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Detecting Plasma Cell Myeloma on the basis of ABO Discrepancy – a Case Study

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ABSTRACT
Plasma cell myeloma is characterised by monoclonal proliferation of plasma cells, causing destructive bone lesions and other systemic manifestations. Plasma cell Myeloma accounts for 1% of all malignancies and 10-20% of all haematological malignancies. The disease spans a clinical spectrum from asymptomatic to highly aggressive disease. M protein caused by myeloma cells results in type III ABO discrepancy, in which rouleaux formation can be misinterpreted as agglutination. We present a case of a 45-year-old female visiting the Outpatient Department (OPD) in our hospital with generalised weakness for five months, tingling and numbness over face and mild back pain. Blood transfusion was advised as the haemoglobin level was low. Initially there was a discrepancy between forward and reverse grouping which was resolved with saline washes. On further investigations like peripheral blood smear and bone marrow examination, the patient was diagnosed as a case of plasma cell myeloma. In our case the blood grouping revealed type III ABO discrepancy which upon further investigations was found to be a case of Plasma Cell Myeloma.

KEY MESSAGE
Plasma cell myeloma has varied presentations. In our case, the patient presented with anaemia, plasma cells in peripheral blood and type III ABO discrepancy. **Rouleaux formation should not be interpreted as agglutination**. Careful vigilance by technicians in blood bank and thorough knowledge regarding various discrepancies will help in the management of the patient.

Keywords: Plasma Cell Myeloma; ABO Discrepancy;

I. INTRODUCTION

Multiple Myeloma (MM) is a plasma cell malignancy in which monoclonal plasma cells proliferate in bone marrow, resulting in an overabundance of monoclonal paraprotein (M protein), destruction of bone and displacement of other hematopoietic cell lines.(1) Plasma cell myeloma (PCM) can present in multiple ways including anaemia, hypercalcemia, renal failure and bone pains or fractures. We report a case of PCM presenting as anaemia and ABO discrepancy.

An ABO discrepancy exists when the results of red cell grouping (forward grouping) do not agree with serum grouping (reverse grouping). Both forward and reverse grouping are necessary as each grouping serves as a check on the other. Myeloma patients have excess of plasma proteins, which cause rouleaux formation. This pseudo-agglutination can be misinterpreted as true agglutination leading to ABO discrepancy.

The Blood bank of our hospital received a request for a single unit of Packed red blood cells (PRBCs) for a 45-year-old Indian female. Her pre-transfusion haematological study revealed a haemoglobin level of 6.7 g/dL. Blood sample was obtained from the patient and we proceeded with pre-transfusion testing of phenotype and antibody screening. The result of the forward and reverse grouping did not match with each other.

CASE PRESENTATION

A 45-year-old female presented to the Outpatient Department (OPD) with fatigue, paraesthesia over face and back pain for about five months. These symptoms were progressive but often improved with rest. No history of fever, mucosal bleeding, or blurred vision. Her social history revealed that the patient was married with two children. She was a non-smoker and non alcoholic. On examination, the patient was oriented to time, place, and person. Conjunctiva were markedly pale and mucous membranes were dry. Cardiovascular system was unremarkable. Lungs were clear bilaterally. Bowel sounds were normal.

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No organomegaly was present on examination. The laboratory findings are discussed below.

LABORATORY PROFILE

Haemoglobin (Hb) was 6.7 gm/dl on presentation. The Red Blood Cell (RBC) count was 1.1 million /mm^3, which was very low. White Blood Cell (WBC) count was found slightly elevated at 11000 cells/mm^3. Platelet count was 95000/mm^3. However, Dengue serology was non-suggestive and NS1 antigen was absent. There was no purpura, petechiae or rash on any parts of the body. Blood and urine cultures were negative. Direct and Indirect Coombs tests were negative. Erythrocyte Sedimentation Rates (ESR) value was high (55 mm/hr). Renal function test, liver function test, serum iron, serum calcium and serum B12 were within normal limits.

