Original Article

Immunoglobulin Pattern in Monoclonal Gammopathy Disorders

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DOI: https://doi.org/10.3329/jafmc.v14i2.45899

Abstract

Introduction: The presence of abnormal monoclonal proteins, the M band, is a frequent characteristic feature of plasma cell dyscrasias and is usually detected as a discrete band in the γ or β region in serum or urine protein electrophoresis. It is characterized and confirmed by immunofixation electrophoresis (IFE). Accurate detection and quantification of monoclonal immunoglobulins are important for the diagnosis and management of monoclonal gammopathies.

Objectives: To find out the pattern of immunoglobulin in monoclonal gammopathy cases and evaluate the role of IFE in the detection of them.

Materials and Methods: This cross-sectional descriptive study was conducted in the Department of Haematology, Armed Forces Institute of Pathology (AFIP), Dhaka from July 2015 to December 2015. Thirty diagnosed cases of monoclonal gammopathies of both sexes were selected. Bone marrow examination, serum protein electrophoresis, skeletal survey, relevant biochemical test and IFE were performed for all the cases.

Results: Out of 30 monoclonal gammopathy cases, M band was identified in 24(80%) cases by serum protein electrophoresis but by the IFE M band was found in all 30(100%) cases. Among the M band pattern of immunoglobulin was characterized by IFE and the result was; 15(50%) cases IgG Kappa, 09(30%) cases IgG Lambda, 02(6.7%) cases IgA Kappa, 02(6.7%) cases IgM Kappa and 02(6.7%) cases light chain kappa monoclonal protein.

Conclusion: Though the number of the patient was limited, it is evident that in 20% gammopathy cases M band was missing by conventional serum protein electrophoresis but IFE could identify M band in all the cases. It is recommended that IFE should be carried out in all monoclonal gammopathy patients.

Key-words: Monoclonal gammopathy, Immunoglobulin, Immunofixation electrophoresis.

Introduction:

Monoclonal gammopathy is defined as the electrophoretically and antigenically homogeneous protein product of a single clone of B lymphocytes or plasma cells. The presence of abnormal monoclonal proteins is referred to as monoclonal gammopathy, is a frequent, characteristic feature of plasma cell dyscrasias. The monoclonal protein is usually detected as a discrete band in the γ or β region in serum or urine protein electrophoresis (M spike). The nature of the monoclonal protein is then characterized and confirmed by an immunofixation electrophoresis (IFE). Accurate detection and quantitation of monoclonal immunoglobulins are important for the diagnosis and management of monoclonal gammopathies[1,2]. In particular, monoclonal immunoglobulins can be used for screening, monitoring and treatment of diseases and for monitoring progression of diseases in monoclonal gammopathy of unknown significance (MGUS). Approximately 30% of monoclonal gammopathy patients (including patients with light chain myeloma, primary or amyloid light chain (AL) amyloidosis, non-secretory myeloma, and light chain deposition disease) produce free light chains (FLC) as the only monoclonal component[3].

The study of monoclonal gammopathy offers an excellent example of how the clinician and the laboratory physician can work together productively. The detection of 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. Samples for serum electrophoresis are analyzed for M band positivity, bone marrow study, biochemical tests and for correlation with clinical profile of the patients. The prevalence of this disease is about 1% of all the cancers but the incidence increases after the age of 60 years. Normally the plasma cells constitute 1% of the cells in the bone marrow, but as the disease progresses, the tumour load in the bone marrow increases up to 80%, depending upon the disease severity. These malignant plasma cells synthesize monoclonal antibodies which are released into the circulation. Therefore, this monoclonal protein (antibody) level in the serum increases[4].

The IFE technique combines zone electrophoresis with immunoprecipitation and is easier and more useful than other techniques. By IFE, proteins of a sample are identified according to their charge separated by electrophoresis on the agarose film, and then they are fixed with monospecific polyclonal antisera. The monoclonal paraproteins can be heavy chain of

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IgG, IgA, IgM, IgD, IgE and or light chain kappa or lambda. IFE is more sensitive and may reveal an immunoglobulin missed out by conventional protein electrophoresis, especially at low concentrations. To know the pattern of immunoglobulin in monoclonal gammopathy cases and evaluate the role of IFE in detection of different immunoglobulin pattern in monoclonal gammopathy cases.

