

Pattern of Serum Protein Electrophoresis Results in a Group of Patients with Plasma Cell Myeloma Confirmed by Bone Marrow Findings at King Hussein Medical Center

*Mansour Al-Hiary MD**, *Baheieh Al-Abbadi MD**, *Nesreen Abu Hazeem MD**,
*Ali Swailmeen MD***, *Najah Aldrous MD**, *Nazmi Kamal MD**

ABSTRACT

Objective: To analyze the serum protein electrophoresis and immunofixation results in a group of patients confirmed to have plasma cell myeloma by bone marrow findings.

Method: The study was conducted at Princess Iman Center for Research and Laboratory Sciences at King Hussein Medical Center. A total of 30 patients were studied in the time period between June 2013 and December 2013. For each patient serum protein electrophoresis and immunofixation were performed. The bone marrow aspirate and biopsy results were reviewed. The patterns of serum protein electrophoresis were analyzed and confirmed by immunofixation electrophoresis.

Results: All the patients' serum protein electrophoresis results showed the presence of monoclonal band. No non-secretory myeloma cases were found. The location of bands was in gamma or beta regions; 23(76.6%) of 30 were found in gamma region, whereas 7(23.4%) in beta region. The largest proportion of IgG paraprotein type was found in gamma region and majority of IgA and IgM types were found in beta region. The most common paraprotein isotype found in beta region was IgA with frequency of 71.4%, 14.3% for IgG and 14.3% for IgM. The most frequent paraprotein (heavy chain) isotype seen was as follows: 80%, 16.6%, and 3.3% for IgG, IgA, and IgM respectively. Regarding light chains, kappa light chain was the most frequent (60%).

Conclusion: The patterns of serum protein electrophoresis and immunofixation of this group of plasma cell myeloma patients showed the presence of M-band in all patients. For early diagnosis patients suspected to have plasma cell myeloma the serum protein electrophoresis and immunofixation remain an easy gold standard test for cases with secretory plasma cell myeloma.

Key words: Electrophoresis, Immunofixation, Plasma cell myeloma.

JRMS March 2015; 22(1): 30-34 / DOI: 10.12816/0009783

Introduction

Plasma cell myeloma is a B-cell malignancy characterized by expansion of a single clone of immunoglobulins secreting plasma cells resulting in increased serum levels of a single

immunoglobulin, which is called monoclonal antibody.⁽¹⁾ These antibodies can be detected by presence of paraprotein called M-protein in serum protein electrophoresis (SPE).⁽²⁾ Plasma cell myeloma constitutes about 10% of

*From the Department of Pathology, Princess Iman Center for Research and Laboratory Sciences, King Hussein Medical Center, (KHMC), Amman-Jordan

Correspondence should be addressed to Dr. M. Al-Hiary, (KHMC), E-mail: Mansourhiary1974@gmail.com
Manuscript received April 2, 2014. Accepted June 19, 2014

hematologic malignancies and 1% of all malignancies,⁽³⁾ with the highest incidence in African-American and Pacific countries.⁽⁴⁾

Serum protein electrophoresis is a laboratory technique used for separation of charged proteins, which is based on movement of these proteins when exposed to an electric current. It is necessary for diagnosis of diseases such as autoimmune diseases, paraproteinaemias, immunodeficiency, and inflammation (acute and chronic).⁽⁵⁾ Bone marrow examination including aspirate and biopsy are gold standards for assessment of bone marrow infiltration by plasma cells, and to study the morphology of these cells.⁽⁶⁾

The WHO diagnostic criteria for symptomatic plasma cell myeloma include presence of M-protein in serum or urine (M-protein in most cases is > 30g/l of IgG or > 25g/l of IgA or > 1g/24 hours of urine light chain), bone marrow clonal plasma cells or plasmacytoma (more than 10% of bone marrow nucleated cells are monoclonal plasma cells, but there are no minimal levels because around 5% of patients with symptomatic myeloma have levels lower than these), and related organ or tissue impairment (hypocalcaemia, renal insufficiency, anemia, bone lesions). While criteria for asymptomatic myeloma (smoldering) include M-protein in serum at myeloma levels (>30 g/l), and 10% or more clonal plasma in bone marrow, and no related organ or tissue impairment.⁽⁷⁾ For these purposes protein electrophoresis and bone marrow examination are mandatory laboratory tests for diagnosis of multiple myeloma. SPE used as screening test for presence of monoclonal immunoglobulin and if present further investigation such as immunofixation is indicated for identification of heavy (α , γ , μ , ϵ , δ) and light (λ , κ) chains.⁽⁸⁾ M-protein can be detected by presence of monoclonal band (M-band) in gamma region (γ) as a narrow sharp spike, and this band may be migrate toward alpha 2(α_2) or beta (β) regions of serum protein electrophoresis densitometry scan.⁽²⁾ IgA M- band in most cases migrate in β region and confused with transferrin, complement 3, and β lipoprotein bands. In these cases if the patients are highly suspicious for having multiple myeloma immunofixation is absolute indication. The rate of monoclonal heavy chain is 55%-60%, 25%, 1-2%, 0.5%, and

very rare for IgG, IgA, IgD, IgM, and IgE respectively. For light chains, kappa light chain is twice more prevalent than lambda. Fifteen percent of multiple myeloma free light chain only secreted without heavy chain, and rare cases presented without M-protein in serum protein electrophoresis and immunofixation known as non-secretory myeloma.⁽⁹⁾

