Excess Free Light Chains in Serum Immunofixation Electrophoresis: Attributes of a Distinctive Pattern

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Abstract Serum and urine protein electrophoresis and immunofixation are essential for identification and categorization of M protein/monoclonal protein. Based on the number of discrete bands identified the condition can be a monoclonal, biclonal or triclonal gammopathy. A subset of cases show an interesting pattern on immunofixation electrophoresis, with a complete immunoglobulin molecule, along with excess free light chains where in one light chain band shows similar mobility as the heavy chain, while the other light chain band of same isotype has a different mobility. Over a 3 year study period, 420 immunofixation electrophoresis gels were studied to select the cases with the typical pattern as described. The clinical records were searched for data of baseline evaluations

done prior to starting therapy, including clinical presentation, biochemical parameters, hemogram and bone marrow examination. Twenty cases (4.7%) were identified from the records, of these 77.8% cases had renal impairment and 33.3% presented with rapidly progressive renal failure. The possible explanation is the toxic effects of excess free light chains, in our cohort. The bound LCs show mobility similar to the HC bands on serum immunofixation gels, however the free light chains, exist in polymeric forms and show a different mobility. The identification and reporting of this pattern provides additional information regarding the high load of light chains, and indicates that patient may have a poor renal outcome/performance.

Keywords Monoclonal band · Multiple myeloma · Immunofixation electrophoresis · Free light chains

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Introduction

Serum and urine protein electrophoresis and immunofixation are imperative tools for identification and classification of M protein/monoclonal protein. The monoclonal protein is seen as a discrete band on the agarose gel in the gamma, beta, or alpha-2 region and can comprise of an intact immunoglobulin, light chain (LC) or heavy chain (HC). Depending on the number of monoclonal protein bands, the condition can be a monoclonal, biclonal or triclonal gammopathy [1–3]. On Serum immunofixation electrophoresis (IFE), we identified a subset of cases with complete immunoglobulin molecule, along with excess free LCs. These cases had a unique appearance on the immunofixation gel, where in one LC band shows similar mobility as the HC, while the other LC band has a different mobility (Fig. 1). These failed to qualify as biclonal gammopathy as

they lacked two distinct isotypes of HCs and did not possess diverse LC subtypes as well. Unlike the biclonal gammopathies which have comparable characteristics and outcomes [2, 3] akin to their monoclonal counterparts, this subgroup has been documented to have a worse prognosis and poor outcome including higher incidence of renal dysfunction and extramedullary plasmacytomas [4]. Limited data is available on this subgroup and none from the Indian subcontinent. In the present study, we describe our observations and analysis of this peculiar subset of cases.

Methods

About 980 serum IFE tests were available for evaluation in a 3 year study period at our institute. Serum protein electrophoresis (SPEP) was performed by agarose gel electrophoresis on Helena Bioscience (Europe) platform using SAS-1 plus and SAS-2 SAS-1SP 24 SB kit. Quantitation of M band was done on densitometric tracing. Serum IFE was performed on SAS-1 IFE-4 kit by using monospecific antisera for the immunoglobulin HC and LC including IgG, IgA, IgM, kappa (κ) and lambda (λ). Results were analyzed using Helena software PT version 3.0.

All serum IFE gels were reviewed by two independent observers. The subtype of HC and LC were identified based on the presence of discrete bands in the respective lanes on the gel. Presence of more than one band was recorded. Cases with one complete immunoglobulin subtype, i.e. single HC and a single LC were classified as monoclonal while those with more than one HC subtype were categorized as biclonal. Cases with two different LC subtypes were considered biclonal as well. Cases with one complete immunoglobulin molecule along with an additional LC of same subclass but different mobility on the gel were

Fig. 1 Serum immunofixation gel shows two distinct light chain bands with different mobilities

selected for evaluation. Cases of monoclonal and biclonal gammopathies were excluded from the study. After selection, corresponding SPEP gels were reviewed for presence, number and location of monoclonal bands. Quantitation of monoclonal protein was performed.

Data of baseline evaluations done prior to starting therapy were retrieved from the medical records, including clinical presentation, biochemical parameters, hemogram and bone marrow examination.

