■ *Short Communication*

BIOCHEMICAL PROFILE OF MULTIPLE MYELOMA IN THE PATIENTS VISITING BPKIHS AS DIAGNOSED BY AGAROSE GEL ELECTROPHORESIS

Nepal AK[1], Shakya PR[1], Gelal B[2], Lal Das BK[3], Shrestha BP[4], Lamsal M[5], BaralN[5], Majhi S[5]
[1]M. Sc.(student), [2]Senior Demonstrator, [3]Assistant Professor, [5] Professor, Department of Biochemistry
[4]Professor, Department of Orthopedics
B. P. Koirala Institute of Health Sciences

Abstract

Multiple myeloma is a proliferative disease of plasma cells. The incidence of the disease increases with age. **Objectives :** To study different biochemical parameters and serum agarose gel electrophoresis patterns of patients consistent with clinical symptoms of multiple myeloma. **Subject and Methods :** A retrospective study was carried out in Department of Biochemistry, B.P. Koirala Institute of Health Sciences, from October 2008 to September 2009. Patients consistent with the symptoms of multiple myeloma were selected for the study. Alkaline phosphatase, total calcium, albumin and globulin levels were estimated. Bence Jones Protein, cell counts of bone marrow and x-ray of skull was carried out. Agarose gel electrophoresis was performed at buffer pH 8.6, 5 mili ampere current and the constant voltage of 200 volts was applied. **Results :** Serum electrophoresis patterns in all the patients showed M-Protein band (M-band) at gamma-region. Serum alkaline phosphatase levels were 216.0 ± 35.7 . Serum total calcium levels were 10.0± 1.5 , serum albumin levels were 4.1 ± 0.5 and serum globulin levels were 3.7 ± 0.9 respectively. Bence Jones Protein was present in urine of the 7 patients out of 14. Plasma cell counts were more than 10% and lytic lesions on skull were present. **Conclusion :** Patients with multiple myeloma showed consistently increased total calcium, decreased serum albumin and normal alkalline phosphatase levels. Electrophoretic patterns showed M-band in all the patients, giving the confirmation of the diagnosis. Though a conventional technique, electrophoresis remains as the gold standard for the diagnosis of multiple myeloma.

Keywords : multiple myeloma, serum agarose gel electrophoresis, M-band.

Introduction

Multiple myeloma is a malignant disorder characterized by proliferation of single clone of plasma cells derived from B-cells in the bone marrow. The plasma cell clone produces a monoclonal M-Protein that can lead to renal failure caused by light chains or hyperviscosity syndrome from excessive amounts of M-protein in the blood.[1] The median age at diagnosis of multiple myeloma is 70 years, and the occurrence increases with age.[2] The diagnosis depends upon the identification of abnormal monoclonal plasma cells in the bone marrow. M-protein in the serum or urine, evidence of end-organ damage and characteristic clinical findings like, fatigue, bone pain, lytic bone disease, renal insufficiency, anemia, hypercalcemia and immunodeficiency are observed in multiple myeloma patients.[3] The best method for detecting monoclonal protein is high resolution agarose gel electrophoresis.

Address for correspondence:
Mr Ashwini Kumar Nepal, Department of Biochemistry
BPKIHS, Dharan
Email: nepalashwini@gmail.com

Proteins are charged molecules that migrate in a solid medium soaked with a buffer under influence of an electrical field. Migration depends upon the net electrical charge of the medium, pH of the buffer, molecular mass and isoelectric point of the protein.[4] M-protein is generally observed as a localized band which is frequently seen on gamma or beta region, it may also be seen on alpha-2-globulin region in rare situations. The gamma fraction is the migration zone of immunoglobulins. Clinical features, age of the patient and biochemical and radiological findings should be carefully considered when interpreting this fraction.[4,5]

Subjects and methods

The study was carried out in Department of Biochemistry B. P. Koirala Institute of Health Sciences, from October 2008 to September 2009. Total of 14 patients admitted to the orthopedic ward, who had clinical symptoms of back or bone pain, tremor and consistent with clinical symptoms of multiple myeloma were chosen for the study. Written consent was obtained from the patients and the ethical clearance was taken as per the guidelines of the institute. Alkaline phosphatase, total calcium, total protein, albumin, globulin in serum were estimated in Vital Lab Selectra E Auto Analyzer. Bence Jones Protein was estimated by heat precipitation method.[8] Bone marrow cell counts and X-ray of skull was performed in Pathology and Radiology departments with standard procedures. Serum agarose gel electrophoresis was carried out with barbitone buffer pH 8.6, current of 5 milli Ampere per slide was applied at 200 volts for 1 hour, with voltage kept constant. Serum protein migration on each slide were compared with the control sample which had consistent serum albumin and globulin levels.[4,5]

