Original Article

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Role of Serum Protein Electrophoresis in Clinically Suspected Cases of Plasma Cell Dyscrasia: A Tertiary Care Center Experience from North India

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ABSTRACT

Background: Serum protein electrophoresis (SPE) and its pattern recognition is an excellent screening technique for the detection of paraproteinemias or monoclonal gammopathies. In this study, we assessed SPE patterns in all clinically suspected cases of plasma cell dyscrasia with usual and unusual clinical presentations and correlated with ancillary investigations.

Method: We analysed serum protein electrophoresis (SPE) patterns in clinically suspected cases of plasma cell dyscrasia in 2 year duration. Based on SPE pattern these cases were divided into two groups: Group I, with M-component and Group II, without M-component. These cases were reviewed with respect to clinical presentation and correlated with immunofixation electrophoresis and bone marrow aspiration/biopsy findings wherever available, especially in the M band positive cases to differentiate multiple myeloma (MM) from the other conditions.

Result: A total of 80 samples were received for SPE, out of these, 56.3% were clinically suspected to have plasma cell dyscrasia. Among these cases 60% were in group I and 40% were in group II. In group II, 39% cases were essentially non neoplastic after relevant investigations. In group I, M-band was observed in 88.9% cases in γ-region and 11.1% cases in β-region. The mean fraction of the M protein was 4.7g/dl with a range of 2.0g/dl to 9.8g/dl. In group II 72.2% showed polyclonal hypergammaglobulinemia, 22.2% showed normal electrophoretic pattern and 5.6% showed hypogammaglobulinemia. Bone marrow aspiration and biopsy were available in 88.9% of all suspected cases. Various haematological neoplasms observed in both the groups, their clinical presentations with interesting facets of the cases have been discussed.

Conclusion: Serum protein electrophoresis is one of the most important diagnostic modality in clinical chemistry. It helps to resolve cases with bone marrow plasmacytosis particularly in differentiating between reactive non-neoplastic plasmacytic proliferations from clonal plasma cell disorders. Moreover, it also facilitates the work-up of cases with atypical presentations and thereby paves way to clinch the diagnosis in these ambiguous cases.

Keywords: *Serum Protein Electrophoresis, Monoclonal Gammopathy, Plasma Cell Dyscrasia.*

Introduction

Serum protein electrophoresis (SPE) is one of the important tools for detection of numerous pathological conditions. There are variations in serum protein levels in disease state; electrophoresis is used for fractionation of these proteins and to elucidate monoclonal paraproteins. These proteins are indicative of diseases of plasma cells and lymphocytes. It is prudent to identify monoclonal gammopathies early to provide monitoring and/or meaningful interventions.

Material and Methods

This retrospective study was conducted in the Department of Pathology, PGIMER, Dr. RML Hospital, New Delhi. All the data from samples received for SPE in last 2 years were analysed and reviewed with respective patient's medical record. SPE was performed with agarose gel electrophoresis (HYDRASYS 2 Sebia) with fractionation of protein in 5 classic zones i.e. albumin, α1, α2, β and γ. Each disease sample was run with a control (normal) sample. The electrophoretic mobility patterns were inferred visually and quantitated by densitometry scan, in which, the relative percentage of each protein fraction, were calculated automatically. Total protein was estimated by biochemical methods and used for aforesaid calculations. Based on SPE pattern all clinically and/or radiologically suspected cases of plasma cell dyscrasia (PCD) were divided into two groups: Group I, with M-component and Group II, without M-component. Patient's clinical profile, radiological findings and laboratory findings were reviewed and correlated with immunofixation electrophoresis and bone marrow aspiration/biopsy findings wherever available, especially in the M band positive cases to differentiate multiple myeloma (MM) from the other conditions.

Result

A total of 80 samples were received for SPE during the 2 years period. Out of these, 56.3% were clinically and/

or radiologically suspected to have plasma cell dyscrasia and 43.7% cases were of liver diseases, renal dysfunctions, unexplained peripheral neuropathies and recurrent infections. Majority of patients of suspected plasma cell dyscrasia were in their 6th decade with male to female ratio (M:F) of 1.4:1.