Because of low Hb levels, blood transfusion was advised and blood samples were sent for blood grouping and crossmatching at our hospital's Blood bank. On forward grouping, agglutination was found with anti-A, anti-B, and anti-D. These results deviated towards assigning the blood group as AB Positive. However, on reverse grouping, the agglutination picture changed. Agglutination was found with A cell and B cell and no agglutination noted with O cell. The results of the forward and reverse blood group were different. Auto control was positive. Pursuant to the discrepancy, we did saline wash for accurate results. Upon washing patient's RBCs with saline 4-5 times, the clumps pooled with anti-A and anti-B sera were no longer demonstrated. The grouping result after saline washes was O Positive blood group. Procedure for cross matching with O positive PRBCs was done. Cross match was compatible with the gel card method. On the tube method the cross match showed rouleaux like pattern mimicking agglutination which disappeared after putting a drop of saline. These variable findings triggered a suspicion for paraproteinemia. Summary of grouping results before and after saline washes is shown in **Table 1**.

Table 1. Results of Blood Grouping

Results of Forward Grouping				
Wash Cycle	**anti-A**	**anti-B**	**anti-D**	**Impression**
Before Wash Cycles	++	++	++	AB Positive
After 4-5 Wash Cycles	-	-	++	O Positive
Results of Reverse Grouping				
Wash Cycle	**A Cells**	**B Cells**	**O cells**	**Impression**
Before Wash Cycles	+++	+++	-	O positive
After 4-5 Wash Cycles	+++	+++	-	O Positive

We had informed the clinician regarding the resolution of blood group discrepancy, obtained the detailed clinical history and examined repeat haemogram.

Peripheral blood smear had a bluish tinge macroscopically. Peripheral smear examination revealed normocytic normochromic RBC with rouleaux formation. These stacks appear evenly dispersed throughout the smear (Figure 1). There was lymphocytic preponderance with plasmacytoid cells and moderate thrombocytopenia. MRI revealed multiple lytic lesions in skull and vertebral bodies. A Bone Marrow study was undertaken to differentiate between metastases and Multiple Myeloma.

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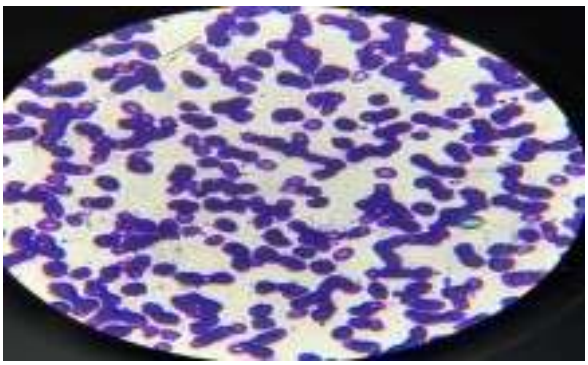
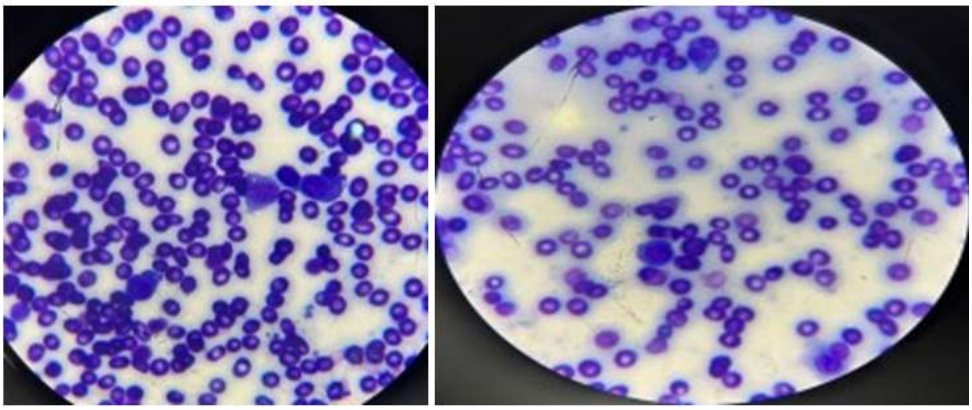


Figure 1- RBCs showing rouleaux formation

Bone Marrow aspirate was diluted with peripheral blood. Bone Marrow aspirate revealed that all hematopoietic cell lines were reduced in number showing proliferation of plasma cells. A total 40% of all nucleated cells were mature and immature plasma cells. Few binucleated and occasional multinucleated plasma cells were also seen (Figure 2). Findings were suggestive of Plasma Cell Dyscrasia with possibility of Multiple Myeloma.



