

Paroxysmal Nocturnal Hemoglobinuria: A Chronic Rare Acquired Hemolytic Anemia in a Middle-aged Female with Thrombotic Complications and Multi-system Involvement

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Abstract:

Paroxysmal Nocturnal Hemoglobinuria (PNH) is an acquired coomb's negative hemolytic anemia, the hallmark of which is chronic intravascular hemolysis, thrombophilia and their sequelae owing to the deficiency of several surface proteins on blood cell membranes. In this case a middle-aged female presents with severe anemia requiring repeated blood transfusion and recurrent deep vein thrombosis (DVT). She had been hospitalized in multiple occasions in multiple centers without any satisfactory diagnosis. The constant laboratory features were that of hemolytic anemia, thrombocytopenia and a negative coomb's test; although reticulocyte count had not been elevated. Lastly Blood flow cytometry revealed PNH clones. Although excellent improvement of the clinical features of PNH can be achieved by treatment with Eculizumab, we could not offer that to our patient due to its unavailability in Bangladesh. She was discharged with advice for Hematopoietic Stem Cell Transplantation (HSCT) after her symptoms improved with symptomatic management. High degree of suspicion and appropriate application of clinical knowledge is necessary to make such a diagnosis early in the disease process, for prompt initiation of treatment and thus minimizing complications.

Key words: Paroxysmal Nocturnal Hemoglobinuria, Intravascular hemolysis, Eculizumab, HSCT.

Introduction:

Paroxysmal Nocturnal Hemoglobinuria (PNH) is a rare Acquired Hemolytic anemia with multisystem involvement, in which certain proteins are deficient not only on RBC membrane but also on all three blood cell membranes that cause unchecked amplification of the complement activation process and the resultant intravascular hemolysis and its consequences, manifesting as hemolytic anemia, thrombocytopenia, pancytopenia, thrombotic and other complications.^{1,2,3} As those proteins are deficient in PNH, there is unchecked complement activation, intravascular hemolysis, hemoglobinuria, vasospasm, pulmonary hypertension, venous and arterial thrombosis and their complications. Patients might present with recurrent DVT, severe anemia, pulmonary hypertension and its complications, hepato- and/or splenomegaly, splenic,

mesenteric or hepatic vein thrombosis (Budd-Chiari Syndrome). There may be bone marrow aplasia even when the peripheral blood counts are normal. The ideal treatment would be Hematopoietic Stem Cell Transplantation (HSCT) but that itself is associated with significant mortality and morbidity. A recently approved humanized monoclonal antibody, Eculizumab, is shown to halt hemolysis and improve symptoms and prevent development of complications.⁴

Here we report a case of Paroxysmal Nocturnal Hemoglobinuria with its clinical features and complications, diagnostic works, management perspectives and shortcomings that will help our clinicians in early diagnosis and treatment in their clinical practice.

Case Report:

A 30-year-old Muslim housewife was admitted in a tertiary hospital on 7th September 2015 with the complaints of breathlessness on exertion, cough, generalized weakness and swelling of legs for 2 months. She experienced gradually increasing shortness of breath on walking for few steps, which worsened on lying flat and caused disturbance of sleep on several occasions. Cough was not associated with sputum production or chest pain, neither did she have any history of asthma or COPD. On admission there was bilateral pitting edema and tenderness in right cuff muscles. She had history

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of hospital admissions on several occasions prior to this. In April 2015, she was admitted due to history of chest pain, fever, cough, headache, vomiting and leg pain for 3 weeks. She was diagnosed as case of Pneumonia with Extra-pulmonary complications (Hematological, Neurological) with DVT with ITP with Severe Anemia with Bilateral Sixth nerve palsy and Retinal Hemorrhage. She was treated with Antibiotics, a short course of low molecular weight heparin (LMWH) and other symptomatic management at that time and was discharged as her pneumonia and other manifestations resolved. In August 2015 she was again admitted and was treated as a case of heart failure and pulmonary hypertension (on the basis of Echocardiography report). The patient had no history of dysuria, dyspepsia, bowel/ bladder disturbance, jaundice, rash, joint pain, oral ulcer or sore throat.

