ORIGINAL RESEARCH PAPER

General Medicine

PAROXYSMAL NOCTURNAL HEMOGLOBINURIA (PNH): A RARE BUT LIFE-THREATENING HEMATOLOGIC DISORDER

KEY WORDS: Paroxysmal Nocturnal Hemoglobinuria, Hemoglobinuria, Flow Cytometry, CD55, CD59, Hemolytic Anemia

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ABSTRACT

Background: Paroxysmal nocturnal hemoglobinuria (PNH) is a rare acquired clonal hematopoietic stem cell disorder caused by a somatic mutation in the PIGA gene, resulting in deficiency of glycosylphosphatidylinositol (GPI)-anchored proteins and complement-mediated intravascular hemolysis. Early diagnosis is essential to prevent life-threatening complications such as thrombosis and bone marrow failure. **Case Presentation:** A 22-year-old male presented with a 3–4-day history of dark-colored urine, predominantly in the early morning hours. He denied fever, dysuria, flank pain, bleeding manifestations, or recent infections. Laboratory investigations revealed severe anemia (Hb 6.0 g/dL), leukopenia (WBC 2.63 × 10^9/L), and thrombocytopenia (106 × 10^9/L). Urinalysis demonstrated significant hematuria and proteinuria. Bone marrow examination showed erythroid hyperplasia with megaloblastic erythropoiesis without dysplasia or infiltration. Flow cytometric analysis demonstrated FLAER/CD24 deficiency in 94% of granulocytes, FLAER/CD14 deficiency in 95.7% of monocytes, and CD59 deficiency in 22.8% of erythrocytes, confirming a large PNH clone. **Conclusion:** This case highlights the importance of considering PNH in young adults presenting with unexplained hemoglobinuria and cytopenias. High-sensitivity flow cytometry remains the diagnostic gold standard, and early recognition facilitates timely initiation of complement inhibitor therapy, improving survival and quality of life.

INTRODUCTION

Paroxysmal nocturnal hemoglobinuria (PNH) is an uncommon acquired hematopoietic stem cell disorder characterized by complement-mediated intravascular hemolysis. The disease commonly presents with hemolytic anemia, hemoglobinuria, fatigue, and dyspnea. Patients may also develop serious complications such as venous thrombosis, renal dysfunction, and progressive bone marrow failure during the course of the disease. PNH results from a somatic mutation in the PIGA gene located on the X chromosome, leading to deficiency of glycosylphosphatidylinositol (GPI)-anchored proteins that normally protect blood cells from complement-mediated destruction.[1][2]

The term *paroxysmal nocturnal hemoglobinuria* was introduced by Enneking in 1925, although descriptions of the disease date back to the late nineteenth century. One of the earliest documented cases was reported by Strübing, who described a young man with recurrent episodes of fatigue, abdominal pain, and intermittent hemoglobinuria. He also observed red discoloration of the patient's plasma following severe episodes, correctly attributing it to intravascular hemolysis. In 1937, Ham demonstrated that erythrocytes from patients with PNH underwent hemolysis when exposed to acidified normal serum, leading to the development of the Ham test, the first diagnostic assay for PNH. Although complement-mediated hemolysis had long been suspected, its definitive role in disease pathogenesis was established only in 1954. Subsequent research identified deficiencies of GPI-anchored proteins on blood cells and ultimately led to the discovery of the underlying genetic defect responsible for the disorder.[1]

Despite its rarity, PNH has a profound impact on patient morbidity and quality of life. Historically, the 10-year survival rate was approximately 50%; however, the introduction of complement inhibitors, particularly eculizumab, over the past 15 years has markedly improved clinical outcomes, increasing the 10-year survival rate to more than 75%.[2]

Case Report

A 22-year-old male presented to the Department of General Medicine at Dr. D. Y. Patil Hospital, Navi Mumbai, with complaints of passage of dark-colored urine for the preceding three to four days. The discoloration was more prominent during the early morning hours and gradually became lighter as the day progressed. The patient denied fever, burning micturition, increased urinary frequency, flank pain, abdominal pain, bleeding manifestations, or recent trauma. There was no history of jaundice, weight loss, night sweats, or previous episodes of similar illness.

The patient had no known history of diabetes mellitus, hypertension, chronic kidney disease, autoimmune disorders, or hematological illnesses. He had never received blood transfusions and reported no recent viral illnesses, medication use, or toxin exposure. His family history was unremarkable for inherited hemolytic disorders or hematological malignancies. There was no history suggestive of connective tissue disorders or thromboembolic disease.

On examination, the patient was conscious, alert, and oriented. He appeared pale but was hemodynamically stable. His pulse rate, blood pressure, respiratory rate, and oxygen saturation were within normal limits. General physical examination revealed pallor without icterus, cyanosis, clubbing, lymphadenopathy, or pedal edema. Cardiovascular, respiratory, abdominal, and neurological examinations were essentially unremarkable. No hepatosplenomegaly or abdominal tenderness was appreciated.

Investigations

Initial laboratory investigations demonstrated pancytopenia. The patient's hemoglobin level was 6.0 g/dL, total leukocyte count was 2.63 × 10^9/L, and platelet count was 106 × 10^9/L. Serial complete blood counts showed gradual improvement during hospitalization, with hemoglobin increasing to 7.8 g/dL on the second day and 8.5 g/dL by the fifth day. Similarly, the leukocyte count improved from 2.63 × 10^9/L to 7.22 × 10^9/L, while platelet counts increased from 106 × 10^9/L to 130 × 10^9/L.

Routine urine examination revealed a pH of approximately 6.5, proteinuria (3+), blood (3+), and numerous red blood

cells, while ketones were absent. These findings, together with the history of early morning dark-colored urine, suggested ongoing intravascular hemolysis.

