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Original Research Article

A study to evaluate Immunoglobulin's fraction by Serum protein electrophoresis for occurrence of Multiple myeloma in a Tertiary care hospital

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Abstract

Background: Serum protein electrophoresis (SPE) is a diagnostic laboratory test that separates and quantifies several classes of serum proteins and that identifies and characterizes the monoclonal gammopathies (M-protein).
Aims and Objective: This study was taken up with an aim to evaluate the relevance and significance of immunoglobulin fraction in discerning multiple myeloma and to assess the morbidity of cases in relation to its clinical diagnosis.
Materials and Method: This study was carried out by analysing data with gamma globulins above biological reference range (0.6-1.6gm/dL) from the samples requested for serum protein electrophoresis by cellulose acetate method during Sept 2013 to Aug 2014 in St. John's medical college biochemistry lab, Bengaluru (Total of 95 cases). Densitometrically estimated M proteins were further divided into 2 groups of below and above 3gm/dL according to the IMWG criteria for classification of Multiple myeloma.
Result: Out of 95 cases, 55(57.9 %) were above 3gm/dL and the remaining 40(42.1%) were below 3gm/dL. Among those 95 cases 18 were confirmed with multiple myeloma. 13 out of 18 cases fall among the group where gamma globulin is > 3g/dL with 81% of them having gamma above 4g/dL. Five cases of multiple myeloma had also been confirmed with their gamma globulins falling below 3g/dL which is compelling.
Conclusion: The significance of elevated gamma globulins value less than the prescribed criteria of IMWG for diagnosing Multiple myeloma gave us importance of clinical correlation before releasing report. Though the morbidity percentage (20%) of multiple myeloma among the group with gamma globulins less than 3gm/dL, is less but nevertheless significant. More number of studies with large sample numbers is required to understand the cases of multiple myeloma with their gamma globulins falling below the IMWG criteria cut off limit.
Keywords: Serum protein electrophoresis; SPE; M band; Multiple myeloma.

1. Introduction

Multiple myeloma is a malignant disorder characterized by proliferation of single clone of plasma cells derived from B-cells in the bone marrow. It results in the secretion of a specific and unique Monoclonal immunoglobulin (M-protein).[1-5] The plasma cell proliferation usually results in extensive skeletal destruction with osteolytic lesions, hypercalcemia, anemia, and, occasionally, plasma cell infiltrationin different organs.

The excessive production of a monoclonal (M) protein can lead to renal failure, hyper viscosity syndrome or recurrent bacterial infections. [6-8]

Protein electrophoresis is advised whenever multiple myeloma is suspected. The monoclonal protein migrates as a single entity in the electric field and is detected by a nonspecific protein stain as a more intensely stained band superimposed on the usual protein pattern. [9] Multiple myeloma accounts for approximately 1% of all the malignancies and about 10% of all the hematological

malignancies. The occurrence of multiple myeloma is 4:100000 worldwide. [10-13]

Serum protein electrophoresis can be routinely used for the diagnosis of multiple myeloma and is well correlated with biochemical, radiological and pathological findings. Therefore, serum protein electrophoresis (SPEP) should be done to evaluate the general manifestations like malaise, weakness, chronic bone pain and anemia, to detect the monoclonal gammopathies. [14-16]

In the interpretation of SPEP, more attention is given to the gamma region, which is mainly composed of immunoglobulin. Many conditions can cause an increase in the gamma region, but those which cause a homogenous spike like a peak in the gamma globulin zone, are of special interest. These proteins are called para proteins or M (monoclonal) proteins. The M protein or the M component is readily detected as a sharp symmetric spike (M spike) usually in gamma or beta region of the electrophoretic strip and rarely in alpha 2 region. [17-19]

In view of these findings a study was taken up to evaluate immunoglobulin fraction by serum protein electrophoresis (CAE) for the occurrence of Multiple myeloma in a tertiary care hospital.

The aims and objective of study is to evaluate the relevance and significance of immunoglobulin fraction in discerning Multiple myeloma and to assess the morbidity of cases in relation to its clinical diagnosis.

