ID: JCDR/2012/3576:0048

Case Report

Haematology Section

A Rare Case of Haemolytic Anaemia: Paroxysmal Nocturnal Haemoglobinuria

DEEPAK MADI, BASAVAPRABHU ACHAPPA, SATISH RAO, MEGHNA RAO, SOUNDARYA MAHALINGAM

ABSTRACT

Paroxysmal Nocturnal Haemoglobinuria (PNH) is a rare disease. It is an acquired chronic haemolytic anaemia. It manifests as a triad of recurrent episodes of haemolysis, thrombotic episodes and pancytopaenia. A 32-year old gentleman presented with a history of gradually progressive generalized weakness. His clinical findings and laboratory investigations were suggestive of haemolytic anaemia and flow cytometry clinched the diagnosis of PNH.

Key Words: Paroxysmal nocturnal haemoglobinuria, Haemolytic anaemia, Complement, CD59

INTRODUCTION

PNH was first described in 1866 in a patient with bouts of "dark coloured urine". Since then, there has been tremendous progress in its diagnosis and management. The major clinical features of PNH are – haemolytic anaemia, venous thrombosis and deficient haematopoiesis [1].

PNH is a rare disease with a prevalence of 1-5 per million. Both men and women are equally affected. The median age of presentation is 42 years (age range 16-75 years). It has a chronic course with a varied presentation. The most common cause of death is venous thrombosis, followed by infection which is secondary to neutropenia.

CASE REPORT

A 32-year old gentleman presented with the complaints of generalized weakness and easy fatigability since 3 months. The symptoms were gradually progressive. There was no difficulty in carrying out activities of daily living. He gave a history of jaundice. There was no history of any chronic illness in the past. He had been previously treated for anaemia and was on iron supplementation. There was no history of previous blood transfusion. There were no similar complaints among his family members. On examination, he he was pale and he had icterus. His systemic examination was normal.

His lab investigations showed Hb -5.8g/dl, Total White Blood Cell count - 4470 cells/cu.mm, platelet count-2,53,000 cells/cu.mm, ESR- 70mm/1st hour, reticulocyte count-6%, Mean Corpuscular Volume (MCV) - 111fL, MCH – 36 pg/cell and MCHC- 33%. Serum LDH was 7205 U/L. Serum Ferritin was normal. His peripheral smear was suggestive of dimorphic anaemia. His biochemical studies showed normal electrolyte and renal functions. His liver functions were deranged with total bilirubin being 7 mg% and direct bilirubin being 0.2 mg%. His liver enzymes were normal. His bone marrow demonstrated erythroid hyperplasia. The initial lab picture was suggestive of haemolytic anaemia and he was further investigated. The ultrasonogram of his abdomen was normal. The random urine sample for haemoglobin was negative. The early morning urine sample for haemosiderin was positive. Coomb's test was negative, the osmotic fragility was normal and Hb electrophoresis was normal. Flow cytometry clinched the diagnosis. It showed that a majority of the RBCs were deficient in CD55 and CD59. Retrospectively, the patient was asked about history of passing dark coloured urine and he gave a history of occasional early morning episodes of urine discolouration.

He was transfused with three units of packed cells and was given oral folic acid supplementation. He was discharged after his haemoglobin levels improved and he is on regular follow up.

DISCUSSION

PNH is an acquired stem cell disorder. PNH is a consequence of the non-malignant clonal expansion of one or several haematopoietic stem cells that have acquired a somatic mutation of the X linked gene, PIG-A(phosphatidylinositol glycan complementation class A gene). The progeny of the affected stem cells are deficient in the glycosyl phosphatidylinositol-anchored protein (GPI-APs) [2]. This results in the deficiency of GPI-AP on the haematopoietic cells (CD16, CD24, CD52, CD55, CD59, CD58, CD73, CD87, CD90) [3,4]. Two of these proteins are CD55 (decay accelerating factor [DAF]) and CD59, which are complement regulatory proteins, the absence of which are implicated in the pathogenesis of PNH [5].

