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RESEARCH ARTICLE

Clinicopathological Diversity of Paroxysmal Nocturnal Hemoglobinuria Clone- A case series.

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Abstract

Introduction: Paroxysmal nocturnal hemoglobinuria [PNH] is a rare hematopoietic stem cell disorder characterized by a somatic mutation in the PIGA gene, leading to a deficiency of proteins linked to the cell membrane via glycophosphotidylinositol [GPI] anchors. It can arise denovo or in a setting of aplastic anemia. We hereby report our experience with six cases of PNH diagnosed by flow cytometry.

Material and methods: The present study was a retrospective study of six cases referred to Kasturba Medical College Hospital, Jyothi circle and Attavar who were diagnosed as Paroxysmal Nocturnal Hemoglobinuria. The details of the cases with regard to age, sex, presenting symptoms, clinical and laboratory findings were obtained from the hospital records. The final diagnosis of PNH was made based on the sensitive flow cytometry test.

Results: Majority had hematuria as one of the presenting symptom. Two of the patients were known cases of aplastic anemia that clonally evolved to PNH. Peripheral smear examination showed pancytopenia in three of the cases while the rest showed anemia. Bone marrow examination was done in three cases which showed hypoplastic marrow in two of them. Flow cytometry of the peripheral blood sample showed more than 5% of granulocytes with PNH clone in all the cases.

Conclusion: Flow cytometry is now widely accepted as the method of choice for detecting GPI-anchor protein-deficient clones in clinical, subclinical PNH and related bone marrow disorders. Clinical and laboratory findings together should raise the suspicion for PNH, and to proceed for diagnosis by flow cytometry.

Introduction

Paroxysmal nocturnal hemoglobinuria [PNH] is a rare acquired clonal stem cell disorder that is characterized by three distinctive clinical features of haemolytic anemia, thrombosis and hemopoietic deficiency, which vary from patient to patient and during the course of the disease.[1-3]The hemolysis is recurrent, episodic and intravascular due to increased sensitivity to complement mediated lysis of the red cells which manifests clinically as dysphagia, lethargy, erectile dysfunction, chronic renal failure, pulmonary hypertension, anemia and hemoglobinuria.[4,5] Episodic hemoglobinuria, although the defining symptom, is reported in only 25% of the patients.[4] The thrombotic tendency can be fatal and is seen not only in the extremities but also in unusual anatomical locations such as hepatic, portal, mesenteric and splenic veins. The hemopoietic deficiency and

bone marrow failure occurs to more or less extent in all patients and results in immune mediated aplastic anemia in its most extreme form. Conversely, the disease may be associated with antecedent history of aplastic anemia[5]. In the present study, six cases of PNH have been evaluated in the laboratory, correlated with the clinical findings and the diagnosis has been confirmed with the help of flow cytometry.

Materials and methods

The present study was a retrospective study of six cases referred to Kasturba Medical College Hospital, Jyothi circle and Attavar who were diagnosed as PNH by flow cytometric analysis. The details of the cases with regard to age, sex, presenting symptoms, clinical and laboratory findings were obtained from the hospital records.

Results

The findings of 6 cases are summarized in the Table 1.
Among the six cases, there were 4 males and 2 females. The age range was 30 – 58 years. Hematuria was the predominant presenting symptom in 5 out of 6 cases and the urine tested positive for hemoglobin. The hemogram showed anemia in all the patients; there was pancytopenia in 3 cases; the bone marrow examination was done in 3 of the cases. Two cases showed hypoplastic marrow with a decrease in all the cell lines and one case showed dimorphic anemia due to co-existing iron deficiency. Serum lactate dehydrogenase levels, which done in 4 cases, was elevated. Only one case developed thrombotic complications and died of sepsis.

Table 1: Clinical and laboratory findings in the six patients

Case	Age in years	Gender	Clinical Presentation	Peripheral smear findings	Bone marrow findings	Serum LDH	Diagnosis
1	45	M	K/C/O MGUS, Dark urine, jaundice, Hepatosplenomegaly	Normocytic normochromic anemia. Hemoglobinuria	Dimorphic anemia	1657	PNH
2	42	F	K/C/O aplastic anemia with intravascular hemolysis and thrombotic complications	Pancytopenia	Hypolpastic marrow	-	Aplastic anemia evolution to PNH
3	58	M	Hematuria,	Anemia with thrombocytopenia	-	2009	PNH
4	35	M	Dark urine, jaundice at the age of 5 yrs, Hepatosplenomegaly	Normocytic normochromic anemia	-	-	PNH
5	30	M	Pancytopenia,	Pancytopenia with a few spherocytes	Hypoplastic marrow	447	PNH
6	32	F	K/C/O aplastic anemia with hematuria	Pancytopenia	-	2715	Aplastic anemia evolution to PNH

PNH- Paroxysmal Nocturnal Hemoglobinuria, M- Male, F- Female, K/C/O- Known case of, MGUS- Monoclonal Gammapathy of Undetermined Significance, LDH- Lactate dehydrogenase.