On examination, she was severely anemic, JVP was raised and had bilateral pitting oedema (more on right) with right cuff muscle tenderness. Her respiratory rate was 20/ min, pulse rate was 96 b/m, temperature was 98° F and blood pressure was 100/60 mmHg. On auscultation there was bilateral basal crepitation. Palpable P2 & Right parasternal heave were present. Other systemic examination, Including nervous, musculoskeletal and alimentary system revealed no abnormality. Fundoscopy showed right sided macular edema.

Complete Blood Count done in this admission showed Hemoglobin 5.8 mg/dL, Total Leucocyte count 7000/ cumm, Platelet count 1,60,000/ cumm and ESR was >140 mm in 1st hour. CBC done on several occasion at past showed severe anemia and normal leucocyte count. Her MCV in this occasion was elevated 113.9 fL, but in previous results it was within normal limit. Though previous blood films showed varying degrees of normocytic/ macrocytic anemia, neutrophilia and thrombocytopenia, PBF on this occasion showed spherocytes, polychromatic macrocytes, nucleated RBCs and roleux formation consistent with autoimmune hemolytic anaemia (AIHA). Serum bilirubin was 1.9 mg/dL (direct 0.7 and indirect 1.2 mg/dL). Previous reticulocyte count, Coombs test, ANA, pANCA, cANCA and Anti-dsDNA results were normal. Bone marrow examination done in April 2015 was consistent with Immune Thrombocytopenic Purpura.

Echocardiogram done in September '15 revealed MR (Gr – I), TR (Gr – I), PR (Gr – II), Moderate Pulmonary Hypertension with RVH, Good LV systolic function (EF – 68%), PASP: 50 mmHg, PADP: 34 mmHg. Duplex Ultrasound of lower limb vessels done in April '15 showed subacute DVT involving distal right superficial, whole of

popliteal and proximal parts of posterior and anterior and posterior tibial veins with signs of minimal recanalization and sapheno-popliteal junction was obscured by thrombus. The study done in August '15 yielded normal study. LDH was 977 U/L and D-dimer in this admission was 3.62 ug/mL whereas in April it was >4 ug/mL. Chest X-ray showed full pulmonary conus with clear lung fields; though varying degrees of pleural effusion were present in previous ones. USG of whole abdomen revealed trace ascites, splenomegaly, and mild pleural effusion.

Her hemolytic anemia and all the features could not be explained by a unifying diagnosis despite repeated hospital admissions. PNH was a possibility considering the coomb's test negative hemolytic anemia, thrombocytopenia and thrombophilia. So, Blood Flow Cytometry was done which revealed PNH clone in 70.30% of WBCs and 25.6% of RBCs which is consistent with a diagnosis of Paroxysmal nocturnal hemoglobinuria.

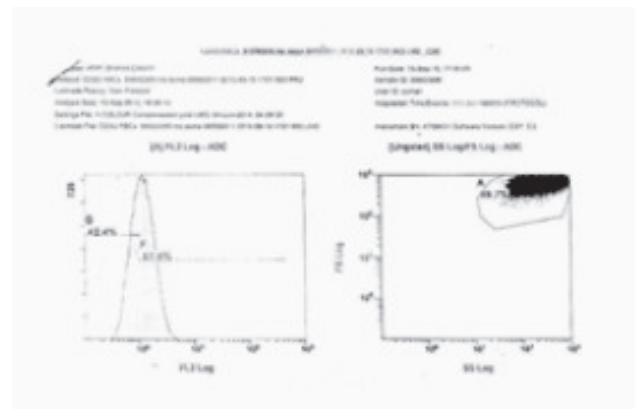


Fig-1: CD55 RBC distribution curve in this patient, flow cytometric analysis.

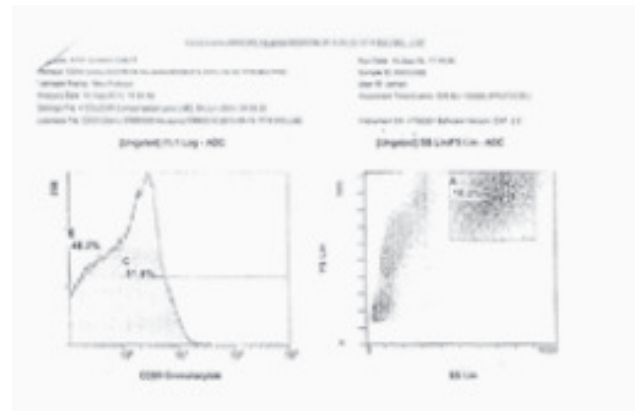


Fig-2: CD59 Granulocyte distribution curve in this patient, flow cytometric analysis.

