Kathmandu University Medical Journal (2006), Vol. 4, No. 4, Issue 16, 513-516

Case Note

Diagnosis of multiple myeloma by demonstration of M protein in bone marrow aspirate by agar gel electrophoresis: A case report

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Abstract
A number of laboratory tests are used to confirm the diagnosis of multiple myeloma, including M protein in the serum. Since M protein in the serum originate from tumour cells in the bone marrow before circulating in the serum, demonstration of M protein in bone marrow aspirate can be added to the batteries of diagnostic parameters.

Keywords: Multiple myeloma, Bone marrow aspirate, M band, Agar gel electrophoresis

Multiple myeloma, a cancer of terminally differentiated plasma cell, typically occurs in elderly patients. Prevalence of the disease is low which constitutes 1% of all cancers but incidence rises after the age of 60 years[1]. The term multiple myeloma describes a characteristic feature, found at *multiple* sites within the bone marrow (*myelo-*) with accumulations of tumor (*-oma*) cells. 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. 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.

A number of laboratory tests and medical procedures are used to confirm a diagnosis of myeloma. Minimum criteria consists of more than 10 % plasma cell in bone marrow[2] and one of the following:

a) M protein in the serum
b) Bence Jones protein (M protein) in the urine
c) Lytic bone lesion on radiological examination

These findings must not be related to metastatic carcinoma, lymphoma, connective tissue disorder or chronic infection. However demonstration of M protein in bone marrow aspirate is not included in the above criteria. Bone marrow aspirate, meant for characterization of plasma cell, was examined to find out the presence of M protein by agar gel electrophoretogram. Agar gel (1%) was used as support media on glass slide for this separation technique. Bone marrow aspirate devoid of cell and artifacts was applied on the slide gently by the help of a spike. Separation was conducted at pH 8.6 using barbitone buffer (ionic strength 0.05) in a horizontal electrophoretic apparatus for 1 hrs. The protocol of electrophoresis technique was followed for remaining procedures. This is an attempt to add a parameter as diagnostic criteria which has not been reported in any literature.

Case report

A 56 year male, farmer by profession, attended Orthopedic OPD with the complaints of productive cough for 3 months duration. He also complained of severe pain in his back of same duration. He had significant weight loss and weakness. He was a non-smoker and occasional drinker. He had no history of pulmonary tuberculosis. He was strongly suspected of suffering from bronchogenic carcinoma. Multiple myeloma was also thought as second probable diagnosis. He was investigated for confirmation of diagnosis.

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Tables showing the various laboratory findings

Table 1: The laboratory findings

Biochemical parameters		
Total Protein	7.3 g /dl	(6.0-8.3 g/dl)
Albumin	2.5 g/dl	(3.5 – 5 g/dl)
Calcium(Ionised)	6.7 mg/dl	(4.2 -5.1 mg/dl)
Alk Phosphatase	576 IU/L	(80 – 300 IU/L)
Hematological parameters		
Hb%	9.5 g/dl	
Hematocrit	30.2 %	
TLC	5,700 /cuml	
DLC	N -66% L- 34 %	
Platelet	332000/cuml	
ESR(mm/1st hr)	63	

Table 2: Bone marrow aspirate findings

Bone marrow aspirate was cellular
Myeloid and erythroid ratio = 3:1
Myelopoiesis was normal
Erythroid series showed normoblastic maturation
Megakaryocytes were seen
Plasma cell comprised of 14 % of all nucleated cells

Table 3: Radiological findings

Chest X-ray (PA view): Lung and pulmonary vasculature shadow was normal
D/L spine showed anterior wedging with decreased vertebral height of D5, D7 and D9 vertebra and degenerative changes as evidenced by osteolyte formation.
There was no sign of secondary metastasis in bone

Table 4: Serum protein electrophoretogram

Sharp spike at γ region (M band)
Normal albumin, α_1, α_2 and β band comparable to control

Table 5: Bone marrow aspirate electrophoretogram

Sharp spike at γ region (M band)
Normal Albumin, thin band visible at α_1 and α_2 region and broad band visible in β region

Discussion

This patient had atypical presentation which mimicked advanced stage of lung cancers with secondaries in bones. There was increased calcium (ionized) level in serum showing osteoclastic involvement which favours diagnosis of Multiple Myeloma. There was mild elevation of alkaline phosphatase which does not favour osteolytic lesion of the disease process but might have originated from unknown osteoblastic involvement3 or hepatic disorders which was not confirmed by laboratory investigation. X ray of thoraco-lumbar vertebral column showed evidence of osteolytic lesions which favours Multiple Myeloma. He had low level albumin in serum but total protein was within the normal range as evidenced from biochemical assay and serum protein electrophoresis (Fig 1 and 2). Albumin band in bone marrow aspirate was similar to serum counter part. Beside all above findings, sharp spike was noticed at the γ region in serum and bone marrow aspirate electrophoresis (Fig 2 and 3).

Fig 1: Agar gel electrophoretogram of control serum showing normal pattern of albumin,α1, α2, β1, β2 and γ band

Fig 2: shows M spike in the γ region

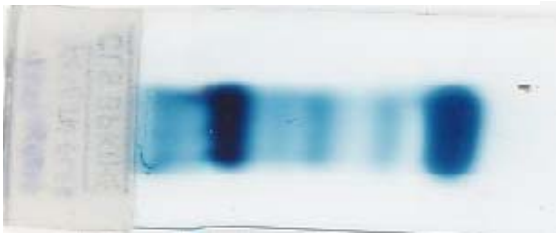

Fig 3: Bone marrow aspirate ectrophoretogram shows albumin band and M spike at γ region which is comparable to serum

Among hematological indices, Hb showed low and ESR moderately high level in first hr which were 9.5 g /dl and 63 mm respectively. Bone marrow aspirate cytology showed 14% plasma cells, but this was supported by demonstration of M band in serum protein electrophoresis for diagnosis of multiple myeloma of stage II[4]. Beside presence of M band in serum protein electrophoresis, diagnosis was also

supported by demonstration of M band in bone marrow aspirate by agar gel electrophoresis which has not been reported earlier. Immunoglobulins are high molecular weight proteins which are synthesized in plasma cells in bone marrows 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 and this study adds a parameter in the diagnostic criteria by demonstration of M proteins in aspirate itself. M proteins in serum is demonstrable in 85% cases of Multiple Myeloma due to low titre[5], but are available in plenty at the vicinity of synthesis in bone marrow. So M band protein in bone marrow aspirate could be demonstrated in serum negative cases.

A diagnosis of multiple myeloma is difficult to make on the basis of any single laboratory test result. Traditionally plasma cell count in bone marrow aspirate cytology is supplemented with presence of M protein in serum electrophoresis and its presence in urine along with lytic lesion of bone in X ray are taken into consideration[6]. Demonstration of M protein in bone marrow aspirate can be added to the batteries of diagnostic parameters.

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