Discussion:

Paroxysmal nocturnal hemoglobinuria is a rare hematopoietic stem cell disorder first described by Dr Paul Strübing in 1882.[3] It is characterized by a somatic mutation in the PIGA gene, leading to a deficiency of proteins linked to the cell membrane via glycophosphotidylinositol [GPI] anchors.[1,3-5] This leads to intravascular hemolysis as a result of sensitivity of red cells to complement mediated hemolysis. The disease is characterized by Coomb's negative hemolytic anemia, marrow failure, or venous thrombotic events [TE]. The non- specific symptoms of the disease make it a hidden disease for a prolonged time in these patients. The pleomorphic clinical presentation of PNH is recognized as two entities: one, predominantly hemolytic without overt marrow failure, is referred to as classical PNH1 and the other, with marrow failure, is often described as the aplastic anemia - PNH syndrome [AA-PNH] [6]

The diagnosis of PNH must be considered in any patient who has the following: (a) signs and symptoms of intravascular hemolysis (manifested by an abnormally high LDH) of undefined cause (i.e., Coomb's negative) with or without macroscopic hemoglobinuria often accompanied by iron deficiency; (b) pancytopenia in association with hemolysis, whether or not the bone marrow is cellular; (c) venous thrombosis affecting unusual sites (d) unexplained recurrent bouts of abdominal pain, low backache or headache in the presence of chronic hemolysis, and (e) Budd-Chiari syndrome.[6]
The disease generally manifests in third to fifth decade. All patients in our series fell in the age range. Males outnumbered the females unlike the usual female preponderance[4].

In our study of six cases, anemia and pancytopenia were the most common presenting features. Parab RB[7] studied 17 cases of PNH and observed that refractory anemia is the most common symptom. Five among six of our cases had typical symptoms of intravascular hemolysis namely hemoglobinuria and hematuria. This is a very consistent symptom as reported by Parab et al as well. The diagnosis was based on clinical features, hemosiderinuria and sucrose lysis test. While this test is sensitive and specific when performed properly, the accuracy depends strongly on the operator.[4]

PNH is associated with an increased risk of thrombosis affecting superficial and deep veins of the body. According to a review, in patients dying of PNH, thrombosis is the leading cause of death. The possible mechanisms include: procoagulant phophatidyl serine exposure due to formation of RBC microvescicles following hemolysis, nitric oxide depletion, complement damage to leukocytes and consequent release of inflammatory mediators.[6] One among six of our patients developed thrombosis of hepatic vein and died of sepsis.

Screening of appropriate patients and arriving at right diagnosis becomes important as the disease can be in subclinical forms. PNH can be a part of other clonal disorders of bone marrow like aplastic anemia[5]. In our series two patients who were known cases of aplastic anemia and on antithymocyte immunoglobulin therapy developed the disease. It was the high levels of serum LDH in one case and presence of classical symptoms of PNH in the other which prompted us to look for PNH clone in them by flow cytometry.

Aplastic anemia has a better prognosis these days because of immunosuppressive therapy (IST). With single or repeated courses of IST, majority of patients respond and have excellent survival. However evolution to clonal diseases like paroxysmal nocturnal hemoglobinuria is one of the serious long-term complication in such patients. According to literature the evolution rate range from 2%-19% over a period of 2-11 years. This progression to PNH did not have significant relation to any therapy. According to other studies this progression made the patients favourably respond to IST. [8,9] It is possible that in these cases the development of PNH clone is an adaptive mechanism to escape the autoimmune attack by lymphocytes in aplastic anemia. The presence of PNH phenotype in patients with aplastic anemia and in those with myelodysplastic syndrome is of clinical importance since it correlates with high probability of these patients responding to immunosuppressive therapy.[9]

In the present day, flow cytometry is the method of choice that is available for diagnosis as well as monitoring the disease.[4,5] By analyzing the expression of GPI-AP namely CD55 and CD59 on hematopoietic cells using

monoclonal antibodies and flow cytometry, even tiny PNH clones can be readily identified. The size of the PNH clone can be best determined by analysis of GPI-AP expression on the granulocytes as the life span of the granulocytes are not shortened unlike that of RBCs.[5,8] The presence of CD55 and CD59 deficient cells above 5% population of the granulocytes is diagnostic criteria for PNH. In all our cases sensitive flow cytometry was used to confirm the diagnosis of PNH.

Bone marrow transplantation is the definitive treatment for the disease. Eculizumab, humanized monoclonal antibody, offers a new hope by reducing the complications of the disease and thus improving quality of life. To conclude, PNH is a rare disease manifesting with myriad symptoms. High index of clinical suspicion coupled with laboratory findings and confirmation by flow-cytometry allows towards diagnosis.

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