2. Materials and Methods

This study was carried out by analysing data with gamma globulins above the biological reference range (0.6-1.6gm/dL)from the samples requested for serum protein electrophoresis by cellulose acetate method during Sept 2013 to Aug 2014 in the St. John's medical college biochemistry lab, Bangalore (Total of 95 cases). SPE was done using Genios electrophoresis instrument which provides the electrophoretic separation of proteins. This mobility pattern was visually interpreted and quantitated by densitometry at 600nm, in which, the relative percentage of each protein fraction is calculated automatically. Densitometrically estimated gamma globulins were further divided into 2 groups of above (group A) and below(group B) 3gm/dL.

Total protein was analysed with modified biuret method and serum albumin using BCP method using Siemens Dimensions RxL instrument. The clinical history and the bone marrow biopsy reports were correlated in the M band positive cases to differentiate Multiple myeloma from other conditions.

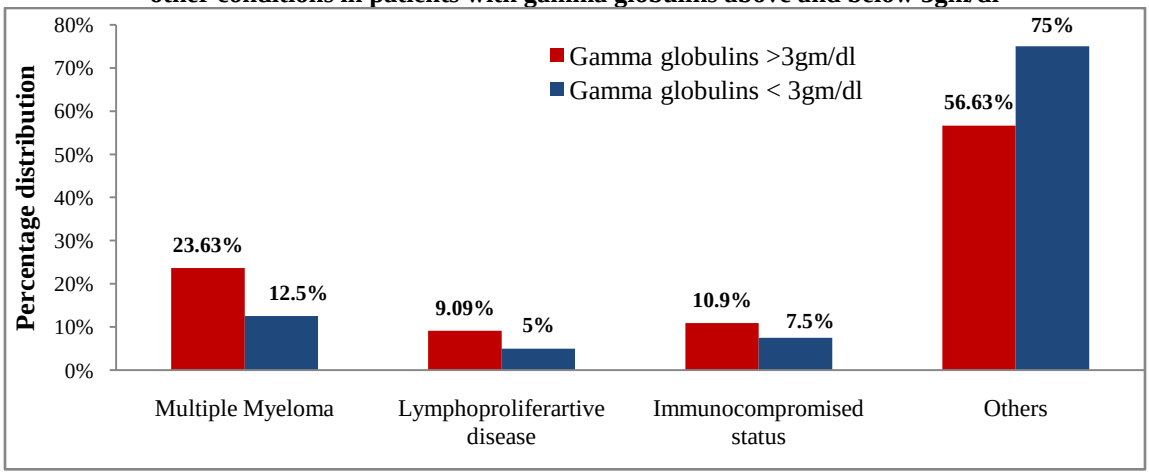
2.1 Statistical Methods

This is a descriptive analytical study where results on continuous measurements are presented on mean $\pm$ SD and results on categorical measurements are presented in number (%). The data was entered on a microsoft excel spreadsheet and analysed using SPSS version 16. Microsoft word and excel have been used to generate graphs and tables.

3. Results

Out of the total 95 cases, 55 cases (57.9%) were having gamma globulins above 3 gm/dL (group A) and remaining 40 cases (42.1%) were having gamma globulins below 3gm/dL (group B). There were 13 (23.63%) cases of multiple myeloma in group A with majority of them (81%) having gamma globulins lying above 4gm/dL. There were 5 (12.5%) cases of multiple myeloma in group B also. 9.09%(5 case) of lymphoproliferative disorders in group A and 5% (2 cases) in group B, 10.90% (6 cases) of immunocompromised cases in group A and 7.5%(3 cases) in group B and 56.36% (31 cases) of other conditions which include acute infections, chronic infections, autoimmune disorders, nutritional anemia in group A and 75% (30 cases) in group B.(Fig 1)

