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# BICLONAL GAMMOPATHIES: RARE PRESENTATIONS

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**ABSTRACT**

**Backgrounds:** Biclonal gammopathies are defined as a clonal proliferation of plasma cells or B-lymphoid progenitors that produces two distinct M bands due to either proliferation of two separate clones of plasma cell, each producing an unrelated monoclonal Ig or it may result from a single clone of plasma cell producing two monoclonal proteins. Biclonal myeloma accounts for approximately 1% of newly diagnosed case of multiple myeloma. These neoplastic clones which secrete one type of M protein might undergo isotype switching resulting in secretion of another subtype of M protein by the same clones producing biclonal spikes. Clinical presentation and treatment are usually similar to that of monoclonal gammopathy with comparable survival outcome. **Aim:** To study rare cases of multiple myeloma with biclonal gammopathies where clinical data is still lacking in the literature. **Methods:**This is a retrospective study including 4 case of biclonal gammopathies Complete blood count along with routine Biochemical investigations were performed. Agarose gel protein electrophoresis was done on Helena Bioscience, Europe using SAS-MX SP-10 SB kit & serum Immunofixation Electrophoresis by SAS-MX IFE 10 SB kit. The results were analysed using Helena software platinum 6.0.100. B-2 Microglobulin, free light chain ratio and immunoglobulin profiles along with Bone marrow aspiration, Bone marrow Biopsy and Flow cytometery were done in all cases. **Results:** Ist case showed two M spike in gamma globulin region on SPE, Second case showed M-spike in Beta and gamma globulin region on SPE and IgA kappa and IgG kappa on IFE. Third case had two M-bands in gamma heavy chain with corresponding bands in kappa light chain region on IFE. Fourth case was with two separate peaks in gamma globulin region on SPE and 2 distinct bands as IgG kappa and IgG lambda on IFE. **Conclusions:** Biclonalitydetermined by presence of para-proteins is considered to represent clonal evolution and may therefore, be a determinant of disease prognosis. The etiology of bi-clonal gammopathy is not yet elucidated; therefore it is necessary to follow the evolution of the clones by more advanced research focused on the genetic study of these clones to better understand the physiopathology and clinical behavior of biclonal Gammopathies.



## INTRODUCTION

Biclonal gammopathies manifestations (BGMs) are defined as a clonal proliferation of plasma cells or B-lymphoid progenitors that produces two distinct M bands due to either proliferation of two separate clones of plasma cell, each producing an unrelated monoclonal Ig or it may result from a single clone of plasma cell producing two monoclonal proteins. Biclonal myeloma is a rare entity and accounts for approximately 1% of newly diagnosed case of multiple myeloma.1 These neoplastic clones which secrete one type of M protein might undergoisotype switching resulting in secretion of another subtype of M protein by the same clones producing biclonal spikes.2Clinical presentation and treatment are usually similar to that of monoclonal gammopathywith comparable survival outcome.

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**Case I: Biclonal M spike IgG multiple Myeloma**

A 69 year old male got admitted at tertiary care Hospital for supportive care. On physical examination, patient was alert, active, oriented to person, place, time. Routine lab investigation along with specialized testing were carried out.

On investigation Complete blood count shows haemoglobin 7.3 g/dL, hematocrit 22.1%, RBC 2.23L $10^6/mm^3$,%, WBC 6.1 $10^3/mm^3$ and Platelet 96 $10^3/mm^3$. Serum protein was 9.72 g/dl and albumin 3.21 g/dl, globulin 6.51 g/dL with A:G ratio 0.49. Serum beta 2 microglobulin was 3.26 mg/L and serum

calcium was 8.29 mg/dl. Serum Urea and creatinine was 40.02 and 4.45 mg/dl respectively. GFR by MDRD

calculator was 13.3 ml/min/1.73 $m^2$ .