Materials and Methods

This cross-sectional descriptive study was conducted in the Department of Haematology, Armed Forces Institute of Pathology (AFIP), Dhaka from July 2015 to December 2015. Total of 30 diagnosed patients of monoclonal gammopathies aged between 40-70 years irrespective of sex were selected for the study. Patients with plasma cell dyscrasia with other malignancy were excluded. Baseline information was collected by face-to-face interview using a standard structured questionnaire. The general medical condition of the patients was evaluated through history, clinical examination and laboratory investigation. Bone marrow aspiration, serum protein electrophoresis, IFE, skeletal survey and relevant biochemical tests were performed for all patients. Protein electrophoresis of the samples was performed by automated capillary electrophoresis machine (Capiflex-2) which identifies the various protein bands and depicts as a graph. The M band is usually found in the gamma globulin region but in a few cases it is identified in the beta region also. The machine identifies the M protein both qualitatively and quantitatively. IFE was also performed by the same capillary electrophoresis machine. IFE identifies the type of heavy (IgG, IgM or IgA) and light chain (either kappa or lambda).

Results

The study revealed that the mean age of the patients was 57.13 ± 9.66 years and majority (56.7%) belonged to 60-70 years' age range. About 63% patients were male with a male-female ratio 1.72:1 (Table-I). Bone marrow study found about 73.3% cases were multiple myeloma and serum protein electrophoresis found M band in 80% cases but IFE found M band in all 30 cases. Among the M band pattern of immunoglobulin was characterized by IFE and the result was; 15(50%) cases IgG Kappa, 09(30%) cases IgG Lambda, 02(6.7%) cases IgA Kappa, 02(6.7%) cases IgM Kappa and 02(6.7%) cases light chain kappa monoclonal protein (Table-II). Biochemical changes according to different immunoglobulin pattern are shown in Table -III. In IgG Kappa renal insufficiency 33.3%, hypercalcaemia 40%, BJP 26.7%. In IgG lambda renal insufficiency 77.7%, hypercalcaemia 44.4%, BJP 44.4%. In IgM Kappa Hypercalcaemia 50%. In IgA Kappa renal insufficiency is 100%, Hypercalcaemia 50%, BJP 100%. In light chain kappa monoclonal gammopathy renal insufficiency 100%, Hypercalcaemia 100%, BJP 100% (Table-III). In IgG Kappa; Multiple myeloma 11, Smoldering multiple myeloma 2, MGUS 2. In IgG lambda; Multiple myeloma 6 and MGUS 3. In IgM Kappa; Multiple myeloma 2. In IgA Kappa; Multiple myeloma 2. In kappa light chain; multiple myeloma 2 (Table-IV).

Table-I: Distribution of patients according to age and sex (n= 30)

Characteristics		Frequency	Percentage
Age (years)	40-49	07	23.3
	50-59	06	20.0
	60-70	17	56.7
	Mean ±SD	57.13 ± 9.66	
Sex	Male	19	63.0
	Female	11	37.0

Table-II: Laboratory findings of patients (n= 30)

Characteristics		Frequency	Percentage
Hb%	Below 9 gm/dl	18	60.0
	Between 9 gm/dl to lower normal range	6	20.0
	Within Normal reference	6	20.0
PBF	Anaemia of chronic disorder	15	50.0
	Microcytic hypochromic blood picture with high ESR	05	16.7
	Neutrophil leucocytosis with high ESR	04	13.3
	None specific findings	03	10.0
	Leuco-erythro-blastic blood picture	03	10.0
Bone Marrow Study	Suggestive of Multiple myeloma (bone marrow plasma cell >20%)	22	73.3
	Plasma Cell dyscrasia (bone marrow plasma cell <20%)	06	20.0
	Secondary Reactive Marrow	02	6.7
Serum electrophoresis	Monoclonal band (M band)	24	80.0
	Normal findings	06	20.0
Type of monoclonal protein by IFE	IgG Kappa	15	50.0
	IgG Lambda	09	30.0
	IgA Kappa	02	06.7
	IgM Kappa	02	06.7
	Light chain Kappa	02	06.7
Osteolytic lesion	Present	22	73.3
	Absent	08	26.7
Serum Creatinine	≤ 2.0 mg/dl	14	46.7
	> 2.0 mg/dl	16	53.3
Urine for BJP	Present	17	56.7
	Absent	13	43.3
Serum Calcium	≤ 11.0 mg/dl	16	53.3
	> 11.0 mg/dl	14	46.7

Table-III: Distribution of biochemical change in different immunoglobulin pattern

Biochemical change	Serum immunoglobulin pattern in monoclonal gammopathy					
	IgG Kappa (n=15)	IgG Lambda (n=9)	IgM Kappa (n=2)	IgA Kappa (n=2)	Light chain Kappa (n=2)	Total
	Frequency (%)	Frequency (%)	Frequency (%)	Frequency (%)	Frequency (%)	
Renal insufficiency	5(33.3)	7(77.7)	0	2(100)	2(100)	16
Hypercalcaemia	6(40.0)	4(44.4)	1(50.0)	1(50)	2(100)	14
BJP	8(53.3)	4(44.4)	0	2(100)	2(100)	16

Table-IV: Diagnosis of monoclonal gammopathy cases according to different immunoglobulin pattern

