Evaluation of Suspected Monoclonal Gammopathies: Experience in a Tertiary Care Hospital

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Abstract

Background : Monoclonal gammopathies occurs in patients with malignant diseases of plasma cells and lymphocytes and in few benign conditions. The objective of this study was to assess the precision, accuracy and confirmation of monoclonal gammopathies on serum protein electrophoresis (SPE) and the clinical relevance of detection and characterization of M component.

Methods : All samples received for serum electrophoresis in the last 3 years were analysed for data on M band positivity and correlating it with clinical profile of the patients. Immunofixation (IFE), Immunoelectrophoresis (IEP) and IgG, IgM estimation were carried out in few cases. The follow up of cases was done by serial monitoring of SPE and β_2 microglobulin levels.

Results : 1155 samples were received during the 3 years period. 282 (24.4%) samples were positive for M component on SPE. Of these, 239 (84.8%) patients had M spike in λ region and 43 patients had M spike in β region. The mean load of the M protein band in the λ region was 37.8% and in β region was 35.8%. IgG with κ chain was seen in 40%, IgG with λ chain was seen in 50%, 5% patients each had IgM with κ and IgA with λ light chain. 246 samples (96.5%) had high levels of β_2 microglobulin. Of the 116 cases of multiple myeloma, IgG levels was more commonly raised (5%) as compared to IgA (6.9%) and IgM (5.2%).

Conclusion : It is recommended that SPE should be performed in patients having unexplained weakness, anaemia, back pain, osteoporosis, osteolytic lesions, fractures, renal insufficiency or recurrent infections.

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Key Words : Serum protein; Electrophoresis; M band; Multiple myeloma

Introduction

Detection of a monoclonal component (M band) may be the result of a clinical suspicion and therefore it will confirm a diagnosis, or more frequently, it represents a casual finding. The clinical indications which raise suspicion are numerous, and range from haematological and bone manifestations to circulatory, renal, infectious or neurological signs. Consequently, serum protein electrophoresis (SPE), has an extremely broad application.

However, for SPE to be effective it must be performed correctly. This was confirmed by a survey in which only 29% of the participating laboratories succeeded in detecting the presence of an M band in a serum sample [1]. A serum M band hidden under the normal electrophoretic band is often missed.

Monoclonal gammopathies, or disorders associated with the production of an abnormal and detectable amount of a monoclonal immunoglobulin (Igs), occurs in patients with malignant diseases of plasma cells and lymphocytes and also in patients with a benign condition. The abnormal immunoglobulins may be detected with SPE, immunoelectrophoresis (IEP) or immunofixation

(IFE). A monoclonal spike referred is seen as a discrete band that usually migrates to the λ or β region of the electrophoretic strip and rarely to α_2 region. A polyclonal increase in Igs produces a broad-band or broad-based peak and is limited to the λ region. Two monoclonal proteins (biclonal gammopathy) occur in 8 to 9% of sera containing monoclonal protein abnormalities [2]. Rarely a triclinal gammopathy (three monoclonal proteins) is found.

Currently recommended techniques for the evaluation of M proteins include high resolution (either gel or capillary based) electrophoresis as well as IFE [3-5]. The study of M band is complex from both analytical and clinical point of view. The objective of this retrospective study was to assess the precision, accuracy and confirmation of monoclonal gammopathies on SPE. Further, the possible clinical relevance of the detection and characterization of M component is also discussed.

Materials and Method

This study was carried out by analysing data of all the samples received for serum electrophoresis in the last 3 years in the Department of Pathology & Molecular Medicine of this hospital. It included, analysing the data for M band

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Table 1**Monoclonal protein positivity in different clinical settings (n=1155)**

Anaemia (n=125)	9 (7.2%)	8 (88.9%)	1 (11.1%)
Renal failure (n=97)	22 (22.7%)	20 (90.9%)	2 (9.1%)
Clinically suspected plasma cell dyscrasia (n=236)	115 (32.9%)	88 (76.5%)	27 (23.5%)
Low backache (n=236)	72 (30.5%)	60 (83.3%)	12 (16.7%)
Haematological malignancies ^a (n=76)	13 (17.1%)	12 (92.3%)	1 (7.7%)
Others ^b (n=272)	51 (18.8%)	46 (90.2%)	5 (9.8%)

a=Cases of NHL, ALL, CLL; b = Cases of PUO, Tuberculosis, GI bleed, weight loss (inv), fracture neck of femur, splenomegaly, carcinoma lung, unstable angina, hypergammaglobunemia, vasculitis, inflammatory bowel disease, MGUS, thrombophilia

positivity and correlating it with clinical profile of the patients. In a few cases of monoclonal gammopathies, IFE and IEP were also carried out and data analysed. In many cases, the estimation of Ig G, Ig A, Ig M was also carried out. The follow-up of confirmed cases of myeloma was done by serial monitoring of SPE and β_2 microglobulin (β_2 M) levels.