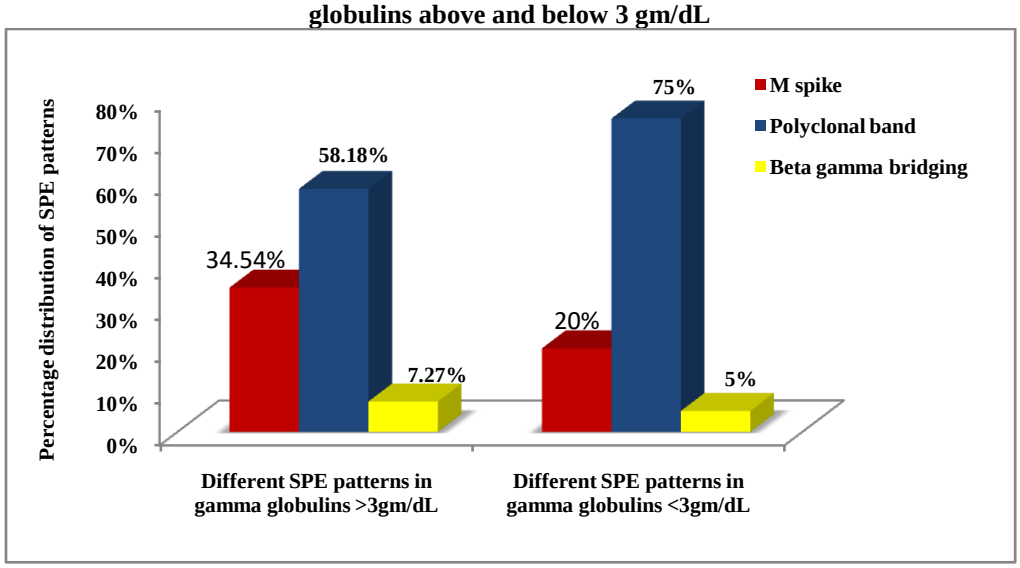
Fig 1: Frequency distribution of Multiple Myeloma, Lymphoproliferative disorders, Immunocompromised state and other conditions in patients with gamma globulins above and below 3gm/dl



Out of the 95 total cases 18 were confirmed with Multiple myeloma, group A getting a share of 13cases (72.2%) and the remaining 5 cases (27.77%) fall in the group B.

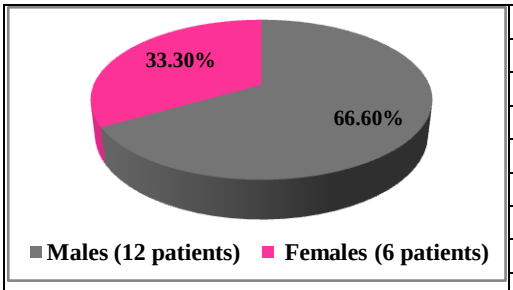
In group A (gamma globulins >3gm/dL), SPE showed spike in 34.54% (19 cases) and 20% (8 case) in group B (gamma globulins <3gm/dL) patients. Group A patients showed polyclonal band in 58.18% (32 cases) &75 %(30 cases) in group B patients. Beta gamma bridging in SPE pattern seen in 7.27% (4 cases) in group A patients & 5% (2 cases) in group B patients (Fig.2)

Fig 2: Percentage distribution of M spike, polyclonal band and beta gamma bridging in patients with gamma globulins above and below 3 gm/dL



Male to female ratio in confirmed multiple myeloma cases is 2:1(Fig 3). Majority of the male patients belonged to age group of 51-60yrs whereas majority of female cases were in the range of 61-70yrs (Fig 3).

Fig 3: Distribution of multiple myeloma cases according to (a) sex (in percentage) and (b) age (in number) with average age of presentation



(a)

Age (in yrs.)	Male	female	Total
30-40	1	1	2
41-50	1	0	1
51-60	4	2	6
61-70	4	2	6
71-80	0	1	1
81-90	2	0	2
Total	12	6	18
Average	**62yrs**	**61yrs**	**62yrs**

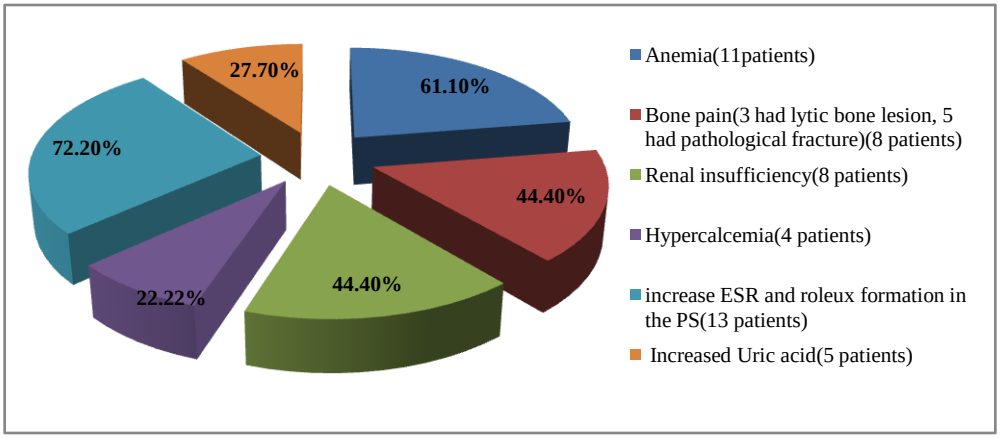
(b)