Among all suspected cases 60% were in group I and 40% were in group II, based on presence or absence of M-component respectively. In group I, M:F was 1.3:1 with mean age of presentation 61.2 years (range 28-80 years). Group II showed M:F 1.6:1 with mean age of presentation 58.8 years (range 22-75 years). In group II, 39% cases were essentially non neoplastic after relevant investigations and mean age of presentation amongst these cases was 58.4 years.

In group I, M-band was observed in 88.9% (24/27) cases in γ-region and 11.1% (3/27) cases in β-region. The mean fraction of the M protein was 4.7g/dl with a range of 2.0g/dl to 9.8g/dl. In group II 72.2% (13/18) showed polyclonal hypergammaglobulinemia, 22.2% (4/18) showed normal electrophoretic pattern and 5.6% (1/18) showed hypogammaglobulinemia. Immunofixation electrophoresis (IFE) was available in some of the cases. In one case from group I, IFE revealed IgM kappa light chain which was diagnosed as Waldenström's macroglobulinemia (WM) after bone marrow examination and immunohistochemistry (IHC). Among cases with M-band in β-region, IFE was done in 2 cases and showed IgA type immunoglobulin.

Bone marrow aspiration and biopsy were available in 88.9% (40/45) of all suspected cases. The distributions of various haematological neoplasms observed in both the groups are depicted in figure 1. In group I, 77.8% cases were diagnosed as MM. Some of these cases (14.3%) were associated with amyloid deposition. Various amyloidotic presentations encountered in this study were subcutaneous nodules, cast nephropathy and colonic amyloidosis. One case of primary renal amyloidosis was also observed which showed M-component on SPE but did not reveal plasmacytosis on bone marrow examination.

Among group I, SPE in one case revealed β-γ fusion with suspicious M band. Bone marrow examination with IHC established the diagnosis of T-ALL.

There was another interesting case of dual malignancy noted in group I, which was a known case of breast cancer post radical mastectomy presented 5 years later with renal dysfunction. Her SPE showed M band in γ-region and after bone marrow examination multiple myeloma was diagnosed.

Among all suspected cases, 11.1% cases of plasma cell myeloma were noted in group II. Five of these cases showed polyclonal hypergammaglobulinemia and one showed hypogammaglobulinemia. In the latter case, free light chain (FLC) assay revealed monoclonal lambda FLC and in conjunction with bone marrow examination and IHC diagnosed as Free light chain only myeloma. Apart from multiple myeloma cases a case of Extramedullary plasmacytoma (EMP) with normal SPE pattern was also noted.

Other than plasma cell dyscrasias, a case of chronic lymphocytic leukaemia (CLL) was also found in which SPE showed hypergammaglobulinemia.

Discussion

Serum protein electrophoresis and its pattern recognition is an excellent screening technique for a wide variety of abnormalities, and for suggesting or confirming a clinical diagnosis. Protein electrophoresis by using a