Agarose gel protein electrophoresis was done on Helena Bioscience, Europe using SAS-MX SP-10 SB kit. On Agarose gel protein electrophoresis total protein: 9.18 g/dl, albumin: 3.19 g/dl, alpha-1: 0.38 g/dl, alpha-2: 0.66 g/dl, beta: 0.49 g/dl, gamma: 4.46 g/dl. Serum protein electrophoresis shows evidence of hypoalbuminemia, low beta globulin with biclonal M spike present in gamma globulin region. Concentration of M spike (Shaded area) is calculated from electrophoretogram is respectively 0.34gm/dl and 3.46 gm/dl.The results were analysed using Helena software platinum 6.0.100. (Figure 1).

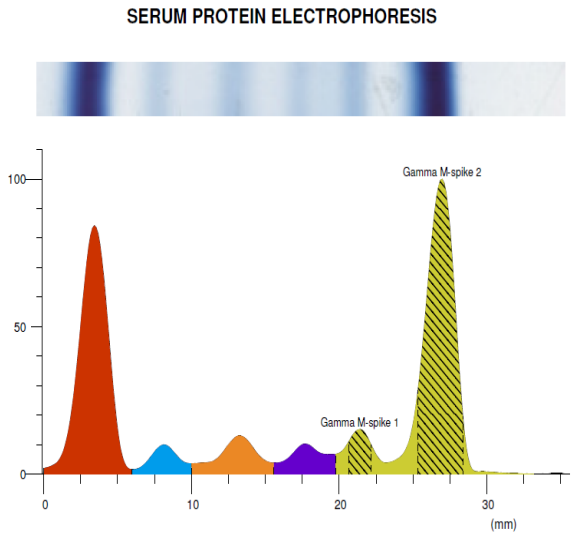


**Figure 1.** Showing two M-spike in gamma globulin region on SPE

Lambda free light chains levels were elevated with value of 36.75 mg/L and kappa free light chain levels were within normal range with value of 8.47 mg/L while Kappa/ Lambda (FLC) ratio was decreased with value of 0.23.

Immunoglobulin panel showed an normal levels IgG and IgM of 42.33 mg/dL and 1464.27 mg/dL respectively. Serum IgA a levels were decreased with value of 46.45 mg/dL.

Peripheral Blood smear shows Normocytic RBCs. TLC is adequate with unremarkable differential count. Platelets were adequate on smear. No hemoparasites seen. No abnormal cells seen.

Bone marrow (BM) aspiration showed markedly hemodiluted marrow showing occasional marrow particles only. Erythroid series shows occasional normoblastic maturation Myeloid series showed neutrophilic maturation. Megakaryocytes were not seen. Plasma cells were predominatly singly scattered accounting for 8-9% of all marrow nucleated cells.

Bone marrow (BM) Biopsy showed a hypercellular marrow for age [Cellularity – 60-80% with trilineagehematopoiesis and sheets & aggregates of mature & immature plasma cells.(75-80% of marrow cells).] Erythroid series show normoblastic maturation. Myeloid series show complete maturation (Figure 2). Megakaryocytes were present. The Reticulin stain was Grade MF-0 while Iron stain was 1+. Immunohistochemistry Markers were performed and the neoplastic cells were found immunoreactive for CD38, CD 138, and lambda light chain. The final morphologic and immunohistochemical diagnosis was of Plasma cell myeloma with lambda light chain restriction.

IMMUNOHISTOCHEMISTRY MARKERS

CD38 - [EP135] - Immuno reactive score 4+ in marrow cells (75 to 80%)

CD138 - [EP201] - Immuno reactive score 3+ in neoplastic cells.

CD56 - [123C3] - Immuno reactive score 4+in plasma cells

Kappa light chain - [ISH] - Non Immuno reactive score '0' in plasma cells

Lambda light chain - [ISH] - Immuno reactive score 4+ in plasma cells

Flow cytometery showed >5% plasma cells coexpress CD38, CD138 and are lambda restricted, CD19 negative but positive for CD117, CD56 & CD 86.