Results

During the study period 420 cases showed monoclonal bands on IFE electrophoresis. Of these 20 cases (4.7%) had a complete immunoglobulin along with an additional band, constituted by excess free LC with a different mobility but same isotype. In the present study, we observed eight cases of IgG λ , λ ; five of Ig G κ , κ ; 3 each of Ig A λ , λ and Ig A κ , κ and one of Ig M κ , κ . The most common immunoglobulin subtype observed was Ig G (65%) followed by Ig A (30%).

We observed two discrete M bands in 9 (45%) cases while 10 (50%) cases had a single M band, in the gamma and beta gamma regions. In addition, no M band was seen on serum electrophoresis in one case. Hypogammaglobulinemia was seen universally. Quantitation of M band ($n = 18$) was performed using densitometric tracing and values ranged from 0.25 to 5.07 gm/dl. The second M spike, corresponded with the additional light chain band, was smaller as compared to the first spike and ranged from 0.18 to 0.48 gm/dl. Quantitation could not be performed in one case owing to a very narrow peak.

Our cohort consisted of eleven males and nine females, and the mean age was 69.5 years (range 39–78 years). Interestingly, the mean age for females was lower than that for male patients (55 vs 71.5 years). There were 15 cases of multiple myeloma, 2 each of B-chronic lymphoproliferative disorder (CLPD) and smoldering multiple myeloma and a lone case of plasma cell leukemia in our cohort.

Of the 18 cases of plasma cell proliferative disorders, anemia was observed in all cases. The mean Hb was 6.8 g/dl (3–10.5 g/dl). Hypercalcemia was seen in 6 (33.3%) cases; mean corrected calcium level was 11.6 mg/dl. Lytic lesions were seen in 11 cases (61.1%). We observed two cases with extramedullary plasmacytoma (11.1%) of ribs, in our series. Beta 2 microglobulin was elevated in 9 of the 10 cases (90%). According to International staging system (ISS) three (30%) of our patients were in stage II, while 7 (70%) in stage III. Serum free light chain assay was done in 7 cases, and the kappa: lambda ratio was deranged in all. The therapy instituted was variable and tailor made, however the most common regime followed was

combination of cyclophosphamide, bortezomib and dexamethasone. The mean duration of follow up was 35 months (17–50 months). Of the patients being followed at our institute, 5 were in complete remission, one in partial remission and 2 patients died. Many of these patients preferred follow up at their respective health centers. None of the patients underwent bone marrow transplant. Additional patient details provided in Table 1.

There were two cases of B cell chronic lymphoproliferative disorder which had involvement of the bone marrow at presentation. Both exhibited anaemia and raised creatinine. One of them was followed up at our institute and had achieved partial remission, with a persistent M band after 7 cycles of lenalidomide and prednisone.

Of the 18 cases of plasma cell proliferative disorders, 14 (77.8%) cases had serum creatinine greater than 1.5 mg/dl at presentation, while 12 cases (66.7%) had creatinine of > 2 mg/dl, indicating a more severe degree of renal impairment at the time of presentation. In addition 6 (33.3%) cases had rapidly progressive renal failure at presentation with decreased urine output or anuria.

Table 1 Plasma cell neoplasia- Patient characteristics

Number of patients	18
Male	10
Female	8
Mean age (years)	59.3 (39–78)
Type of M component	
IgG KK	5
IgG- λ , λ	7
Ig A KK	3
IgA- λ , λ	3
International staging system (n = 10)	
I	None
II	3
III	7
Creatinine (mg/dl)	4.33 (0.6–14.8)
Hb (gm/dl)	6.8 (3–10.5)
Calcium [corrected (mg/dl)]	11.7 (7.6–16.8)
Serum albumin (g/dl)	3.5 (2.04–5.7)
Lytic lesions	11
Plasma cell % bone marrow (n = 14)	53.3 (12–85)
Extra medullary plasmacytoma	2 (rib)
Serum β 2 microglobulin (mg/l)	13.78 (1.22–34.11)
Mean follow up (months, n = 10)	35 (16–50)