3.1 Clinical presentation

61.1 % (11patients) of MM patients presented with anemia. 44.4% (8 patients) of MM patients presented with bone pain. 44.4% (8 patients) of MM patients presented with renal insufficiency. 22.22% (4 patients) of MM patients presented with hypercalcemia (Total calcium and ionized calcium). 72.2% (13 patients) of MM patients had increased ESR and roleux formation in the PS. 27.7% (5 patients) of MM had increased uric acid level at the time of admission.

88.88% (16 patients) of Multiple myeloma patients had plasma cells >30% in the bone marrow biopsy. Only in 2 patients (11.1%), immunoelectrophoresis was done to confirm multiple myeloma. Bence jonce protein (BJP) was negative in all the patients.

Fig 4:



Majority of the multiple myeloma cases had M spike in the gamma region (61.1%) except 7 cases, in which 6 cases had M spike in beta region and beta gamma region (33.33%) and in 1 case it was a polyclonal band (5.55%).

Densitometric images of serum protein electrophoresis showing M spike (Fig 5) and Normal QC: Lot no: 14441(Fig 6).

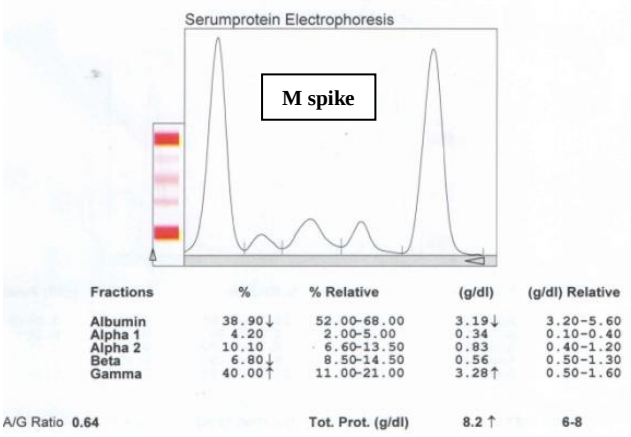


Fig 5: Densitometric images of serum protein electrophoresis showing M spike

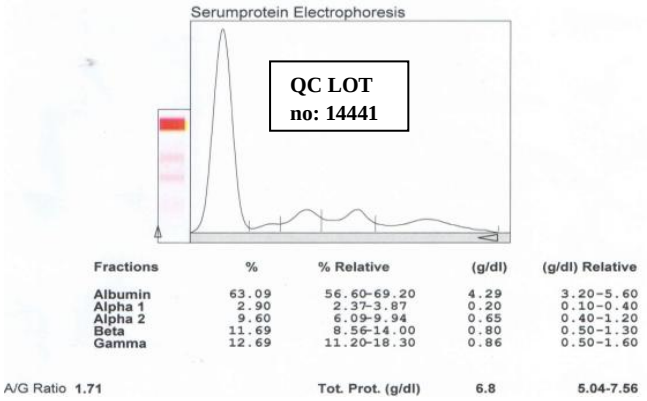


Fig 6: Densitometric images of serum protein electrophoresis showing Normal QC: Lot no: 14441

Densitometric images of serum protein electrophoresis showing polyclonal band (Fig 7) and beta gamma bridging (Fig 8)

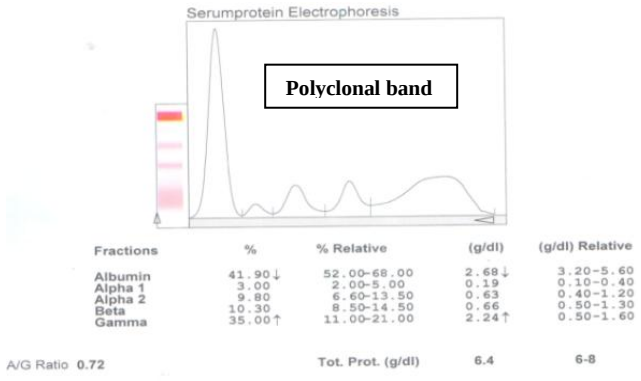


Fig 7: Densitometric images of serum protein electrophoresis showing polyclonal band