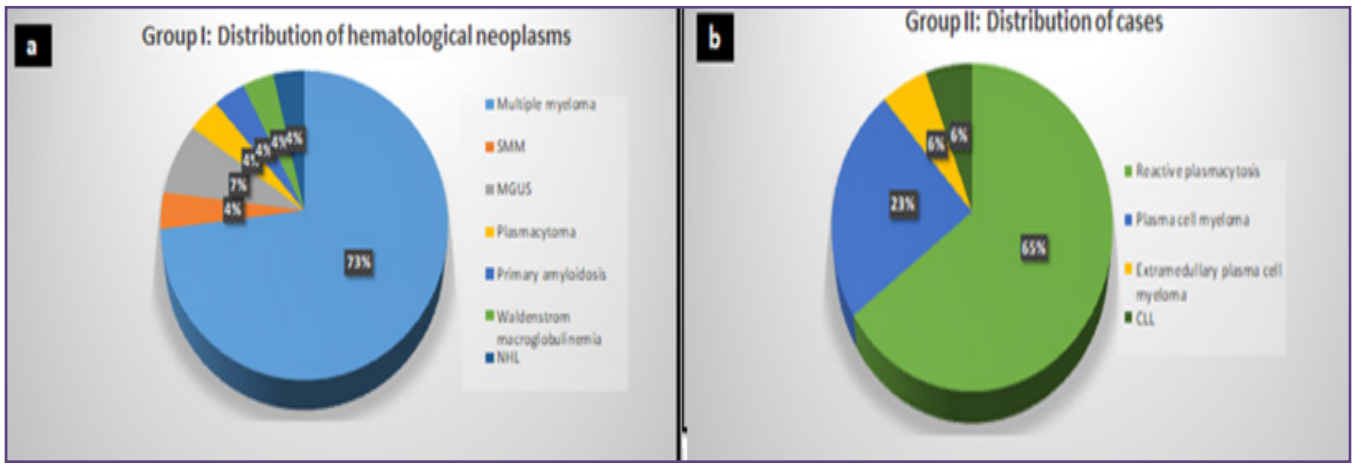


Fig.1: (a) Distribution of haematological neoplasms in Group I, (b) Distribution of cases in Group II.