SPE was done using Paragon SPE kit (Beckman Coulter Inc. Fullerton, CA) which provides the electrophoretic separation of proteins. This mobility pattern was visually interpreted and quantitated by densitometry at 600nm, on a Beckman APPRAISE densitometer in which, the relative percent of each protein fraction is calculated automatically.

Immunoelectrophoresis was performed using Paragon IEP kit (Beckman Coulter Inc., C.A.). It provides for the electrophoretic separation of proteins in a buffered agarose gel (0.8% agarose, 0.6% barbital buffer). After electrophoresis specific antiserum was applied to designated antiserum troughs running parallel to the axis of the electrophoretic migration and immunodiffusion was allowed to occur. The simultaneous diffusion of the proteins in the sample and the antisera in the troughs resulted in the formation of precipitin arcs. Interpretation was made by visual examination of the size, shape and position of the precipitin arcs.

Immunofixation was performed using Paragon IFE kit (Beckman Coulter Inc, C.A.). After electroporesis, specific antiserum was overlaid directly onto the gel surface along the axis of the electrophoretic migration and immunofixation was allowed to occur. Interpretation is made visually by comparing the specific precipitin band to the (SPE) reference pattern.

IgG, IgA, IgM, were estimated using MININEPHTM HUMAN kit (Binding Site Ltd., Birmingham, UK). It involves a reaction with specific antiserum to form insoluble complexes. Concentrations are automatically calculated by reference to a calibration curve stored within the instrument.

Estimation of Beta-2-microglobulin was carried out by ELISA using commercial kit (ORGENTEC Diagnostika GmbH, Mainz).

Results

A total of 1155 samples were received during the 3 years period. Out of these, 349 samples were clinically and or radiologically suspected to have plasma cell dyscrasia. Of the total cases, 282 (24.4%) samples were positive for M component on SPE. Of these 282, 239 (84.8%) had M spike in

λ region and 43 had M spike in β region. No patient had M spike in the α_2 region. The mean load of the M protein band in the λ region was 37.8% with a range of 23.6% to 68.4% (normal: 9.0 to 23.0%) and in β region was 35.8% with a range of 16.9% to 45.5% (normal: 9.0 to 16.2%) (Table 1 and Fig 1). The male to female ratio was 1.2:1.

This department established its own reference intervals of SPE. Accordingly, 40 apparently healthy ambulatory patients (20 male and 20 female) were used to generate the central 95% reference intervals for the serum protein fractions as shown in Table 2.

20 patients with positive M bands were subjected to IFE and IEP. IgG with κ chain was seen in 08 patients (40%), Ig G with λ chain was seen in 10 patients (50%), 01 patients (5%) had IgM with κ light chain and one patient (5%) had Ig A with

Fig. 1 : Images of Serum protein electrophoresis showing M band in lanes 5, 6, & 8. Inset (left) image shows normal distribution of protein and right inset show M spike in λ region.

Table 2

SPE reference range [n=40 (20 males and 20 females)]

Protein fraction		Mean	Std dev	95% range
Albumin	%	60.0	4.04	51.9 - 68.0
	g/dL	4.2	0.38	3.5 - 5.0
Alpha 1	%	2.7	0.62	1.5 - 3.9
	g/dL	0.2	0.05	1.0 - 0.3
Alpha 2	%	8.9	1.43	6.0 - 11.8
	g/dL	0.6	0.10	0.4 - 0.8
Beta	%	12.6	1.81	9.0 - 16.2
	g/dL	0.9	9.12	0.6 - 1.1
Gamma	%	16.0	3.54	9.0 - 23.0
	g/dL	1.1	0.27	0.6 - 1.7
A/G ratio		1.5	0.24	1.0 - 2.0

λ light chain (Fig 2, 3).

Estimation of β_2 microglobulin was carried out on 255 serum samples of confirmed multiple myeloma that had positive M band on SPE. Of these, 246 samples (96.5%) had high levels of β_2 microglobulin (range: 2.01 to 28.73 μ g/dl). Serial monitoring of representative 10 patients of multiple myeloma for β_2 microglobulin and SPE was done over a period of one

Fig. 2 : Immunofixation studies done to characterize the monoclonal protein as a monoclonal immunoglobulin G λ protein. The arrow indicates the lanes that show reactivity with the specific antibodies.