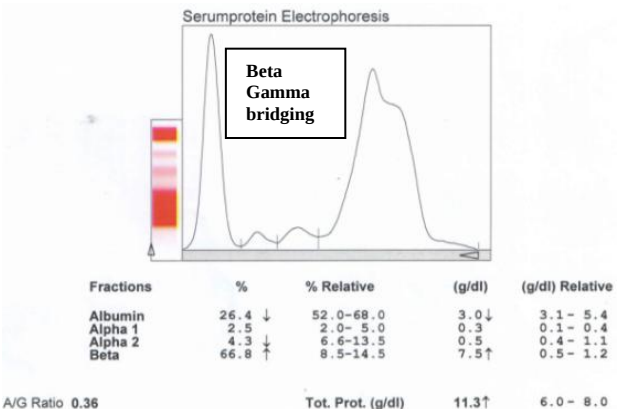


Fig 8: Densitometric images of serum protein electrophoresis showing beta gamma bridging

The maximum numbers of quantified gamma globulins were showing above 4 gm/dl. Mean concentration of M protein in the gamma region in group A was 5.45gm/dl with a range of 3.21gm/dl – 10 gm/dl and 2.76

gm/dl for group B with a range of 2.23 -2.98gm/dl. All the multiple myeloma cases were co-related with bone marrow biopsy reports and the clinical features. The % of the myeloma cells in the bone marrow was variable and it ranged from 15% to 90% for group A and from 30% to 60% for group B.

In patients with gamma globulins less than 3gm/dL all the patients with multiple myeloma had >30% of plasma cells in the bone marrow biopsy signifying the importance of M spike which leads to the early detection and initiation of prompt treatment, therein reducing the morbidity. (Fig 10)

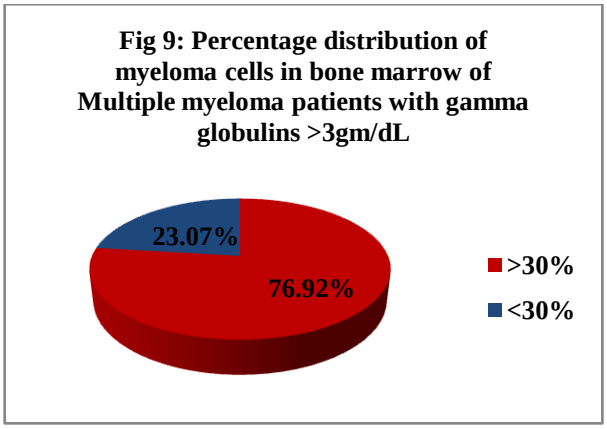


Fig 9: Percentage distribution of myeloma cells in bone marrow of Multiple myeloma patients with gamma globulins >3gm/dL

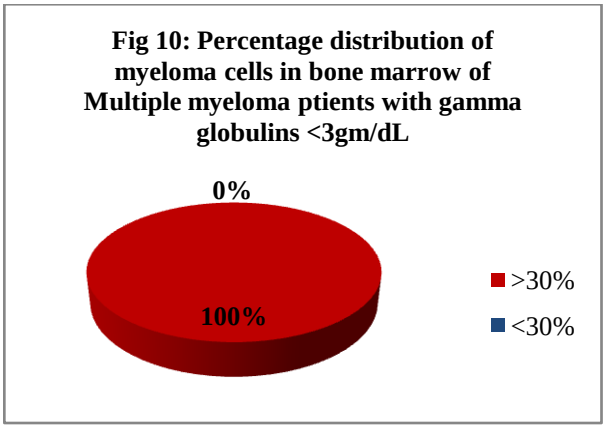


Fig 10: Percentage distribution of myeloma cells in bone marrow of Multiple myeloma ptients with gamma globulins <3gm/dL

Only one patient with multiple myeloma expired during the study whose gamma globulins was 4.8 gm/dL and others were on chemotherapy regimen.