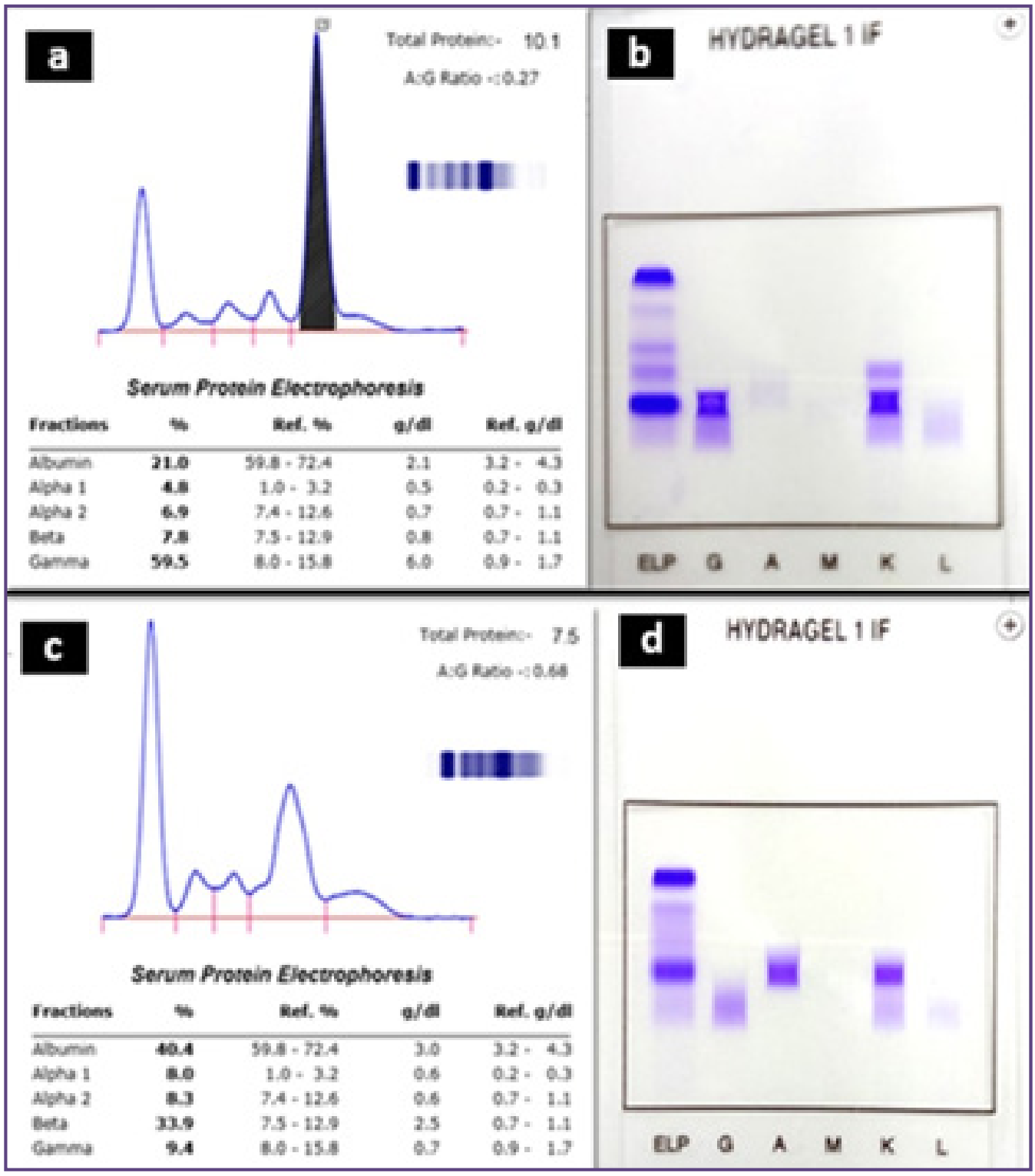


Fig. 2: Cases of Multiple myeloma (a) SPE showing M-band in γ-region, (b) IFE showing monoclonal IgG kappa light chain, (c) SPE