Fig. 3 : Immunoelectrophoresis studies done to characterize the monoclonal protein as a monoclonal immunoglobulin G κ protein. The arrows indicates the lanes that show reactivity with the specific antibodies

Fig. 4 : Serial monitoring of β_2 microglobulin in multiple myeloma cases showing various levels. These did not correlate with M band on SPE.

Table 3

Serum immunoglobulins estimation (n=116)

Immunoglobulin (normal)	Increased (%)	Normal (%)
IgG (6.56 - 13.5 g/L)	58 (50%)	58 (50%)
IgA (0.86 - 3.20 g/L)	8 (6.9%)	108 (93.1%)
IgM (0.52 - 1.79 g/L)	6 (5.2%)	110 (94.8%)

year and results are shown in Fig 4.

Estimation of Ig G, Ig A and Ig M was done in 116 cases of multiple myeloma. It showed IgG myeloma was more common (50%) as compared to Ig A (6.9%) and Ig M (5.2%) myeloma (Table 3).

Discussion

The study of monoclonal gammopathy offers an excellent example of how the clinician and the laboratory physician can work together productively. The detection of a M band is often a casual finding in a routine workup and can point the clinician towards the diagnosis; on the other hand, the search for M band is often suggested by the clinical picture. The monoclonal gammopathies include malignant conditions such as plasma cell dyscrasias, rarely Non-Hodgkin's lymphoma, chronic lymphatic leukaemia and benign idiopathic forms of unknown significance, probably not related to B – cell disorders e.g. carcinoma, aging, viral and parasitic infections, after cardiac surgery, and associated with drug treatment (diphenylhydantoin, sulfonamides, penicillin). In this study, 24.4% of the serum samples were positive for M – band on SPE. These patients presented with features of anaemia (7.2%), renal failure (22.7%) and low backache (30.5%). In the “other” subgroup where 18.8% of cases showed positive M – band; were cases of benign chronic inflammatory disorders, carcinomas, collagen vascular diseases and gastrointestinal disorders.

After monoclonal protein is identified to be abnormal, the heavy – and light - chain isotypes are identified by using IEP or IFE. If there is bowing in the light chain arc without the corresponding abnormality in the IgG,

IgA or IgM areas, the possibility of an IgD or IgE paraprotein should be investigated [6]. In our study, none of the patients were diagnosed as a case of IgD or IgE myeloma. In this study, IgG λ myeloma was more frequent (50%) as compared to IgG κ myeloma (40%), IgM κ and IgA λ myeloma were seen in 5% each.

IFE is more sensitive than IEP and therefore is particularly useful for the assessment of small residual M components following treatment. Appropriate sample dilution is critical to distinguish a sharp monoclonal component from dense polyclonal band. In this study, one case of Ig A κ was subjected to IFE on treatment and the samples were diluted before IFE.

β_2 M levels above 5 μ g/ml, identify a subgroup of patients with a median survival of only two years [14]. In this study, of the 246 samples who had high β_2 M levels, 81 (33.5%) had β_2 M levels more than 5 μ g/ml. β_2 M is not helpful for monitoring the course of disease, because patients relapse without a preceding increase in β_2 M levels [7]. Additionally, β_2 M levels are elevated in patients on interferon even when the disease is in remission. In our study, it was seen that β_2 M and quantification of M protein in SPE were not corroborating in most cases.

Quantitation of immunoglobulin performed by radial immunodiffusion in the past was imprecise for Ig A and Ig M paraproteins and rate nephelometry for immunoglobulin quantitation is the method of choice [8,9, 15-17]. The amount of M protein can also be assessed with the densitometer tracing of SPE. The two methods frequently give discrepant results, with higher values being obtained with nephelometry [6,14–16]. So, when evaluating response to treatment, it is important to select only one technique. In this study, Ig estimation was done in 116 cases of multiple myeloma and 72 cases (62.1%) were positive. Ig G levels were most frequently raised (50%) followed by IgM (10.3%) and Ig A (6.9%). Similarly, quantification of M proteins was performed, which corroborated with the diagnosis of gammopathies and was helpful in monitoring the therapeutic modalities.

It is concluded that high level of precision and resolution of SPE, IFE and IEP leads to improved detection and follow-up of patients with monoclonal gammopathies. It is recommended that SPE should be performed in patients having unexplained weakness, fatigue, unexplained anaemia, back pain, osteoporosis, osteolytic lesions, fractures, renal insufficiency or recurrent infections. However, it is emphasised that many monoclonal proteins are associated with non malignant conditions and in many never associated with complications or evolution to malignancy. Rare cases of light chain disease can still be missed by the above

investigations, in whom tests for serum and urine free light chains, measuring both free κ and λ light chains are required.

Conflicts of Interest

None identified

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