CD55 is an inhibitor of C3 convertase and CD59 prevents the formation of the Membrane Attack Complex (MAC) by inhibiting the incorporation of the C9 polymers into the membrane. Of these 2 proteins, CD59 is considered to be more important. Thus, the red cells are rendered exquisitely sensitive to the complement, being activated either through the alternate pathway or through an antigen-antibody reaction. This results in intra-vascular haemolysis. The pathophysiology of the thrombophilia in PNH is poorly understood. It has been postulated that elevated levels of procoagulant microparticles which are released from the activated platelets could lead to an increased thrombotic risk in these patients [6]. PNH can occur in patients with a previous history of Aplastic Anaemia (AA) or sometimes, a patient of PNH can progress to Aplastic anaemia [7].

A scheme of classification has been proposed which incorporates variation in history, presentation and clinical manifestations. The three types of PNH are – classical PNH, PNH in the setting of another bone marrow disorder and subclinical PNH (PNH-Sc) [8]. Classical PNH has the manifestations of intravascular haemolysis

both clinically and as suggested by the investigations. There is no evidence of bone marrow abnormality except for erythroid hyperplasia. PNH in the setting of specified bone marrow disorder has the evidence of both intravascular haemolysis and a specific bone marrow abnormality.

Patients with subclinical PNH have no clinical or lab evidence of haemolysis. Small populations of GPI-AP–deficient haematopoietic cells (peripheral blood erythrocytes, granulocytes, or both) are detected by very sensitive flow cytometric analysis.

Various tests are used in the diagnosis of PNH. Traditionally, lytic tests like the Ham's test and the sucrose haemolysis tests were used. Flow cytometric analysis by using antibodies which are directed against GPI-AP is the most sensitive and informative test which is available for the diagnosis of PNH [9]. For the initial studies, the quantitation of at least 2 GPI-APs is recommended, to exclude the possibility that the clinical process was a consequence of an inherited, isolated deficiency of a single GPI-AP . Erythrocytes with a complete deficiency of the GPI-APs are called PNH III, those with a subtotal deficiency (usually 10% of the normal expression) of the GPI-APs are called PNH II, and those with normal expression of the GPI-APs are called PNH I [10].

The minimal essential criterion which is required for the diagnosis and categorization are:

1. Evidence of a population of peripheral blood cells (erythrocytes, granulocytes, or preferably both) which are deficient in GPI-APs. Flow cytometric analysis of peripheral blood erythrocytes, granulocytes, or both by using primary antibodies against GPI-APs or the FLAER (fluorescently labeled inactive toxin aerolysin) assay reveals a population of haematopoietic cells which are partially or completely deficient in all the GPI-APs.
2. Complete blood count, reticulocyte count and serum concentrations of lactate dehydrogenase (LDH), bilirubin (fractionated) and haptoglobin.
3. Bone marrow aspiration, biopsy, and cytogenetics.

Our patient had clinical and lab features which were suggestive of classical PNH. He was pale and icteric. His haemoglobin was low, with a high reticulocyte count. His high unconjugated bilirubin and elevated serum LDH levels pointed towards haemolytic anaemia. The detection of haemosiderin in urine and the flow cytometric evidence that a majority of the RBCs were deficient in GPI-AP, sealed the diagnosis of classic PNH in our patient.

The management of patients with PNH is by supportive treatment only,, which includes the use of filtered red cells, whenever required. Folic acid supplements are mandatory. Iron supplementation should be given cautiously and the serum iron levels should be checked periodically. Corticosteroids are advocated at the times of acute exacerbation of the haemolysis. They do not have any long term use. The only form of treatment that can provide a cure is allogenic bone marrow transplant whenever an HLA identical sibling is available. Long term anti-coagulation is necessary in patients who present with thrombotic events.

A major advance in the management of PNH is the advent of a monoclonal antibody which is directed against complement C5, Eculizumab. It is safe and well tolerated in patients with PNH. The major adverse effect of the eculizumab therapy includes an increased risk of infection with capsulated organisms mainly, N. meningitides. The main problem with the long term therapy with eculizumab is the cost factor [11].