**Case 2: Biclonal Gammopathywith IgA kappa and IgG kappa (IgG-IgA isotype combination)**

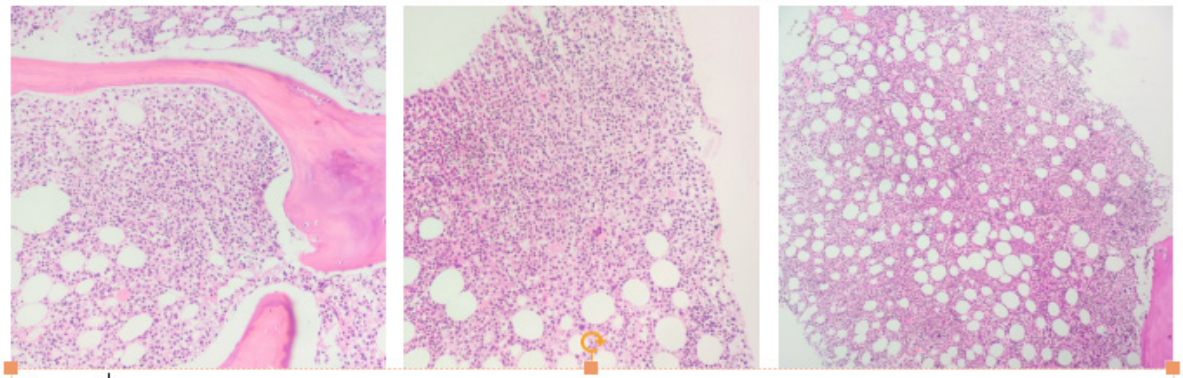

Figure 2:-
Photomicrogrpahs show a hypercellular marrow for age [Cellularity – 60 to 80%] with trilineage hematopoiesis & sheets & aggregates of mature & immature plasma cells (75-80% of marrow cells).

A 53 year old female presented in OPD of tertiary care with complaint of swelling in bilateral limb. On physical examination, patient was alert, active, oriented to person, place, time. Routine lab investigation along with specialized testing were carried out.

| Table 1: Showing Demographic and Clinical Investigation of cases | | | | | |
|---|---|---|---|---|---|
| **S.No.** | **Parameters** | **Case 1** | **Case 2** | **Case 3** | **Case 4** |
| 1 | Age/Sex | 69Y/M | 53Y/F | 41Y/M | 59Y/M |
| 2 | Hb (g/dL) | 7.3 | 6.8 | 14.2 | 8.3 |
| 3 | RBC (106/mm3) | 2.23 | 3.53 | 4.74 | 2.36 |
| 4 | WBC (103/mm3) | 6.1 | 19.8 | 4.3 | 2.1 |
| 5 | Platelet (103/mm3) | 96 | 68 | 135 | 90 |
| 6 | Total Protein (g/dL) | 9.72 | 5.9 | 9.16 | 7.5 |
| 7 | Albumin (g/dL) | 3.21 | 2.8 | 4.71 | 3.62 |
| 8 | Serum Calcium (mg/dL) | 8.29 | 8.29 | 9.60 | 8.45 |
| 9 | Urea(mg/dL) | 40.02 | 122.41 | 13.06 | 41.74 |
| 10 | Creatinine(mg/dL) | 4.45 | 8.63 | 0.71 | 1.07 |
| 11 | Beta 2 microglobulin (mg/L) | 3.26 | 4.73 | 1.71 | 5.76 |
| 12 | Kappa light chain (mg/L) | 8.47 | 186.0 | 41.25 | 103.44 |
| 13 | Lambda light Chain (mg/L) | 36.75 | 515.45 | 10.81 | 70.72 |
| 14 | IgA (mg/dL) | 46.45 | 164.19 | 49.06 | 139.133 |
| 15 | IgM (mg/dL) | 42.33 | 45.1 | 37.2 | 94.92 |
| 16 | IgG (mg/dL) | 1464.27 | 1503.67 | 1624.1 | 1770.96 |

On investigation Complete blood count shows haemoglobin 6.8 g/dL, hematocrit 21.9%, RBC 3.53 L $10^6$/mm$^3$,%, WBC 19.8 $10^3$/mm$^3$ and Platelet 68 $10^3$/mm$^3$. Serum protein was 5.9 g/dl and albumin 2.8 g/dl, globulin 3.1 g/dL with A:G ratio 0.9. Serum beta 2 microglobulin was 3.26 mg/L and serum calcium was 8.29 mg/dl. Serum Urea and creatinine was 122.41 and 8.63 mg/dl respectively. GFR by MDRD

calculator was 4.9 ml/min/1.73 m$^2$ .