showing M-band in β-region, (d) IFE showing monoclonal IgA kappa light chain.

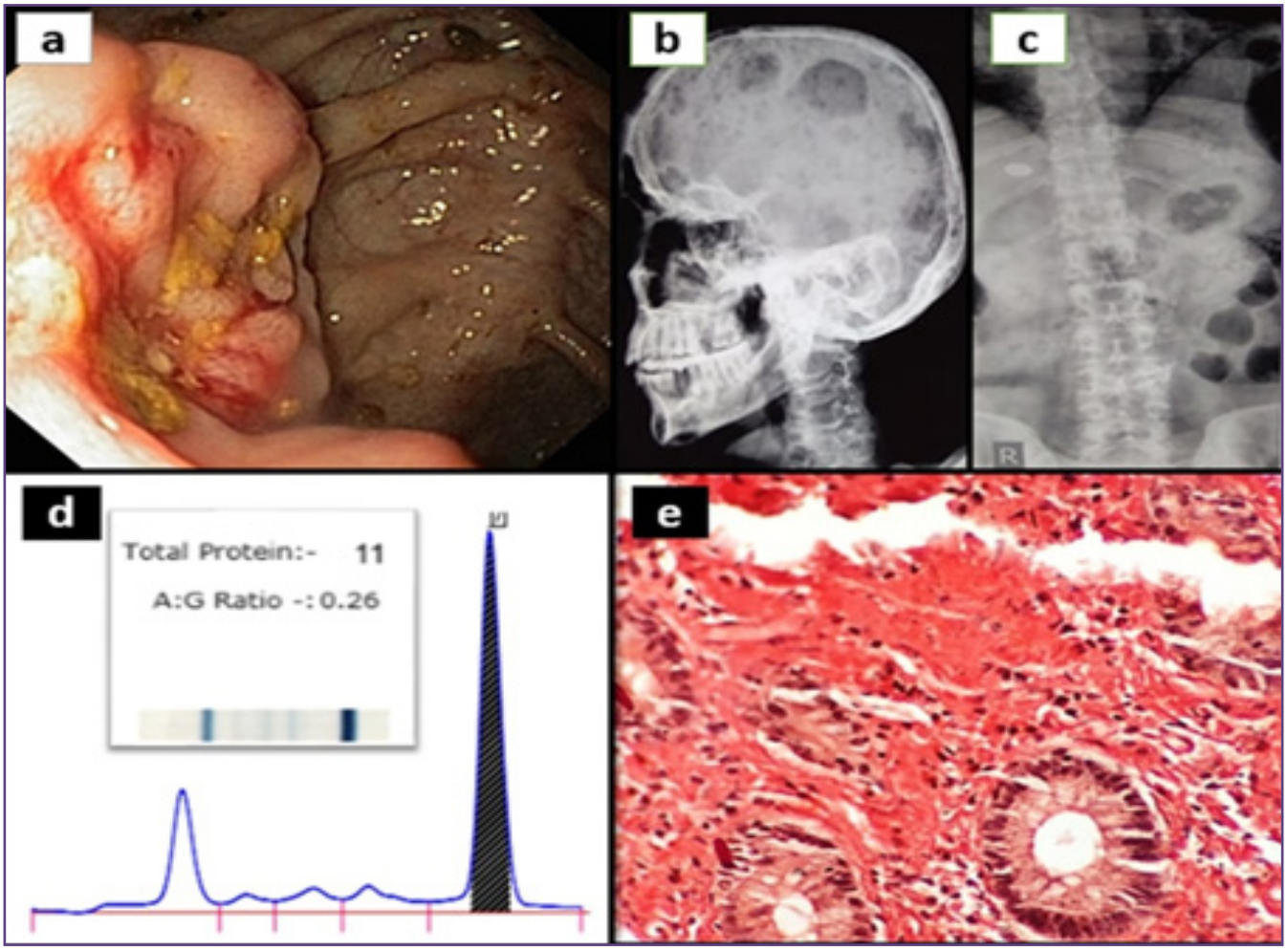
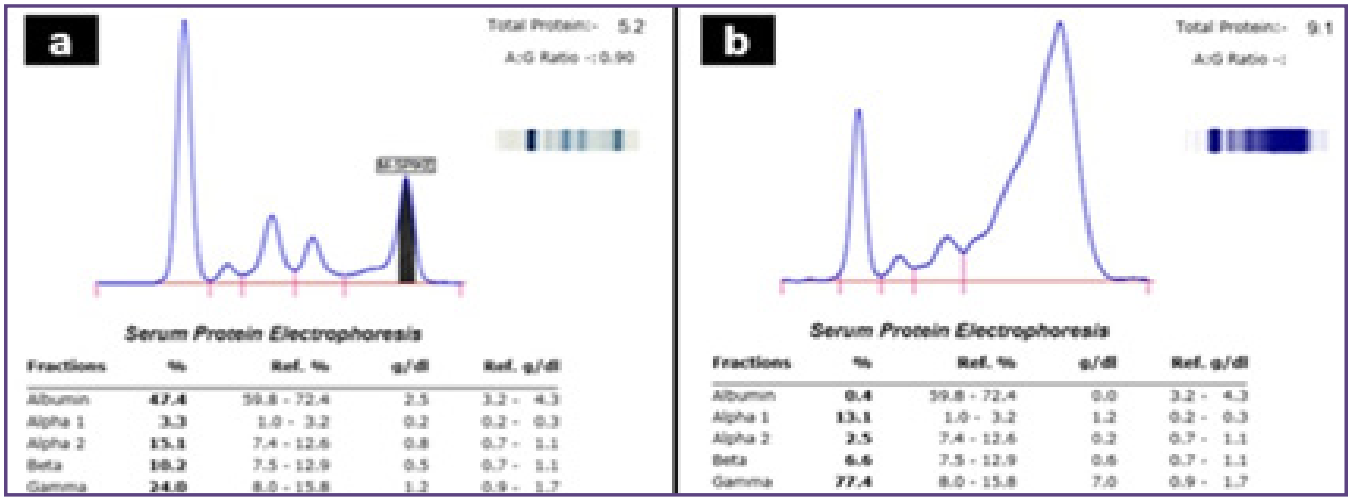