Method

The results of 30 patients were reviewed in the time period between June 2013 and December 2013 in Princess Iman Center for Research and Laboratory Sciences at King Hussein Medical Center. Nineteen of 30 were males and 11 were females. Ages of individuals ranged between 36 years and 83 years with a median age of 61 years. For each individual serum protein electrophoresis, and immunofixation were performed, and the results of bone marrow examination (aspirate and biopsy) were reviewed. For serum protein electrophoresis and immunofixation 10ml of blood was collected from the patients, then the serum was separated by centrifugation.

Electrophoresis of serum proteins was performed by agarose gel (Hydrasys from Sebia, UK). The samples were further investigated by immunofixation electrophoresis with antisera to heavy chains isotypes (α , γ , μ) and light chains isotypes (λ , κ).

Samples for bone marrow examination including aspirate and biopsy were obtained from posterior iliac crest and sternum. Up to 300 μ l of aspirate, which is the optimal volume was collected, and 2 mm wide with 2 cm long solid piece of bone marrow also obtained, both samples were examined under light microscope.

Results

A total of 30 patients had a diagnosis consistent with multiple myeloma based on bone marrow findings. The mean age of plasma cell myeloma patients in the study was 61 years, with age ranging from 36 years to 83 years which is consisted with peak incidence ranging between 50 and 70 years. One patient had myeloma at 36 years of age.

The percentage of plasma cells in bone marrow ranged from 12% to 90% on aspirate smear with large aggregate of plasma cells on biopsy.

Table I: Related organ or tissue impairment. Total number = 30

| Clinical criteria | Anemia (pale) | Bone lesion (bone pain) + Anemia | Renal insufficiency | Renal insufficiency + Anemia | Hypercalcemia + Anemia |
|-------------------|---------------|----------------------------------|---------------------|------------------------------|------------------------|
| Number | 19 | 6 | 2 | 2 | 1 |
| Percentage | 63.3% | 20% | 6.6% | 6.6% | 3.5% |

Table II: Distribution of M-band in SPE regions. Total number = 30

| SPE M-band region | Number | Frequency |
|-------------------|--------|-----------|
| B | 7 | 23.4% |
| Γ | 23 | 76.6% |

Table III: Frequency of paraprotein types in γ region and β region. Total number = 30

| Paraprotein Type | β region | γ region |
|------------------|----------------|-----------------|
| IgG | 1 | 23 |
| IgA | 5 | ---- |
| IgM | 1 | ---- |

Table IV: Frequency of paraprotein isotypes. Total number = 30

| Paraprotein isotype | | Number | Frequency |
|---------------------|-------------|--------|-----------|
| Heavy chain | Light chain | | |
| IgG | | 24 | 80% |
| IgA | | 5 | 16.6% |
| IgM | | 1 | 3.3% |
| | K | 18 | 60% |
| | Λ | 12 | 40% |

Table V: Paraprotein fractions frequency. Total number = 30

| Paraprotein fraction | Number | Frequency |
|----------------------|--------|-----------|
| IgG κ | 17 | 56.6% |
| IgG λ | 7 | 23.6% |
| IgA κ | 2 | 6.6% |
| IgA λ | 3 | 10% |
| IgM λ | 1 | 3.3% |

All patients presented with related organ and tissue impairment (Table I). The most frequent one was anemia in 28 patients (93.4%).

Serum protein electrophoresis gel shows presence of paraprotein (M-band) in two regions including beta (β) and gamma (γ) regions. Twenty-three (76.6%) out of 30 were found in γ region, whereas 7 (23.4%) in β region (Table II). The level of paraprotein in patients reviewed was more than 30g/l for IgG and more than 25 g/l for Ig A. The most common paraprotein isotype found in beta region was IgA with frequency 71.4%, 14.3% for IgG and 14.3% for IgM. The largest proportion of IgG paraprotein type was found in γ region and majority of IgA and IgM types were found in β region (Table III).

The most frequent paraprotein (heavy chain) isotype seen was as follows: 80%, 16.6%, and 3.3% for IgG, IgA, and IgM respectively. Regarding light chains, κ light chain was the most frequent (60%) (Table VI). As seen, the most frequent paraprotein type was IgG, followed by IgA and IgM; and kappa (κ) light chain more frequent than lambda (λ).

Depending on results obtained from the study there were five different paraprotein fraction types with highest frequency (56.6%) for IgG κ fraction (Table V).

Discussion

Electrophoresis is used for analysis of serum proteins particularly gamma globulins. The main

important finding is the presence of a paraprotein in the gamma (γ) region or in the beta (β) region.