CONCLUSION

The diagnosis of Paroxysmal nocturnal haemoglobinuria should be considered in all the patients who present with haemolytic anaemia in the absence of splenomegaly.

REFERENCES

[1] Rosse WF, Nishimura J. Clinical manifestations of paroxysmal nocturnal haemoglobinuria: present state and future problems. *Int J Haematol* 2003; 77: 113-20.
[2] Takeda J, Miyata T, Kawagoe K, Iida Y, Endo Y, Fujita T, et al. Deficiency of the GPI anchor which is caused by a somatic mutation of the PIG-A gene in paroxysmal nocturnal haemoglobinuria. *Cell* 1993;73:703-11.
[3] Hillmen P, Richards SJ. Implications of recent insights into the pathophysiology of paroxysmal nocturnal haemoglobinuria. *Br J Haematol* 2000;108:470-9.
[4] Karadimitris A, Luzzatto L. The cellular pathogenesis of paroxysmal nocturnal haemoglobinuria. *Leukemia* 2001;15:1148-52.
[5] Rosse WF, Ware RE. The molecular basis of paroxysmal nocturnal haemoglobinuria. *Blood* 1995; 86: 3277-86.
[6] Hugel B, Socie G, Vu T, Toti F, Gluckman E, Freyssinet JM, et al. Elevated levels of circulating procoagulant microparticles in patients with paroxysmal nocturnal haemoglobinuria and aplastic anemia. *Blood* 1999; 93 (10): 3451-56.
[7] Dunn DE, Tanawattanacharoen P, Boccuni P, Nagakura S, Green SW, Kirby MR, et al. Paroxysmal nocturnal haemoglobinuria cells in patients with bone marrow failure syndromes. *Ann Intern Med* 1999; 131: 401-08.
[8] Parker C, Omine M, Richards S, Nishimura J, Bessler M, Ware R, et al. Diagnosis and management of paroxysmal nocturnal haemoglobinuria. *Blood* 2005; 106 (12): 3699-709.
[9] Richards SJ, Rawstron AC, Hillmen P. Application of flow cytometry in the diagnosis of paroxysmal nocturnal haemoglobinuria. *Cytometry* 2000;42: 223-33.
[10] Fujioka S, Yamada T. Varying populations of CD59-negative, partly positive and normally positive blood cells in different cell lineages in the peripheral blood of paroxysmal nocturnal haemoglobinuria patients. *Am J Haematol* 1994;45:122-27.
[11] Hillmen P, Hall C, Marsh J C , Elebute M, Bombara M P, Petro B E, et al. Effect Of eculizumab on the haemolysis and transfusion requirements in patients with paroxysmal nocturnal haemoglobinuria. *N Engl J Med* 2004; 350: 552-59.

AUTHOR(S):
1. Dr. Basavaprabhu Achappa
2. Dr. Deepak Madi
3. Dr. Satish Rao
4. Dr. Meghna Rao
5. Dr. Soundarya Mahalingam

PARTICULARS OF CONTRIBUTORS:
1. Associate Professor.
2. Associate Professor.
3. Associate Professor.
4. Physician.
5. Associate Professor.

NAME OF DEPARTMENT(S)/INSTITUTION(S) TO WHICH THE WORK IS ATTRIBUTED:
Kasturba Medical College, Mangalore, India - 575001.

NAME, ADDRESS, TELEPHONE, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Deepak Madi,
Assistant Professor, Department of General Medicine,
Kmc Hospital, Attavar, Mangalore 575001, India.
Phone: 919845609148; E-mail: bachu1504@yahoo.co.in

FINANCIAL OR OTHER COMPETING INTERESTS:
None.

Date of Submission: **Nov 14, 2011**
Date of Peer Review: **Dec 06, 2011**
Date of Acceptance: **Dec 28, 2011**
Date of Publishing: **May 31, 2012**