Results

Table 1 shows different biochemical parameters estimated of the patient suspected of multiple myeloma. Total protein and albumin levels were 7.9 ± 0.6 and 4.1± 0.5 respectively. Total protein to albumin ratio were decreased in all patients. Serum total calcium levels were 10.0 ± 1.5.

Table1: Biochemical Profile of the patients

S. No.	Total Protein (6-8.3) g/dl	Albumin (3.5-5) (g/dl)	Globulin (2.5-3.3) (g/dl)	Albumin/ Globulin Ratio (1.00-1.51)	Total Calcium (8.4-10.2) mg/dl	Alkaline Phosphatase (80-300) (IU/L)	Bence Jones Protein
1.	8.3	3.4	4.9	1: 1.44	11.4	183	Present
2.	7.3	4.1	3.2	1.28: 1.0	11.1	250	Present
3.	7.9	3.5	4.4	1: 1.257	10.0	226	Absent
4.	7.8	4.2	3.6	1.166: 1	11.0	254	Present
5.	8.1	5.1	3.0	1.7: 1	7.2	266	Absent
6.	7.3	4.4	2.9	1.51: 1	7.5	165	Present
7.	7.4	4.6	2.8	1.64: 1	11.0	233	Absent
8.	8.1	4.4	3.7	1.18: 1	10.3	244	Present
9.	8.6	4.5	4.1	1.09: 1	7.0	183	Absent
10.	9.1	2.9	6.2	1: 2.13	9.9	150	Absent
11.	7.5	4.3	3.2	1.34: 1	10.9	189	Absent
12.	8.1	4.5	3.6	1.25: 1	11.1	223	Present
13.	8.3	4.4	3.9	1.12: 1	10.3	225	Absent
14.	6.8	4.3	2.5	1.72: 1	11.5	234	Present

Alkaline phosphatase levels of the patients were 216 ± 35.7. Bence Jones Protein in urine was present in 7 out of 14 patients.

Table 2 shows serum electrophoresis patterns, radiological and bone marrow findings. Serum electrophoresis patterns of all patient showed sharp spike at gamma-region (M-band). Lytic lesions on X-ray findings were found on 10 out of 14 cases. Normocellular pattern of bone marrow was seen in 3 out of 14 cases, 11 cases showed plasma cells more than 10 % of all nucleated cells.

Table 2: Serum electrophoresis, radiological and bone marrow findings

S. No.	Serum electro-phoresis (M-band)	Radiological Findings (Lytic lesions)	Bone Marrow findings (more than 10% plasma cells of all nucle-ated cells)
1.	Present	Present	Absent
2.	Present	Absent	Absent
3.	Present	Absent	Present
4.	Present	Present	Present
5.	Present	Present	Present
6.	Present	Present	Absent
7.	Present	Absent	Present
8.	Present	Absent	Present
9.	Present	Present	Present
10.	Present	Present	Present
11.	Present	Present	Present
12.	Present	Present	Present
13.	Present	Present	Present
14.	Present	Present	Present

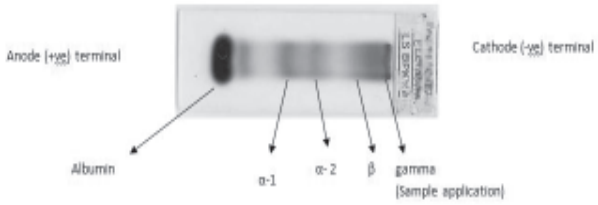

Fig A: Normal electrophoretic pattern showing showing Albumin, á-1, á-2, â and gamma-globulin fractions

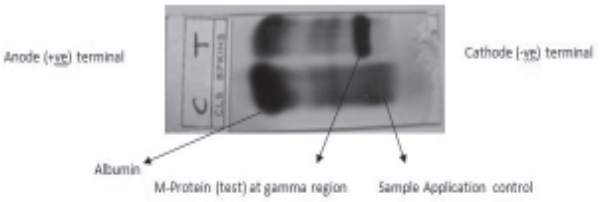

Fig B: Electrophoretic pattern of case as compared with control

Fig A and Fig B shows the electrophoretic pattern of control showing Albumin, á-$_1$, á-$_2$, â and gamma-globulin fractions respectively and electrophoretic pattern of case as compared with control.