Agarose gel protein electrophoresis was done on Helena Bioscience, Europe using SAS-MX SP-10 SB kit. On Agarose gel protein electrophoresis total protein: 5.23 g/dl, albumin: 2.03 g/dl, alpha-1: 0.33 g/dl, alpha-2: 0.70 g/dl, beta: 1.25 g/dl, gamma: 0.92 g/dl. Serum protein electrophoresis shows

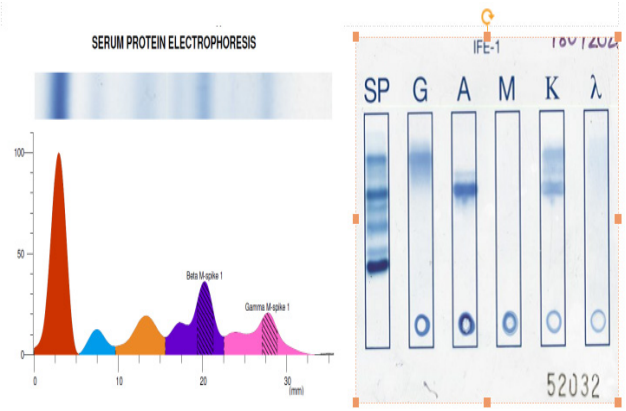


**Figure 3.** Showing M-spike in Beta and gamma globulin region on SPE and IgA kappa and IgG kappa on IFE

Lambda free light chains levels were elevated with value of 515.45mg/L and kappa free light chain levels were also raised with value of 186.0 mg/L while Kappa/ Lambda (FLC) ratio was normal with value of 0.36.

| **Table 2**. IHC (Immunoreactive score in marrow cells) | | | | |
|---|---|---|---|---|
| **Marker** | **Case 1** | **Case 2** | **Case 3** | **Case 4** |
| CD 38 | 4+ | 4+ | 4+ | 4+ |
| CD 138 | 3+ | 4+ | 3+ | 4+ |
| CD 56 | 4+ | 4+ | 0 | 0 |
| kappa light chain | 0 | 4+ | 4+ | 3+ |
| lambda light chain | 4+ | 3+ | 0 | 4+ |

**Case 3: Gammopathy with 2 monoclonal bands both identified as IgG kappa (IgG-IgG isotype combination)**

A 41 year old male presented with complaint of pain in back since last one month. On physical examination, patient was alert, active, oriented to person, place, time. Routine lab investigation along with specialized testing were carried out.

On investigation Complete blood count shows haemoglobin 14.2 g/dL, hematocrit 43.8%, RBC 4.74 L $10^6$/mm$^3$,%, WBC 4.3 $10^3$/mm$^3$ and Platelet 135 $10^3$/mm$^3$. Serum protein was 9.16 g/dl and albumin 4.71 g/dl, globulin 4.45 g/dL with A:G ratio 1.06. Serum beta 2 microglobulin was 1.71 mg/L and serum calcium was 9.60 mg/dl. Serum Urea and creatinine was 13.06 and 0.71 mg/dl respectively. GFR by MDRD

calculator was122.3 ml/min/1.73 m$^2$ .

Agarose gel protein electrophoresis was done on Helena

Bioscience, Europe using SAS-MX SP-10 SB kit. On Agarose gel protein electrophoresis total protein: 9.16 g/dl, albumin: 4.75 g/dl, alpha-1: 0.19 g/dl, alpha-2: 0.67 g/dl, beta: 1.14 g/dl, gamma: 2.41 g/dl. Serum Protein Electrophoresis shows evidence of hypergammaglobulinemia with slight notch in gamma globulin region.The results were analysed using Helena software platinum 6.0.100. (Figure 1). To reveal the nature of clonality or suspicious band, serum Immunofixation study was performed. High resolution serum immunofixation electrophoresis shows two bands in IgG heavy chain region with 2 corresponding bands in kappa light chain region suggestive of biclonal gammopathy (Figure 4). Such pattern is very rare if we consider the various immunofixation patterns observed in different gammopathies.