Our study presents the patterns of serum protein electrophoresis in patients diagnosed with plasma cell myeloma by bone marrow plasma cells infiltration of more than 10%, and with parallel electrophoresis performed for each individual. All patients had M-band in SPE with a concentration of the paraprotein ranging between 31-39 g/l for IgG and 26-27 g/l for IgA and measuring of paraprotein quantity is required for differentiate plasma cell myeloma from other diseases presented with M-band on SPE.⁽¹⁰⁾

In gamma region the frequency was 76.6% (23) and 23.4 %⁽⁷⁾ in the beta region. A study conducted in India showed the presence of M-band in 87.5% and 12.5% of all cases in gamma and beta regions respectively.⁽²⁾ The most common Paraprotein isotype found in the beta region was IgA with 71.4% frequency; however IgM and IgG were 14.3% for each. As shown from results the IgA paraprotein more frequently migrates into the beta region, which was also seen in a study conducted in USA.⁽¹¹⁾

Regarding the frequency of M- protein the most common one was IgG (80%), followed by IgA (16.6%), and IgM (3.3%); with 60% having kappa and 40% having lambda light chain. Our frequency rate when compared with other studies was nearly equal 75.47% for IgG, 16.98% for IgA, 64.16% for kappa, 35.84% for lambda.⁽¹²⁾

IgG isotype and kappa light chain was the most frequent in our population, and the less frequent was IgM. However, IgM isotype was common in other populations such as France,⁽¹³⁾ but not detected in the study conducted in India.⁽¹¹⁾

The study reports five different patterns of paraprotein fractions, the IgG κ constitute 56.6% which was most common, IgG λ (23.3%), IgA κ (10%), IgA λ (6.6%), and IgM λ (3.3%).

Our study showed high sensitivity of serum protein electrophoresis and immunofixation in patients diagnosed with plasma cell myeloma (100%). The sensitivity was high in Netherlands (90.1%),⁽¹⁴⁾ and United State of America (94.4%).⁽¹⁵⁾

The sensitivity was higher in our study because of the fact that the number of patients reviewed was less than the other studies.

Conclusion

The patterns of serum protein electrophoresis and immunofixation of 30 patients diagnosed as multiple myeloma based on bone marrow findings showed the presence of M-band in all patients. For early diagnosis patients suspected to have multiple myeloma the serum protein electrophoresis and immunofixation remains an easy gold standard screening test for cases with secretory plasma cell myeloma.

References

1. **Ahmed KY.** Clinical and Laboratory Profiles of 109 Patients diagnosed as Multiple Myeloma in Erbil City. *J Fac Med Baghdad* 2013; 55: 2.
2. **Sunita T.** The role of Serum Protein Electrophoresis in the Detection of Multiple Myeloma: An Experience of a Corporate Hospital. *Journal of Clinical and Diagnostic Research* 2012; 6: 1458-1461.
3. **Joseph RM, Craig BR, Francis KB.** Management of newly diagnosed symptomatic multiple myeloma: updated mayo stratification of myeloma and risk-adapted therapy (mSMART) consensus guidelines. *MayoClin Proc* 2013; 88:360-376.
4. **Meenakshi G, Rana AK, Deepika T.** Multiple myeloma: the disease and its treatment. *IJBCP* 2013; 2: 103.
5. **Waqar A, Saad A, Khalid A.** Diagnostic significance of serum protein electrophoresis. *Biomedica* 2004; 20: 40-44.
6. **Harousseau JL, Dreyling M.** Multiple myeloma: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Annals of Oncology* 2012; 21: 155-157.
7. **Steven HS, Elias C, Nancy LH.** World Health Organization Classification of Tumors. 4th ed. Lyon cedex 08, France; 2008.
8. **Jesus FS, Bruno P, Norma CG.** New Tools for Diagnosis and Monitoring of Multiple Myeloma. *ASCO educational book* 2013.p. 313-318.
9. **David FK.** Protein Electrophoresis in Clinical Diagnosis. 1st ed. 338 Euston Road, London; 2003.
10. **Theodore XO, Timothy JH.** Understanding and interpreting serum protein electrophoresis. *American Family Physician* 2005; 71: 105-112.
11. **Howard T, Chin YC, Kevin SL.** Accuracy of serum IgM and IgA monoclonal protein measurements by densitometry. *Annals of Clinical & Laboratory Science* 2003; 33: 160-166.

12. **Kalpana S, Bhawna S, Sarika A.** Immunological evidence of monoclonal gammopathy in North India: a hospital based study. *Pathology and Laboratory Medicine International* 2010; 2: 107–111.
13. **Saleun JP, Vicariot M, Deroff P, Morin JF.** Monoclonal gammopathies in the adult population of Finistère, France. *J Clin Pathol* 1982; 35: 63–68.
14. **Andries JB, Ageeth BR, Coby E.** Screening for M-proteinemia: serum protein electrophoresis and free light chains compared. *Clin Chem Lab Med* 2009; 47:1507–1511.
15. **Jerry AK.** Screening panels for monoclonal gammopathies: time to change. *Clin Biochem Rev* 2009; 30: 105-111.