Discussions

Electrophoresis has widely been used to show changes in the serum proteins particularly in the globulin fractions. It has been used as quantitative assessment of the changes in relative amounts of globulin fractions present and quantitative assessment to obtain the percentage of bands formed by each of the fractions present, i.e. albumin, á-$_1$, á-$_2$, â and gamma-globulin fractions respectively.[5] Varied patterns are obtained in multiple myeloma, the most characteristic finding is the presence of a band of abnormal protein termed M-protein or "M-Band." It occurs as intense, narrow band most often found with the gamma-globulins, then in a diminishing frequency between gamma and the â-globulin and rarely between the â and á$_2$ regions. There is often dissociation between the presence of the M-Protein in serum and of Bence Jones Protein in the urine. Occasionally both are seen in the same patient at least by ordinary qualitative urine tests.[4-6]

Due to various outcomes of patient with multiple myeloma, determining the prognostic factor is important for predicting disease outcome and determining the treatment method. In most cases of multiple myeloma, there is a malignant proliferation of plasma cells producing either IgA or IgG.[3] The abnormal protein is all of one electrophoretic mobility because it has been formed by the progeny of one plasma cell. Decreased levels of albumin may result from decreased synthesis, increased catabolism or combination of these. Serum albumin reflects the interleukin-6 levels, the liver function and the nutritional status. Albumin levels are consistently decreased on above cases with decrease in total

protein and increase in serum globulin levels.[3] A normal serum alkaline phosphatase level and increased calcium levels in patients with multiple myeloma is a suggestive finding towards the support of diagnosis.[6] Alkaline phosphatase is increased when bone regeneration is taking place. It is not found unless there is simultaneous formation of new bone or osteoid tissue. In multiple myeloma, the lesion is almost purely destructive and due to osteoclastic involvement of bone, Alkaline phosphatase is usually within normal limits.[6,7]

A proportion of multiple myeloma cases are found to have a moderately raised serum calcium which is usually normal and upto 17 mg/dl. Although the plasma proteins are increased in this condition, the correlation of this with serum calcium is much less close than that of the latter with increase in urinary calcium excretion. The rise in serum calcium appears to be due to increased removal from the bones.[3,6] Immunoglobulins are high molecular proteins which are synthesized in plasma cells in bone marrow and are available in plenty at the vicinity of the synthesis. Study of bone marrow aspirate is mandatory for diagnosis and staging of the disease process. Normally plasma cells constitute 1% in the bone marrow but as the disease advances, tumor load in bone marrow increases up to 80 % depending on severity.[6,7] These malignant plasma cells synthesize monoclonal antibody and release it to the circulation. As a result high concentration of monoclonal antibodies is present in bone marrow as well as in serum.[7]

Limitations

Few number of samples due to few patients of multiple myeloma coming to this hospital and the lack of differentiating the protein fractions by densitometer is the limitation of this study. Characterization of immunoglobulin (IgG or IgA) and sub-typing can be done if immunoelectrophoresis will be performed Further study is needed in this part, with more sample size and including the recent tests like immunoelectrophoresis and densitometry to support the diagnosis of multiple myeloma.

Conclusion

Based on the results obtained above, serum electrophoretogram showing M-band in the gamma region is a diagnostic test for multiple myeloma. Serum electrophoresis can be routinely used for the diagnosis of multiple myeloma and is well correlated with biochemical, radiological and pathological findings. The diagnosis of multiple myeloma is not simple to proceed as there are many confounding factors. Though, being the conventional technique serum electrophoresis is still widely used for the demonstration of M-Protein in the myeloma patient, which remains a gold standard. It adds batteries to the diagnosis to other supportive factors including presence of clinical symptoms like bone pain, hypercalcemia, normal alkaline phosphatase, bone marrow plasma cells more than 10% and Bence Jones Proteinuria.

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