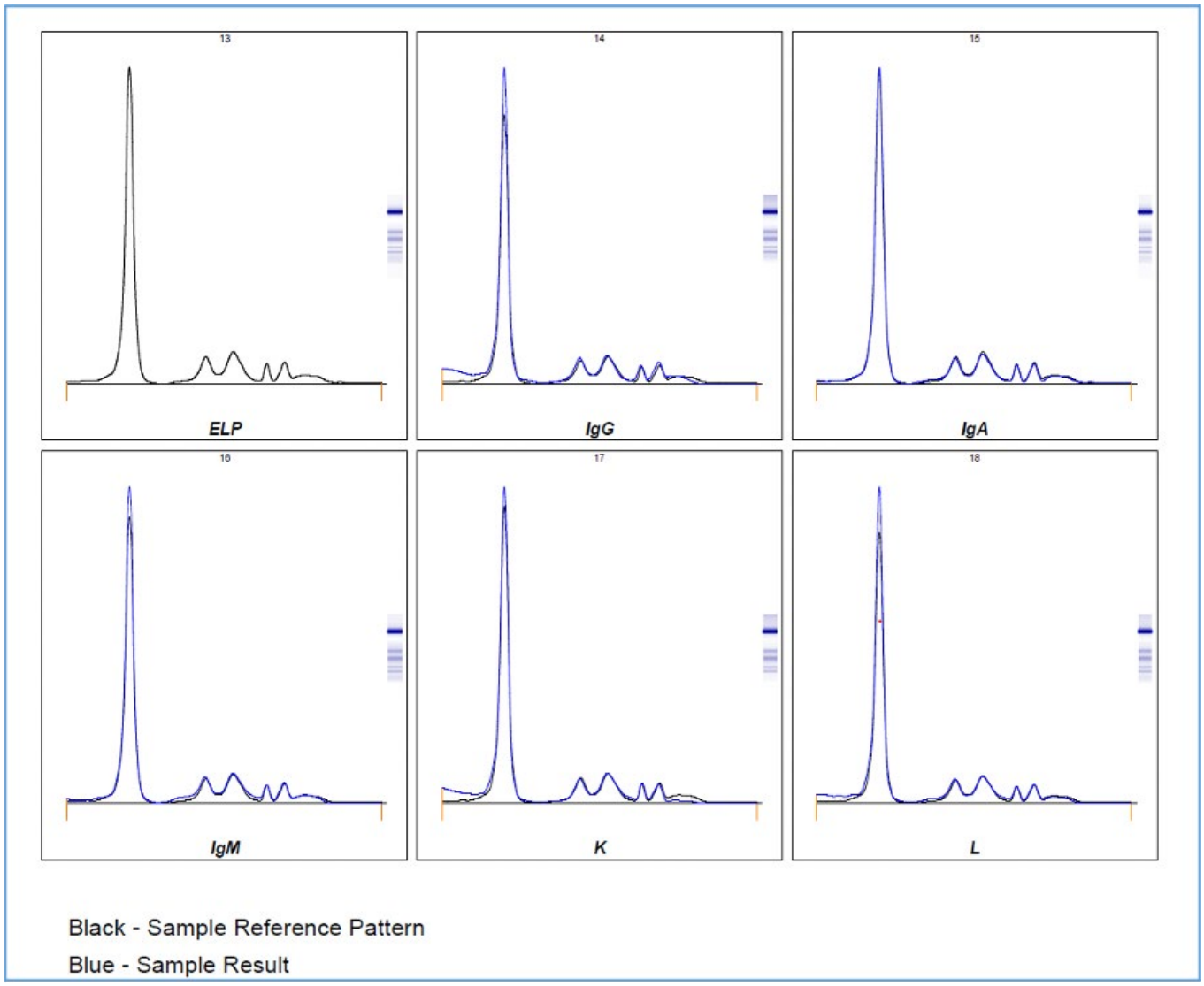

Figure 4: Serum protein immunotyping (post initiation of first cycle of chemotherapy) using Sebia minicap shows absence of monoclonal gammopathy.

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