She was being treated with Antibiotics, Ambrisentan and Sildenafil for her pulmonary hypertension and LMWH to help her current DVT features. Her symptoms improved with these treatment and she was subsequently discharged in stable and symptom free conditions with further advice for HSCT. Although according to current guidelines the first treatment that should be offered is Eculizumab, it is not available in Bangladesh and the patient party is not well off enough to afford treatment in abroad.

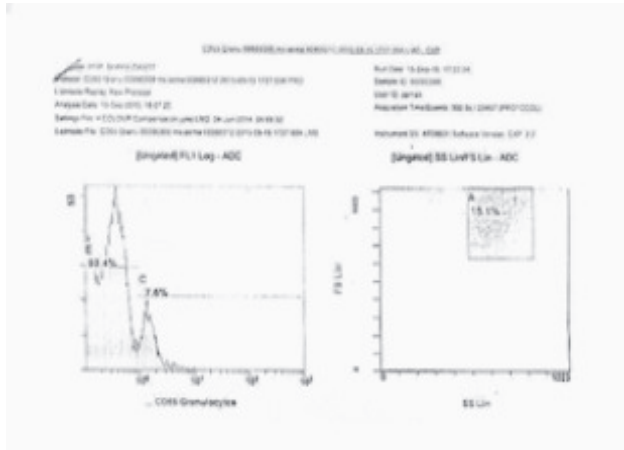


Fig.-3: CD59 Granulocyte distribution curve in this patient, flow cytometric analysis.

FLOW CYTOMETRY REPORT-PNH EVALUATION

Specimen : Peripheral blood
 Antibodies : Immunophenotypic analysis was performed using gating antibodies CD59 and CD55

Flow Results :

Cell type	Deficiency	Result
Red blood cells	Type-I (Normal CD59 expression)	91.2 %
	Type-II (Partial CD59 deficiency)	4.8 %
	Type-III (Complete CD59 deficiency)	2.0 %
Granulocytes	Type-I (Normal CD59 expression)	51.8 %
	Type-II (Partial CD59 deficiency)	48.2 %
	Type-III (Complete CD59 deficiency)	0.0 %
Red blood cells	Type-I (Normal CD55 expression)	37.6 %
	Type-II (Partial CD55 deficiency)	42.4 %
	Type-III (Complete CD55 deficiency)	19.9 %
Granulocytes	Type-I (Normal CD55 expression)	7.6 %
	Type-II (Partial CD55 deficiency)	92.4 %
	Type-III (Complete CD55 deficiency)	0.0 %

Diagnosis : WBC : PNH clone identified (70.3 %)
 RBC : PNH clone identified (25.6 %)

Comment : Flow cytometric analysis shows a 48.6 % PNH clone within the RBCs and granulocytes. These findings are consistent with a diagnosis of paroxysmal nocturnal hemoglobinuria (PNH). The difference in clone size between the white blood cells and the red blood cells may be due to hemolysis and/or recent transfusion.

Fig.-4: Report of Flow Cytometric Analysis of patients blood for CD59 & CD55 deficient clone.

Discussion:

Paroxysmal Nocturnal Hemoglobinuria is known to be a rare acquired hemolytic anemia in which RBCs undergo chronic persistent complement mediated hemolysis owing to the deficiency on its membrane of several important proteins which in normal conditions happen to halt the process of complement activation. Later it was found out that those proteins are missing in other cells of blood as well as RBCs. No hereditary case has been reported and prevalence is equal in men and women. The biochemical defect is a genetic mutation that inhibits the synthesis of Glycosyl-phosphatidylinositol (GPI) anchor that binds these proteins to cell membranes. The gene responsible is the PIG-A (Phosphatidylinositol Glycan Class A) gene on the X chromosome, and there is substantial variability in the pattern of mutation in the gene from patient to patient.^{5,6} The membrane proteins that are deficient in PNH are complement –regulating surface proteins and includes CD55 (Decay Accelerating Factor; DAF), CD59 (Membrane Inhibitor of Reactive Lysis; MIRL) and C8 binding protein (Homologous Restriction Factor; HRF). These proteins interact with complement proteins, particularly C3b and C4b, and inhibits the complement activation process. Most important of these is CD59, which hinders the insertion into the membrane of C9 polymers. The absence of these proteins causes unchecked amplification of the complement activation process and consequent cell lysis. The clinical features of PNH are more or less all related to the cell lysis. Lysis of RBC causes hemolytic anemia, which may be mild, moderate or severe at presentation, the hemoglobin that is released due to intravascular hemolysis depletes serum haptoglobin and remaining free hemoglobin goes on to be excreted by the kidneys with the urine (hence the name hemoglobinuria), which in the longer term can cause chronic renal failure.