Fig. 3: (a) Colonoscopy showing ulcerated and thickened mucosa in caecum, (b) & (c) X-ray skull and spine show multiple lytic lesions, (d) SPE showing M spike in γ-region, (e) Colonic biopsy showing amyloid deposition in lamina propria, Congo red stain x400.



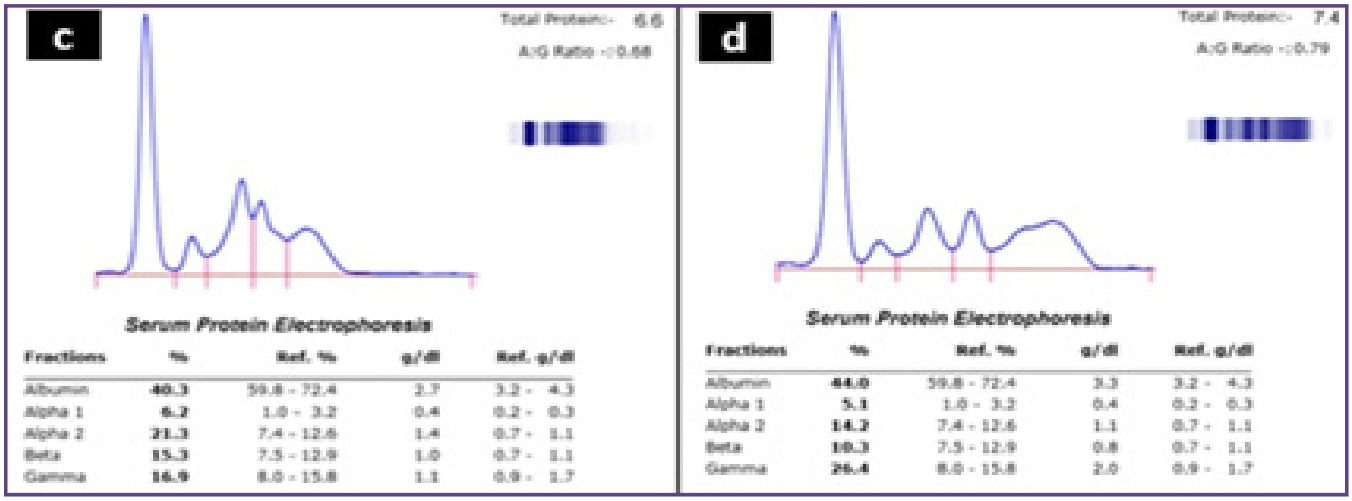


Fig. 4: Electrophoretic patterns: (a) M spike in γ-region in a case of renal amyloidosis, (b) β-γ fusion with suspicious M spike in a case of T-ALL, (c) Increased fractions of α1,α2,β and γ globulin without M spike in a case of Multiple myeloma, (d) polyclonal hypergammaglobulinemia in a case of CLL.

liquid medium was first developed in the 1930s by a Swedish biochemist Arne Tiselius and since then various evolutions have been done in methods of electrophoresis such as cellulose acetate, agarose, high-resolution agarose gel electrophoresis, capillary electrophoresis and protein electrophoresis with immunofixation.[1]

Serum protein electrophoresis is an indispensable aid in the diagnosis of many diseases including a variety of chronic infectious & inflammatory disorders, protein-losing enteropathies, renal disorders, immunodeficiency states and paraproteinemias caused by haematological neoplasms. It is of utmost importance in detection of paraproteinemias or monoclonal gammopathies.[2] It is extremely important to differentiate monoclonal from polyclonal gammopathies. Monoclonal gammopathies are associated with a clonal process that is malignant or potentially malignant. In contrast, polyclonal gammopathies are caused by reactive or inflammatory processes. In this study, monoclonal gammopathies were identified through SPE in 60% of all suspected cases out of which, 91.3% showed M-band in the γ-region. In other studies, Chopra et. al[3] and Tripathy et. al[4] have also reported γ-region M-spike in 84.8% and 87.5% respectively.

In our study, we found most of the cases in their 6th decade with slight male predominance. The mean age of presentation in monoclonal gammopathy cases was relatively higher than polyclonal gammopathy cases. These findings were in accordance with other Indian studies.[3][5]

Among the cases of monoclonal gammopathy (group I), multiple myeloma was diagnosed in 69.5% cases after bone marrow examination. In all these cases there were marked increase in total protein especially the γ-globulin with a mean γ-globulin level of 4.7g/dl (range 2.0-9.8g/dl). (Figure 2a)

In one of the cases from group I, SPE showed decreased albumin and β-globulin with M-spike in the γ-region (M-protein 4.2g/dl). Immunofixation electrophoresis (IFE) identified IgM immunoglobulin kappa light chain. IgM paraproteinemia is considered to be the major defining feature of WM, but it may also occur in other B-cell lymphoproliferative disorders such as CLL, diffuse large B-cell lymphoma, extra nodal marginal zone lymphoma, mantle cell lymphoma, IgM myeloma etc.[6] Although paraprotein concentrations are generally higher in WM, it has limited diagnostic value and immunophenotypic criteria are thus essential for the accurate diagnosis of WM.[6][7] Indeed, accurate determination of the IgM levels is crucial to evaluate response in these patients. M-band quantitation by SPE can be challenging due to propensity of IgM paraproteins to form higher-order complexes or multimers in serum. In such cases, IgM heavy chain nephelometry, can be done for estimating paraprotein concentration.[8] Further, with bone marrow aspiration, biopsy and immunohistochemical findings final diagnosis of Waldenström's macroglobulinemia (WM) was given in our case.