4. Discussion

Multiple Myeloma (MM) is a malignancy of plasma cells that results in their accumulation in the bone marrow (BM). These cells produce monoclonal immunoglobulins and cytokines that are responsible for the anemia, bone pain, hypercalcemia, renal insufficiency, and infections that occur in these patients. The prevalence of multiple myeloma is low, about 1% of all the cancers, but the incidence increases after the age of 60yrs. The term, 'multiple myeloma' describes a characteristic feature which is found at multiple sites within the bone marrow (myelo), with the accumulation of the tumor (oma) cells. Normally, the plasma cells constitute 1%of the cells in the bone marrow, but as the disease advances, the tumor 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, the monoclonal protein (antibody) level in the serum increases. The study of monoclonal gammopathy offers an excellent example of how the clinician and the laboratory physician can work together productively. The detection of an 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.

Age of presentation and percentage of cases below 30yrs and above 45yrs well correlated with the mayo clinic studies. [7,8] Increased number of cases around the age of 60yrs might be because of frequent visits to the doctor due to multiple health issues.

Multiple myeloma was more common in males compared to females. Male to female ratio was 2:1, which was consistent with the Sunita Tripathy *et al* (1.7:1) and Col GS Chopra *et al* studies (1.2:1).

61.1 % (11patients) of MM patients presented with anemia. The mechanism in most patients is due to marrow replacement by myeloma cells or inadequate production of red blood cells due to either erythropoietin deficiency from accompanying renal failure.[25] In some patients, anemia is disproportionate to renal failure or marrow involvement and is thought to be related to cytokine-mediated marrow suppression [26] or shortened red blood cell survival; however, overt immune hemolytic anemia is rare.

44.4% (8 patients) of MM patients presented with bone pain and lytic bone lesions. Lytic bone lesions in multiple myeloma represent uncoupling between osteolytic and osteoblastic activities favoring more to osteolytic activities. Variety of mediators like IL-1, IL-1β, IL-6, sIL-6R, TNFα, MIP-1α, receptor activator of NF-κB ligand, macrophage inflammatory protein 1 alpha, dickkopf 1, osteoprotegerin and parathyroid hormone related protein has been implicated for this uncoupling. [27,28]

44.4% (8 patients) of MM patients presented with renal insufficiency with serum creatinine more than 2mg/dL. The major causes of renal failure are myeloma kidney (precipitation of monoclonal light chains in distal and collecting tubules) and hypercalcemia. Other causes include dehydration, hyperuricemia, and primary amyloidosis (AL).[25]

22.22% (4 patients) of MM patients presented with hypercalcemia (Total calcium and ionized calcium) and is one of the causes of treatable renal insufficiency. Hypercalcemia is due to increased destruction of bones.

72.2% (13 patients) of MM patients had increase ESR and roleux formation in the peripheral smear and is mainly due to fibrinogen, mono- or polyclonal increase of IgG, IgA, IgM alone or in combinations. 27.7% (5 patients) of MM had increased uric acid level at the time of admission in our study.

International staging system (ISS)[31] was used in comparison to the older clinical staging system of Durie & Salmon[30] for classification of patients with multiple myeloma and their prognosis/survival.

The treatment of myeloma is divided into supportive care with the goal of preventing or ameliorating complications of myeloma; these are most commonly renal, skeletal, infective and treatment related to bone marrow failure. The second aspect is specific treatment directed against the malignant plasma cells. [31,32] In recent years the introduction of 3 novel drugs oriented on basic mechanisms of multiple myeloma cells proliferation and survival has improved patients' outcome. These drugs are: thalidomide, its new analog, lenalidomide, and proteasome inhibitor, bortezomib. All three are highly effective both in newly diagnosed and relapsed/resistant patients. [33-36]

5. Conclusion

- The significance of elevated gamma globulins value less than the prescribed criteria of IMWG for diagnosing Multiple myeloma gave us importance of clinical correlation before releasing report.
- Though the morbidity percentage (20%) of multiple myeloma among the group with gamma globulins less than 3gm/dL, is less but nevertheless significant.

6. Limitations

Immunofixation (IFE) is more sensitive than SPEP for detecting the monoclonal immunoglobulins and to identify the heavy or light chain isotype. We could not detect the immunoglobulin isotype due to a lack of IFE in our laboratory.

Acknowledgement

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