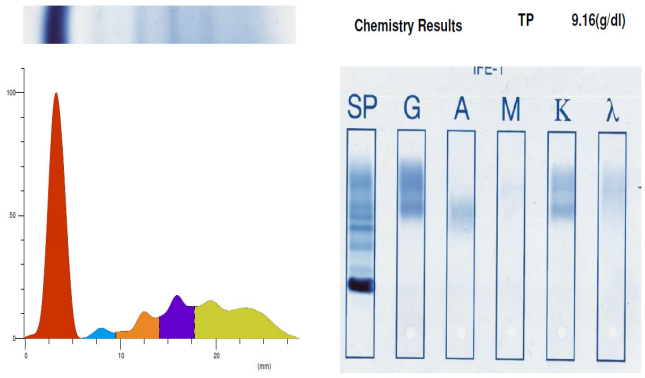


**Figure 4.** Showing two M-bands in gamma heavy chain with corresponding bands in kappa on IFE

Lambda free light chains levels were within normal range with value of 10.81 mg/L and kappa free light chain levels were elevated with value of 41.25 mg/L while Kappa/ Lambda (FLC) ratio was increased with value of 3.81.

Immunohistochemistry- Tumor cells are positive for CD138. Kappa high chain restriction is seen. Lambda is negative. LCA, CD3, CD20, PLAP and Synaptophysin are negative.

BM aspiration report showed trilineage hematopoesis with ~01% plasma cells.

BM biopsy showes mildly hypocellular marrow with trilineage hematopoiesis with no increase in plasma cells..

**Case 4: Biclonal Gammopathywith IgG lambda and IgG kappa(IgG-IgG isotype combination)**

A 59 year old male palready diagnosed with multiple myeloma presented in OPD on follow up visit. On physical examination, patient was alert, active, oriented to person, place, time. Routine lab investigation along with specialized testing were carried out.

On investigation Complete blood count shows haemoglobin 8.3 g/dL, hematocrit 25.5%, RBC 2.36 L $10^6$/$mm^3$,%, WBC 2.1 $10^3$/$mm^3$ and Platelet 90 $10^3$/$mm^3$. Serum protein was 7.5 g/dl and albumin 3.62 g/dl, globulin 3.9 g/dL with A:G ratio 0.93. Serum beta 2 microglobulin was 5.76 mg/L and serum calcium was 8.45 mg/dl. Serum Urea and creatinine was 41.74 and 1.07 mg/dl respectively. GFR by MDRD

calculator was 70.8 ml/min/1.73 $m^2$ .

Agarose gel protein electrophoresis was done on Helena Bioscience, Europe using SAS-MX SP-10 SB kit. On Agarose gel protein electrophoresis total protein: 7.5 g/dl, albumin: 3.12 g/dl, alpha-1: 0.39 g/dl, alpha-2: 0.84 g/dl, beta: 0.93 g/dl, gamma: 2.22 g/dl. Serum Protein Electrophoresis shows evidence of hypergammaglobulinemia with two small separate peaks in gamma globulin region.The results were analysed using Helena software platinum 6.0.100. (Figure 1). To reveal the nature of clonality or suspicious band, serum Immunofixation study was performed. High resolution serum immunofixation electrophoresis shows two distinct bands in IgG heavy chain region with corresponding bands in kappa as well as lambda light chain region suggestive of biclonal gammopathy (Figure 5). Such pattern is very rare if we consider the various immunofixation patterns observed in different gammopathies.