Another notable finding in the present study was clone of myeloma protein associated with the β-region in three cases. IFE was done in two of these cases which showed IgA immunoglobulin with kappa light chain. (Figure 2c, d) A related technical pitfall in such cases is inability to quantify the M-protein, often with monoclonal IgA protein, since its anodal electrophoretic migration may be

masked by other bands such as transferrin and complement component 3 (C3) that co-migrates in β region.[9][10]

In addition to technical pitfall in the interpretation of SPE, clinical presentation in these cases can be quite deceptive which we noted in one of our cases in which, a young male, with history of chronic renal disease for past 2 years presented with multiple subcutaneous swellings on face, upper arm and bilateral gluteal region with hypopigmented skin lesions. X-ray showed multiple lytic lesions in skull, bilateral humerus and bilateral femur. His kidney function test (KFT) was deranged and calcium levels were raised. SPE showed M-spike in β region. A diagnosis of "amyloid tumor" was given after histological examination. Bone marrow aspiration revealed features of plasma cell myeloma. Multiple myeloma patients are prone to develop amyloidosis due to overproduction of one type of abnormal immunoglobulin. Not all but, approximately 35% of myeloma patients may develop amyloidosis at some point, however only 10-15% may experience symptoms associated with it.[11] Skin manifestations in myeloma associated amyloidosis depend upon the site of amyloid deposition. A similar case with multiple, non-itchy, papular lesions in lower eyelids & lower chest wall was reported by Kumar et al.[12]

Similarly, in one of the interesting cases, a young male presented with complaints of pain in abdomen, vomiting and hematemesis. His lab investigations revealed raised ESR, hypoalbuminemia with reversal of A:G ratio and M-band in γ-region on SPE. X-ray skull and spine showed multiple lytic lesions. Bone marrow findings were suggestive of MM. However, Colonoscopy showed evidence of pancolitis with few ulcers in ileum along with thickened folds in caecum. After histological examination an impression of amyloidosis was given. (Figure 3)

In cases of MM one of the common renal complications is cast nephropathy. We also noted one such case in an elderly female presented with chronic renal disease and deranged KFT. Her calcium and ESR levels were found to be within normal limit; however, M-band was noted on SPE. Renal biopsy was suggestive of cast nephropathy. Bone marrow examination showed 40-45% plasma cells with few immature forms and plasmablasts. Her γ-globulin level was high (4.8g/dl). On the other hand, in case of primary amyloidosis the quantity of the circulating monoclonal protein is usually lower than in MM. We observed another case of renal amyloidosis with M component in γ-region (0.7g/dl) on SPE. (Figure 4a) Bone marrow examination did not reveal any increase in plasma cells. IFE is rather required in these cases for detection of the monoclonal protein. In our case, renal biopsy was done, amyloid was demonstrated by congo red stain and IHC showed monoclonal kappa light chain.

There were two cases with M component, identified as monoclonal gammopathy of undetermined significance (MGUS) after complete work up for multiple myeloma. Traditionally, MGUS has been considered a relatively benign entity with low likelihood of progress to myeloma. From the hematologic stance, the small plasma cell clone responsible for MGUS may not progress to myeloma but nonetheless, may still promote kidney injury.[13]In another interesting case, an elderly female presented with papulonodular skin lesions and multiple lymphadenopathies. Her biochemical analysis showed increased total protein with hypoalbuminemia and decreased calcium ion levels. SPE revealed β-γ fusion with suspicious M-band in γ region.(Figure 4b) Bone marrow examination revealed atypical lymphoid cell aggregates positive for CD3 and negative for CD20 along with reactive plasmacytosis and a final diagnosis of T-cell ALL was rendered. Association of a monoclonal gammopathy with B-cell NHL is a well-described phenomenon although the frequency varies with histological subtypes. However, its association with T-cell neoplasm is infrequent. On data search very few case reports of T cell ALL associated with monoclonal gammopathy were found.[14]