2(a) **2(b)**

Figure 2 : Microscopic View of Bone Marrow Aspirate

2(a) :- Plasma cells with eccentric nucleus
2(b) :- Plasma cells with binucleated cell

Trephine biopsy revealed hematopoietic tissue being replaced by interstitial and diffused pattern of myeloma cells. (Figure 3).

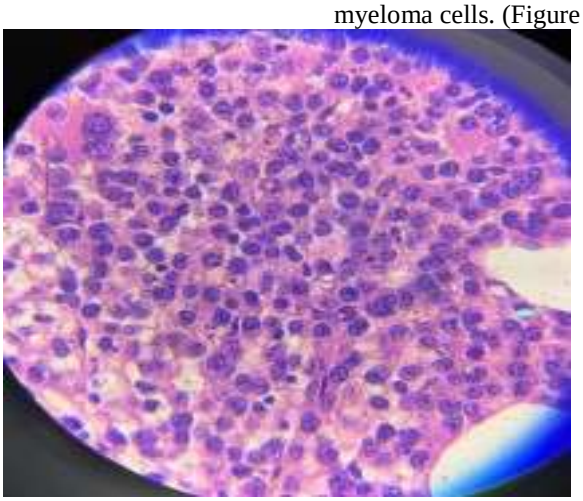


Figure 3: Microscopic View of Bone Marrow trephine biopsy

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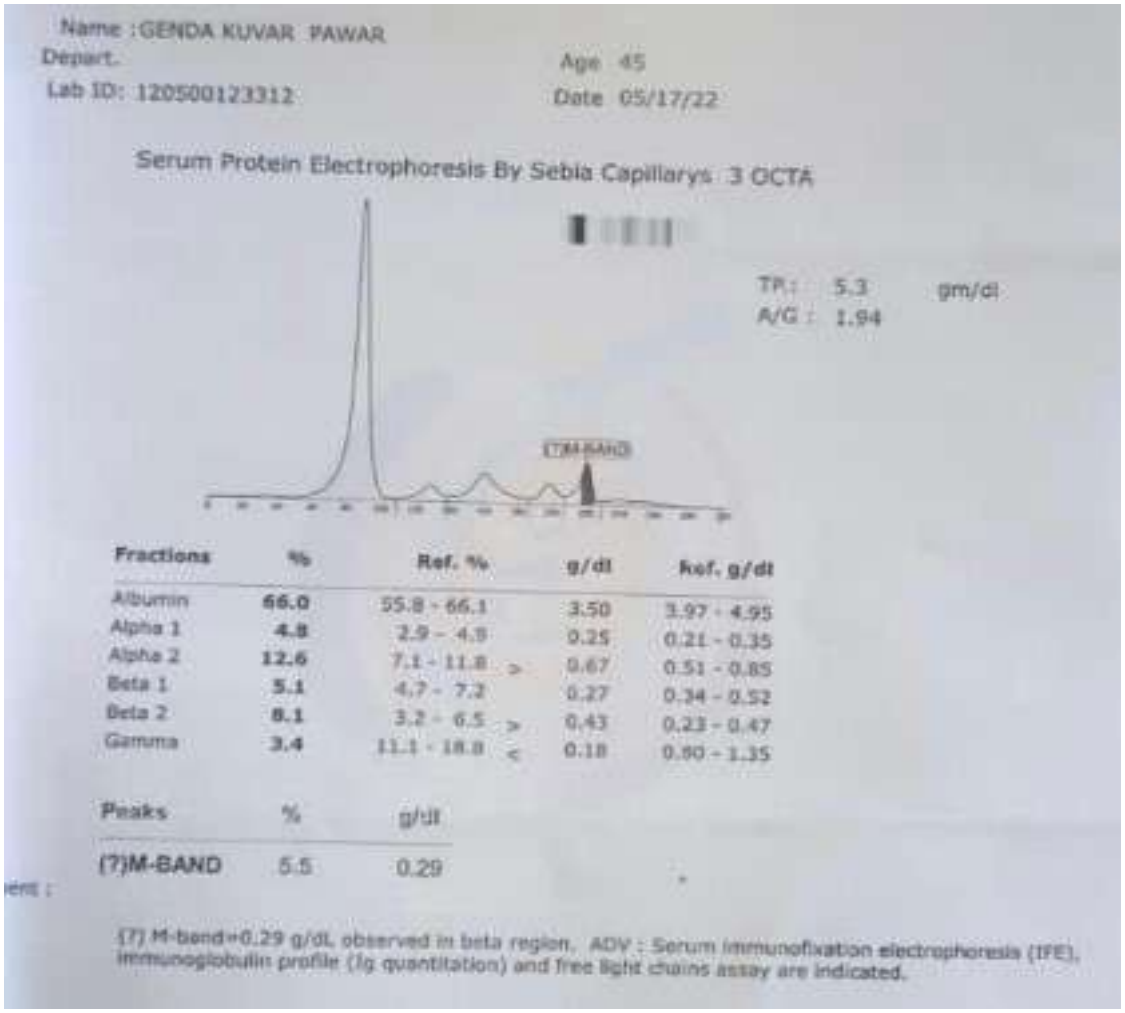
Patient was further advised for Immunohistochemistry. Serum protein electrophoresis revealed monoclonal protein spike and depressed synthesis of normal immunoglobulins (Figure 4). Immunofixation electrophoresis revealed monoclonal gammopathy. Test for urine Bence-Jones protein was negative.