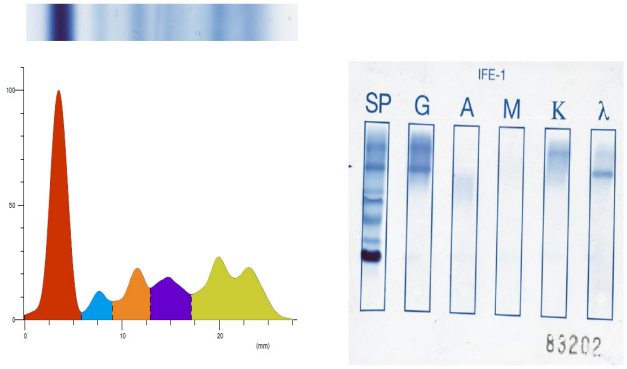


**Figure 5.** Showing two separate peaks in gamma globulin region on SPE and 2 distinct bands as IgG kappa and IgG lambda on IFE

Lambda free light chains levels were elevated with value of 70.72 mg/L and kappa free light chain levels were also raised with value of 103.44 mg/L while Kappa/ Lambda (FLC) ratio was normal with value of 1.46.

Immunoglobulin panel showed an normal levels IgA and IgM of 139.133 mg/dL and 94.92 mg/dL respectively. Serum IgG levels were elevated with value of 1770.96 mg/dL.

Peripheral Blood smear shows normocytic hypochromic RBCs with anisocytosis & few macrocytes. TLC is mildly reduced. Platelets are reduced. No hemoparasites or abnormal cells seen.

BM aspiration showed Normocellular marrow with trilineage hematopoiesis with plasmacytosis.Erythroid series showed normoblastic maturation. Myeloid series showed complete neutrophilic maturation.Megakaryocytes were adequate with unremarkable morphology. Plasma cells were seen singly scattered accounting to 8% of all marrow nucleated cells. Few binucleated forms are also seen.

BM Biopsy shows a cellular marrow for age [Cellularity – 35-40 % with trilineage hematopoiesis showing erythroid hyperplasia and plasmacytosis.(M:E ratio 2:1).] Erythroid series show normoblastic maturation. Many megaloblastoid forms are seen. Myeloid series show complete maturation. Megakaryocytes were occasionally seen. Singly scattered plasma cells are seen accouting to 8-9% of all marrow nucleated cells. Few binucleated forms are seen.

Reticulin: Grade MF-0

Iron stain: 2+

IMMUNOHISTOCHEMISTRY MARKERS

CD38 - [EP135] - Immuno reactive score 4+ in marrow cells (15 to 20%)

CD138 - [EP201] - Immuno reactive score 4+ in neoplastic cells(15 to 20%) .

CD56 - [123C3] - Non Immuno reactive score '0' in plasma cells

MUM1-[EP190] - Immuno reactive score 3+ in plasma cells

Kappa light chain - [ISH] - Non Immuno reactive score 3+ in plasma cells

Lambda light chain - [ISH] - Immuno reactive score 4+ in plasma cells

## DISCUSSION

Biclonal gammopathy is a very rare presentation of disease in patients with multiple myeloma. The present study reported Biclonal gammopathy in four different cases (as shown in Graphs above)

Biclonal gammopathies are defined by simultaneous appearance of different M components, two distinct or different monoclonal proteins. Biclonal gammopathy is relatively rare in M proteinemia and accounts for approximately 1-5% of all myelomas. The most commonly encountered combination is IgG and IgA (53%), followed by IgM and IgG (24%) as per literature.3 Biclonal gammopathy results from the proliferation of two clones of plasma cells, each producing an unrelated monoclonal Ig or it may result from the production of two monoclonal proteins by a single clone of the plasma cell. It is also presumed that the neoplastic clone which secretes one type of M protein might undergo isotype switching resulting in the production of the second type of M protein resulting in biclonal gammopathy. 4