Again in group I, one more curious finding observed was occurrence of multiple malignancies in same patient. An elderly female who underwent radical mastectomy for carcinoma breast 5 years back, presented with renal dysfunction. Her protein levels were elevated with raised globulin levels. In view of deranged KFT and A: G reversal, SPE was done which showed evidence of M-band in γ-region. Bone marrow examination showed increase in plasma cells (>45%), suggestive of MM. Diagnosis of multiple malignancies in a single patient is challenging. After extensive literature search, it has been found that very few cases have been reported with diagnosis of MM and breast cancer.[10]

Sporadically, plasma cell dyscrasia cases can be non-secretory due to defect in immunoglobulin synthesis or secretion. In one series, SPE showed M-band in only 82% of patients with multiple myeloma and remainder had hypogammaglobulinemia or a normal-appearing pattern.[2]

In this study, 11.1% (5/45) cases of plasma cell myeloma did not show M-spike (figure 4c) rather SPE displayed polyclonal hypergammaglobulinemia in four cases and one showed hypogammaglobulinemia. Most of these cases often fall into a more recently identified group of "free light chain–only" myeloma or oligo-secretory myeloma. These are patients in whom disease can be revealed only with the

free light assay (FLC), which is known to more accurately detect kappa and lambda light chains in the blood.[15]

We also got one such case, an elderly male presented with complaints of persistent low-grade fever, easy fatigability and lower backache. Radiological examination did not reveal any lytic lesion. Serum chemistry showed mild alteration in the A:G ratio. SPE and urine electrophoresis revealed no M-component and urine Bence Jones proteins were negative. Peripheral blood smear revealed pancytopenia. Bone marrow displayed predominantly plasmablasts with paucity of normal haematopoietic elements. A panel of IHC was put up on bone marrow biopsy, which revealed strong CD138 expression with lambda restriction and cytoplasmic positivity for IgM. Those cells were found to be negative for leukocyte common antigen, CD20 and CD3. An FLC assay was done subsequently which revealed monoclonality of plasma cells showing marked excess of lambda FLC (>1000 mg/L). A final diagnosis of FLC myeloma (plasmablastic type) was made.

In addition to multiple myeloma cases a case of extramedullary plasmacytomas was also found in group II, patient presented with left tonsilar growth and histomorphologic differentials given were, EMP and diffuse large B-cell lymphoma (plasmacytoid type). Immunohistochemistry showed positivity of CD38, CD79b, IgG and lambda light chain restriction. EMPs are uncommon tumours and comprise only a small percentage of all plasma cell malignancies.[17] Some of the observers have reported the presence of M protein in 24–75% of patients in different series.[18][19] In our case, SPE revealed normal electrophoretic pattern. The frequency of M protein probably depends on the level of sensitivity of the tests used as the levels of paraproteins were generally low.

We also found a case of CLL with bone marrow lymphocytosis. SPE showed increased γ- globulin fraction (2.0g/dl). (Figure 4d) CLL is usually associated with hypogammaglobulinemia.[20]

This is due to impaired function of neoplastic B cells and regulatory abnormalities of T and NK cells. On literature search, limited cases of CLL with polyclonal hypergammaglobulinemia were found.[21]

These findings indicate that SPE is helpful in the diagnosis of both common diseases with unusual presentations and rare disorders with common presentations and thereby represents a pertinent diagnostic tool.

Conclusion

Serum protein electrophoresis is one of the most important diagnostic modality in clinical chemistry as it helps in sub-fractionation of different types of proteins. It helps to resolve cases with bone marrow plasmacytosis particularly in differentiating between reactive non-neoplastic plasmacytic proliferations from clonal plasma cell disorders like plasma cell myeloma. Moreover, it also facilitates the work-up of cases with atypical presentations and thereby paves way to clinch the diagnosis in these ambiguous cases. The aforesaid study highlights the role of non-invasive investigations like SPE and IFE in the evaluation of such cases so as to impart definitive diagnosis, better characterisation and optimum patient management.

Statement of Informed Consent

Identifying information, including patients' names, initials, or hospital numbers, have not been revealed in written descriptions or photographs.

For this type of study (retrospective) formal consent is not required.

Statement of Human and Animal Rights

This article does not contain any studies with animals performed by any of the authors.

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