Name :GENDA KUVAR PAWAR
Depart.
Age 45
Lab ID: 120500123312
Date 05/17/22

Serum Protein Electrophoresis By Sebia Capillarys 3 OCTA

TP: 5.3 gm/dl
A/G : 1.94

Fractions	%	Ref. %		g/dl	Ref. g/dl
Albumin	66.0	55.8 - 66.1		3.50	3.97 - 4.95
Alpha 1	4.8	2.9 - 4.9		0.25	0.21 - 0.35
Alpha 2	12.6	7.1 - 11.8	>	0.67	0.51 - 0.85
Beta 1	5.1	4.7 - 7.2		0.27	0.34 - 0.52
Beta 2	8.1	3.2 - 6.5	>	0.43	0.23 - 0.47
Gamma	3.4	11.1 - 18.8	<	0.18	0.80 - 1.35

Peaks	%	g/dl
(7)M-BAND	5.5	0.29

(7) M-band=0.29 g/dL observed in beta region. ADV : Serum immunofixation electrophoresis (IFE), immunoglobulin profile (Ig quantitation) and free light chains assay are indicated.

Figure 4 – Protein Electrophoresis report

Serum free light chain quantification was done. Results showed free Kappa Chain-15.50 mg/L, free Lambda Chain-20300.00 mg/L (which was markedly elevated). Free Kappa chain Lambda chain ratio was 0.001.

Diagnosis of Multiple Myeloma was based on a combination of the presence of multiple criteria including 40% of plasma cells in the bone marrow, multiple lytic bone lesions, monoclonal protein spike in serum protein electrophoresis, depressed synthesis of normal immunoglobulins and markedly raised free Lambda chain.

II. DISCUSSION

Multiple myeloma is a haematological malignant neoplasm of the bone marrow. It is a neoplastic disease characterised by the infiltration of bone and bone marrow by myeloma cells forming multiple tumour masses. [(2)] Production of normal immunoglobulins is impaired with a significant increase in the number of abnormal plasma cells.[(3)] The condition is usually progressive and generally fatal. The disease causes pain, fractures, anaemia, hypercalcemia, kidney failure, bacterial infections, nerve compression with paralysis, skeletal deformities, and changes in mental status ranging from mild to severe confusion. [(4,5)]

The aetiology of multiple myeloma is unknown. However, genetics, radiation exposure and chronic antigenic stimulation have been suggested as predisposing factors.[(6)] Increase in the incidence of multiple myeloma during this past

century implicate environmental factors as important causal agents. A single insult is not thought to be sufficient to induce the disease. However, continued exposure results in the clonal expansion of plasma cell after cumulative mutational damage has altered its genetic makeup. [7]The molecular and cytogenetics of cells in multiple myeloma are under investigation, but the precise causes of these abnormalities are largely unknown. [8]