Discussion

The presence of complete immunoglobulin along with an additional band constituted by excess free LC with a different mobility but similar isotype was observed in 4.7% cases. Yang et al. [4] made similar observations and have reported this finding in 5.4% of monoclonal gammopathies in their series. A study from India reported a much higher number of such cases, 25.1% [5]. We observed eight cases of IgG λ , λ ; five of Ig G κ , κ ; 3 each of Ig A λ , λ and Ig A κ , κ and one of Ig M κ , κ . The most common immunoglobulin isotype observed was Ig G (65%) followed by Ig A (30%), this observation is similar to that reported by other larger studies on monoclonal proteins [6, 7]. Yang et al. [4] have observed presence of only excess free λ chains in their cohort. The distribution of cases in their cohort was IgG λ , λ (53.8%), IgA λ , λ (26.9%) and IgD λ , λ (19.2%). Another series by Misra et al. [5] observed cases with IgG κ , κ (8.1%) and IgG λ , λ (17%). Surprisingly the reason behind this observation remains unexplained.

The presence of monoclonal proteins is well known in lymphoproliferative disorders besides plasma cell dyscrasias, where the former results from clonal B cell proliferation and the later represent clonal proliferation of plasma cells. MGUS is most common cause of paraproteinemia, followed by multiple myeloma while lymphoproliferative disorders account for a small number of cases (4%) [8]. In our series, multiple myeloma was the predominant presentation, this was concordant with our case selection as these have excess free LCs at presentation.

Lytic lesions were observed in nearly half of our cases. Yang et al. [4] have reported presence of extramedullary plasmacytomas in 38.5% cases in a similar cohort. We found two cases with extramedullary plasmacytoma (11.1%) in ribs, in our series.

The most significant difference in this cohort of patients was the renal presentation. Renal failure was seen in 30–50% cases of MM when the serum creatinine cut off of 1.5 mg% was used [9, 10], however the numbers reduce to about 20% if the limit is set at 2 mg% [7]. In the present study population 77.8% cases had serum creatinine greater than 1.5 mg/dl at presentation, while 66.7% had creatinine of > 2 mg/dl. Also 33.3% had rapidly progressive renal failure at presentation with decreased urine output or anuria. Similar findings have been reported by Yang et al. [4] in a comparable cohort. The cause appears to be the toxic effects of excess free light chains, in our cohort. Other contributing factors that add to the renal impairment in this clinical setting may include hypercalcemia, hyperuricemia, dehydration etc.

The cause for the presence of distinct pattern could be ascribed to the synthetic properties of plasma cells and the biochemical properties of LCs. Normal plasma cells produce slight excess of LCs over HCs. These are either excreted or catabolized by the tubular cells. These excess free light chains (FLC) exist in monomeric and dimeric forms. The λ chains have a higher tendency towards dimerisation and oligomerisation, as compared to κ chains [11].

Increased levels of dimeric and multimeric forms of LCs have been demonstrated in plasma cell neoplasms. This could plausibly be attributed not only to an excess production but alterations in the structure of the LCs as well [12]. These forms have a different size and charge as expected from their difference in mobility in comparison to monomeric LCs observed on gel electrophoresis. The bound LCs show mobility similar to the HC bands on serum IFE gels, while the free LCs, which are expected to exist in polymeric forms show a different mobility. This leads to the development of an interesting pattern on the gel, visualized in form of presence of one complete Ig molecule along with an additional band in the LC lane of same isotype but with different mobility.

Our findings are in sync with established knowledge of renal toxicity occurring due to excess free LCs. We hereby comprehend that the presence of this excess of LC must be highlighted in the reporting of routine serum IFEs. This would provide additional information to a clinician that the patient is carrying high load of LCs and is likely to have a poor renal outcome/performance. This could likely affect the treatment protocol. This should further be followed up by a 24 h urine electrophoresis and IFE on a concentrated aliquot to provide a measurement of the size of the LC spike.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical Standard All procedures performed in studies involving human participants were in accordance with the ethical standards of

the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed Consent This was a *retrospective study* and for this type of study formal consent is not required.

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