The free hemoglobin also binds irreversibly to Nitric Oxide and depletes NO in peripheral blood, causing vasospasm with resultant gut ischemia, pulmonary hypertension and other vasospastic complications.⁷

One of the most prominent presenting features in PNH is thrombophilia, the mechanism of which has yet not been delineated fully. Patients present with thrombotic symptoms ranging from DVT to hepatic and mesenteric vein thrombosis (e.g. Budd-Chiari syndrome) to cerebro-vascular accidents.⁸⁻¹⁰ Pulmonary hypertension in PNH can be either due to NO depleted state or due to widespread formation of thrombuses in pulmonary microvasculature.¹¹ In our patient however, the possibility of chronic pulmonary thromboembolism could not be excluded as CTPA was not done.

Varying degrees of Bone Marrow Failure is almost always present in patients with PNH. 10-20% patients with PNH

goes on to develop Aplastic Anemia and 5% patients with aplastic anemia develops PNH. The relation between these and the pathogenesis is not yet fully elucidated.^{12,13}

Patients present with passage of dark red urine – specially in the morning, features of thrombosis and the presentation can be varied. There may be varying degrees of anemia, thrombocytopenia, markedly elevated LDH level, increased Reticulocyte count, elevated serum bilirubin - specially the indirect fraction, decreased level of serum Haptoglobin. Anemia may be normo-macrocytic or may be microcytic if the patient is iron deficient. Bone marrow may show erythroid hyperplasia with varying degrees of dyserythropoietic features. In the long run bone marrow may become hypocellular or frankly aplastic.

The acidified serum (Ham) test is highly reliable in making a definitive diagnosis but the gold standard now is Blood Flow cytometry.¹⁴⁻¹⁷ If 5% or more RBCs and 20% or more granulocytes are CD59 and CD55 deficient, the diagnosis of PNH can be definitively made.¹⁸

Apart from symptomatic management with blood transfusion, antithrombotic prophylaxis, pulmonary hypertension management, the definitive curative treatment is Allogenic Hematopoietic Stem Cell Transplantation (HSCT).^{19,20} But HSCT itself carries risks of transplant related mortality of 42% and graft failure in 8% cases.²¹ The availability of HLA-matched donor is also a concern. With advent of a new drug, approved in 2007, namely Eculizumab, which is a humanized monoclonal antibody against complement component C5, the indication for allogenic HSCT has changed significantly.²²⁻²⁵ The initial treatment of patients with classic PNH should be Eculizumab, which shows significant reduction in mortality and morbidity, rate of hemolysis and development of thrombotic complications. There are concerns regarding increased susceptibility of infection specially *Neisseria meningitidis* for which prophylactic vaccination with tetravalent meningococcal vaccine is indicated. There is also small risk of developing meningococcal septicemia in vaccinated patients for which prophylactic antibiotic therapy with Penicillin or Erythromycin is indicated.²⁶⁻²⁸

Paroxysmal Nocturnal Hemoglobinuria was first described in 18th century. “Paroxysmal” “nocturnal” hemoglobinuria is a misnomer in the sense that, it is now well known that the hemolysis and resultant hemoglobinuria is neither ‘paroxysmal’ nor purely ‘nocturnal’. Hemolysis occurs throughout the day and the passage of dark coloured urine in the morning is due to overnight increment in concentration of hemoglobin in urine. There is persistent amplification of complement cascade activation via the alternative pathway

in PNH and there can be ‘paroxysmal’ acceleration of complement activation and hemolysis during a course of viral or bacterial infection through an antigen-antibody reaction.¹⁸

PNH is a multifaceted disease that has the potential for significant mortality, morbidity and can drastically reduce the quality of life in affected patients. Appropriate awareness of the rare condition among clinicians is therefore of paramount importance.

Conflict of interest: None.

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