Chronic stimulation of the immune system has been a suspected trigger of multiple myeloma with certain medical conditions such as rheumatoid arthritis, chronic allergic conditions, and chronic infections as they are implicated in the stimulation of the aberrant production of plasma cells. Anticipation, a phenomenon in which an inherited disease is diagnosed at an earlier age in each successive generation of a family, has been demonstrated in multiple myeloma. [9] The incidence of myeloma is five cases per 100,000 persons each year and males have approximately 50% greater risk than females. There is greater incidence in black individuals than white individuals. Persons of Japanese and Chinese descent experience the least incidence. Age increases the risk of multiple myeloma as the disease is rarely seen in persons less than 40 years of age with the mean onset at age 60. [10]

The malignant plasma cells produce immunoglobulins resulting in an overproduction of intact immunoglobulins (IgG, IgA, IgD, or IgE) or Bence Jones protein. Plasmacytoma produce IgG in about 55% of myeloma patients and IgA in about 20%. Of these IgG and IgA patients, 40% also have Bence Jones proteinuria. Light chain myeloma is found in 15% to 20% of patients and their plasma cells secrete only free monoclonal light chains, and a monoclonal spike is usually absent on serum electrophoresis. [3]

During the different stages of the disease, almost all patients develop anaemia. The peripheral smear shows rouleaux formation as the result of elevated globulins or fibrinogen in the plasma. These stacks appear evenly dispersed throughout the smear. Rouleaux formation correlates with a high erythrocyte sedimentation rate. A few abnormal plasma cells may be seen in later stages on the peripheral blood differential. [11] The leukocyte and platelet count are usually normal in early stages of the disease until overpopulation of the marrow with abnormal plasma cells occurs. This may produce pancytopenia or elicit a leuko-erythroblastic response. Serum creatinine, Blood Urea Nitrogen (BUN), Lactate dehydrogenase (LDH), calcium, protein, and serum uric acid are frequently elevated. [5]Monoclonal peaks of immunoglobulin can be found in serum protein electrophoresis. The immunoglobulin type can be determined by immune-electrophoresis or immunofixation electrophoresis. Bence-Jones protein or light chain proteins can be identified in urine in 80% of myeloma patients. Bone marrow aspiration and biopsy usually indicate increased numbers of plasma cells at various stages of maturation.[12]

ABO discrepancies occur when the red cell testing does not agree with the expected serum testing.[12] Washing the patient's red blood cells with saline can usually resolve the ABO discrepancy. ABO discrepancies are divided into four groups.[13]

- Group I are discrepancies between forward and reverse groupings because of weaklyreacting or missing antibodies.
- Group II are discrepancies between forward and reverse groupings resulting from weaklyreacting or missing antigens.
- Group III discrepancies are found between forward and reverse groupings caused by protein or plasma abnormalities and result in rouleaux formation or pseudo-agglutination. -

-Group IV discrepancies are between forward and reverse groupings encompassing miscellaneous problems such as warm or cold auto-antibodies and poly-agglutination.

M protein produced by plasma cells leads to elevated levels of globulin or light chains of immunoglobulin. Abnormally elevated paraprotein causes the rouleaux formation in which stacks or aggregations of RBC give stacked coin appearance. This is due to reduction in zeta potential (charge on RBC membrane). Multiple myeloma elevates the globulin level resulting in rouleaux and Group III ABO discrepancies. Because rouleaux formation causes the red blood cells to adhere to one another as in stacked coins, it can be mistaken for agglutination by a new or inexperienced laboratory technician. Phenotyping can usually be accomplished by washing the patient's red cells several times with a saline solution. In true agglutination, red cells will continue to clump even after the washing with saline. In our case, the positivity with anti-A and anti-B before saline wash was not true agglutination but due to rouleaux formation.

III. CONCLUSION

Large amounts of monoclonal protein and a loss of functional antibody production can cause immunological dysfunction and distinct laboratory findings. In Multiple Myeloma, an ABO

discrepancy can result from protein abnormalities causing rouleaux formation or pseudo agglutination in the blood group test. ABO blood group change can occur in multiple myelomas, so the blood group should be checked thoroughly in patients with haematological malignancies. Mismatched blood transfusion can be fatal.

Whenever an ABO discrepancy is encountered, repeat tests should be done on the same sample using washed red cells before carrying out additional investigations. If discrepancy persists, tests are done on new blood specimens obtained from the donor unit or patient to resolve discrepancies. If such discrepancies are diligently approached with available resources, most of them can be resolved serologically even before resorting for high end investigations.

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