Pattern of monoclonal immunoglobulin	Diagnosis				
	Multiple myeloma	Smoldering multiple myeloma	MGUS	kappa light chain multiple myeloma	Total
IgG Kappa	11	2	2	0	15
IgG Lambda	6	0	3	0	9
IgM Kappa	2	0	0	0	2
IgA Kappa	2	0	0	0	2
Light chain Kappa	0	0	0	2	2
Total	21	2	5	2	30

Discussion

In this study, the mean age was 57.13±9.66 and majority (56.7%) was 60-70 years' age group. Male predominance (63%) male to female ratio was 1.72: 1. A similar study by Shaheen et al[5] reported that the mean age of the patients was 58 years and male to female ratio was 1.35:1, which is consistent with this study. In a study by Talerman, anaemia was present on admission in 74% of cases and was severe (below 9 gm/dl) in 50%[6] and also consistent with the study by Shaheen et al[5], where haemoglobin was below normal in 90% and <8.5 gm/dl was in 39% patients. In this study, the peripheral blood film at diagnosis, 15(50%) revealed anaemia of chronic disorder, 05(16.7%) were microcytic hypochromic blood picture with high ESR, 04(13.3%) were neutrophil leucocytosis with high ESR, 03(10%) were none specific findings and 03(10%) were Leuco-erythro-blastic blood picture. This study shows bone marrow findings at diagnosis, majority 22(73.3%) were found suggestive of multiple myeloma, followed by 06(20%) were found plasma cell dyscrasia and 02(6.7%) were secondary reactive marrow. Yasseen MK et al[7] reported that in serum protein electrophoresis: monoclonal M-band was found in 30(93.75%) patients of the studied group, in present study serum protein electrophoresis at diagnosis found in 24(80%).

In the present study, out of 30 monoclonal gammopathy cases, M band (monoclonal protein) identified 24(80%) by conventional serum protein electrophoresis but by the IFE method found the presence of M band (monoclonal protein) 30(100%) cases. Among the M band (monoclonal protein) also categories/ pattern by immunofixation method. The present study detected immunoglobulin pattern by IFE, which indicated predominance of IgG Kappa monoclonal protein (50%), followed by IgG Lambda monoclonal protein (30%), IgA Kappa monoclonal protein (6.7%), IgM Kappa monoclonal protein (6.7%) and Light chain kappa monoclonal protein (6.7%), and Giti S et al[8] also reported IgG kappa monoclonal protein in 80% of cases and IgG lambda monoclonal protein in 20% of cases which is almost similar to the study by Katzmann et al[9]. The study found IFE confirmed all 169 serum M-spikes in his study as monoclonal proteins which had 100% specificity.

Serum IFE method is considered the Gold standard[10,11] with high sensitivity and specificity to detect a small monoclonal protein early and to distinguish the heavy chains from light chains present in the serum and urine of a patient with monoclonal gammopathy. In this study, monoclonal band was detected in all (30) cases by serum IFE technique, whereas serum protein electrophoresis (SPEP) method could detect 24(80%) cases only. So in the remaining 06 cases (20%), a small sharp spike of monoclonal band was found by IFE method whereas, SPEP technique could not detect those cases. A study by Tate et al[12] reported that serum protein electrophoresis detected M protein 74.3%- 87.0%. But in addition of IFE increased this proportion to 97.4%, but the majority of those missed M-proteins MGUS at low risk of progression to Multiple myeloma. A study by Riccaracdi et al[13] reported that out of 561 monoclonal gammopathies patients, IgG was found 71.47%, IgA was 18.36% and IgM 4.27%. A study by Khan and Bina confirmed that IFE is the only method required to identify IgM paraproteins, with a detection limit of ≤0.25 g/L even when the concentration of polyclonal immunoglobulin is increased[14]. A study by Youinou et al[15] reported that out of 219 monoclonal gammopathies patients, IgG was found 51.40%, IgA was 21.49% and IgM 22.89%. A study by Steingrimsdottir et al[16] revealed that the type of paraprotein was IgA in 33.5%, IgG in 57% and IgM in 8.5% of cases. This study shows the urine for Bence Jones protein was present among 17(56.7%) among the study population. Youinou et al[15] study reported Bence Jones proteinuria was detected 51.1% of cases, which is similar to the findings of this study. This study shows serum albumin level at diagnosis 70% were <3.5 mg/dl and 30% were >3.5 mg/dl which is similar to Shaheen et al[5] and Lakshminarayan et al[17].

Conclusion

In spite of the limited number of patients from this study, it was evident that in 20% gammopathy cases M band was missing by conventional serum protein electrophoresis but IFE could identify M band in all the cases. It is recommended that IFE should be carried out in all monoclonal gammopathy patients. The simplicity and economy of the method and the clarity of final results suggest that immunofixation should be considered as a possible successor for the study of monoclonal proteins.

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