These patient are more symptomatic than monoclonal gammapathy, although there is no significant difference in respect to clinical features and presentation and treatment.5 The therapeutic response in biclonal myeloma has been reported to be similar to that of monoclonal disease. Some studies have reported that the second clone has a protective role and is associated with better therapeutic response, remission rates, and slower progression. The determinant of the behavior of the disease is thought to be the dominant clone which increases over time. 6 Mullikin et al. has reported that 23 out of 393 patients with biclonal gammopathy of unknown significance progressed, with the dominant clone being the determinant throughout the course.7

Gallart et al. have reported a case of primary IgG with minor IgD shifting to predominant IgD after chemotherapy.Another case with the shift from IgG κ to IgG κ and IgD κ has also been reported.The phenomenon of biclonal gammopathy is not restricted to plasma cell neoplasms. Certain B cell lymphomas with plasmacytic differentiation, such as lymphoplasmacytic lymphoma, can also produce paraproteins which can rarely result in biclonal gammopathy.2

Biclonal gammopathies are more symptomatic than its monoclonal counterpart. About 40% of the patients with biclonal gammopathies present with suggestive clinical signs and symptoms. A significant percentage of the symptomatic patients have underlying lymphoproliferative disease most commonly waldenstrom macroglobulinaemia. Nafia Al-Riyami et al., had recently described a rare occurence of biclonal gammopathy in a patient with Chronic Lymphocytic Leukemia (CLL). Rest of the symptomatic patients either have multiple myeloma or have some underlying non-haematolgical disorder. Nae Yu Kim et al., described a rare case of biclonal gammopathy accompanied by prostate cancer.

Ideal biclonal cases show two different Ig Light chain rather than two different Ig Heavy chain subtypes because most commonly a single myeloma clone can express a single type of light chain only. Hence, such true biclonal myeloma cases expressing both kappa and lambda are extremely rare. Between 1966 and 1979, a study was conducted on 57 patients detected with biclonal gammopathy. Out of these 57 patients, 53% patients had IgG, IgA components whereas 26% had IgG, IgM components. Of the 115 light chains, 70% were kappa and 63% were both kappa and lambda. A large study comprising of 1027 plasma cell myeloma patients showed only 2% cases of biclonal gammopathy on serum protein electrophoresis.8 However, there was no mention regarding the combinations of biclonal M-proteins detected in the study. There are few other reports where combinations of IgG/IgM, IgA/IgG, and kappa/lambda light chain biclonal gammopathies have been described. Study of Nafia Al-Riyami et al., described a rare case of biclonal gammopathy with CLL which showed biclonal immunoglobin A (IgA) kappa and IgA lambda in serum immunofixation electrophoresis. 9Another study observed similar case of IgG Kappa and IgA Lambda type biclonal gammopathy, detected by appearance of two bands in the gamma region on serum protein electrophoresis. 10

According to the various studies in the literature, the plasma level of immunoglobulins is often elevated due to the lymphocyte proliferation in the BG. Immunoglobulin is used as a tumor marker for the recognition of the clinical disorder and for monitoring the progression, response or relapse of the disease, but also at the time of diagnosis to point out the dominant immunoglobulin since in the literature it is found that the dominant clone is the main actor in the evolution of the disease.7

Conality studies was most important to unfold whether this exceptional combination belongs to a truly biclonal population or rather a single neoplastic clone suffered two hits was the major limitation of our case series. It was seen that anti-myeloma therapy was more effective against multiple myeloma clones rather than MGUS clones in patients with biclonal gammopathy.1

Conclusion: In conclusion, the long-term tracking of these unusual cases may give us insight into the pathogenesis and clinical behavior of biclonal Gammopathies. Investigations related to genetics of these clones can indicate markers that lead to more aggressive conditions. The etiology of bi-clonal gammopathy is not yet elucidated; therefore it is necessary to follow the evolution of the clones by more advanced research focused on the genetic study of these clones to better understand the physiopathology of the bi-clonal peak. Biclonality, whether determined by molecular-genetic methods or by presence of para-proteins is considered to represent clonal evolution and may therefore, be a determinant of disease prognosis. To

conclude more prospective research work needs to be done for better realization of the underlying diseases biology, pathogenesis and behavior of this rare disorder. During follow-up, both paraproteins have to be addressed.

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