To evaluate the underlying cause of pancytopenia, a bone marrow biopsy was performed. Histopathological examination demonstrated erythroid hyperplasia with megaloblastic erythropoiesis. There was no evidence of dysplasia, marrow infiltration, fibrosis, or hematological malignancy.

Considering the patient's clinical presentation and persistent evidence of hemolysis, flow cytometric analysis for PNH was performed using fluorescent aerolysin (FLAER) and monoclonal antibodies directed against GPI-linked proteins. Flow cytometry demonstrated FLAER/CD24 deficiency in 94% of granulocytes, FLAER/CD14 deficiency in 95.7% of monocytes, and CD59 deficiency in 22.8% of erythrocytes. The presence of large GPI-deficient clones confirmed the diagnosis of classical paroxysmal nocturnal hemoglobinuria.

Differential Diagnosis

Several differential diagnoses were considered in view of the patient's presentation with hemoglobinuria and pancytopenia. Autoimmune hemolytic anemia was considered but was less likely because of the characteristic flow cytometric findings and absence of supportive clinical features. Glucose-6-phosphate dehydrogenase deficiency, thrombotic thrombocytopenic purpura, hemolytic uremic syndrome, aplastic anemia, acute glomerulonephritis, and other inherited hemolytic anemias were also considered. However, the demonstration of large PNH clones on flow cytometry definitively established the diagnosis of paroxysmal nocturnal hemoglobinuria.

Management

The patient was managed conservatively during hospitalization with supportive therapy, including correction of severe anemia and close monitoring of hematological parameters. Renal function and clinical status remained stable throughout the hospital stay. After confirmation of the diagnosis, the patient was referred for hematology consultation for evaluation of definitive treatment with complement inhibitor therapy, including eculizumab or ravulizumab, depending on availability and affordability. He was advised regular follow-up for monitoring of blood counts, renal function, disease progression, and thrombotic complications.

Outcome and Follow-up

The patient's clinical condition improved gradually during hospitalization. His urine discoloration subsided, and serial blood counts demonstrated progressive improvement. At the time of discharge, he remained hemodynamically stable without evidence of thromboembolic events or worsening renal function. He was advised lifelong hematology follow-up, periodic assessment of PNH clone size by flow cytometry, and evaluation for initiation of complement inhibitor therapy. He was also counseled regarding warning symptoms suggestive of thrombosis and the importance of prompt medical attention should these occur.

DISCUSSION

Paroxysmal nocturnal hemoglobinuria (PNH) is a rare, acquired clonal hematopoietic stem cell disorder caused by a somatic mutation in the *PIGA* gene, resulting in deficiency of glycosylphosphatidylinositol (GPI)-anchored proteins, particularly CD55 and CD59. The absence of these complement-regulatory proteins renders erythrocytes highly susceptible to complement-mediated intravascular hemolysis, producing characteristic manifestations such as hemolytic anemia, hemoglobinuria, fatigue, dyspnea, and varying degrees of cytopenia. The disease exhibits marked clinical heterogeneity, which may delay diagnosis, particularly in young patients presenting with nonspecific symptoms.[1,2]

Our patient presented with the classical manifestation of recurrent early-morning dark-colored urine associated with severe anemia and pancytopenia. Although hemoglobinuria is a hallmark of PNH, not all patients exhibit the complete classical triad of hemolysis, bone marrow failure, and thrombosis at presentation. The presence of leukopenia and thrombocytopenia suggested underlying bone marrow involvement, reflecting the close association between PNH and bone marrow failure syndromes such as aplastic anemia.[1,3]

Flow cytometry remains the gold standard for diagnosing PNH because of its high sensitivity and specificity. The demonstration of FLAER-negative granulocytes and monocytes, together with CD59-deficient erythrocytes, confirmed the diagnosis in our patient. Granulocyte and monocyte clone sizes of 94% and 95.7%, respectively, represented a large PNH clone and indicated extensive involvement of the hematopoietic stem cell compartment. Granulocyte clone size is generally considered more reliable than erythrocyte clone size because PNH erythrocytes are selectively destroyed by complement-mediated hemolysis, and the measured erythrocyte clone may also be affected by previous transfusions.[1,3]

Bone marrow biopsy demonstrated erythroid hyperplasia with megaloblastic erythropoiesis, without evidence of dysplasia or malignant infiltration. Erythroid hyperplasia likely represented a compensatory response to chronic intravascular hemolysis, whereas megaloblastic changes may have reflected increased erythropoietic stress or an associated nutritional deficiency. The absence of marrow dysplasia or malignant infiltration supported the diagnosis of PNH and helped exclude other marrow disorders.

Thrombosis is one of the most serious complications of PNH and remains a major cause of disease-related mortality. Thrombotic events may occur at unusual venous sites, including the hepatic, portal, mesenteric, and cerebral veins. Their pathogenesis is multifactorial and involves complement activation, platelet activation, endothelial dysfunction, release of free hemoglobin, nitric oxide depletion, and activation of coagulation pathways. Although our patient did not develop thrombosis during hospitalization, the presence of a large PNH clone may place him at considerable future risk, necessitating close follow-up and timely institution of disease-modifying therapy.[4,5]

Increasing hemolysis and recurrent red blood cell transfusion requirements may be associated with greater disease burden and clonal evolution, although clinically significant hemolysis can also occur in patients with smaller PNH clones.[6] Assessment of disease activity, hemolytic burden, transfusion requirements, symptoms, and clone size is therefore important when determining the optimal timing of therapy. Early recognition may allow complement inhibition to be initiated before life-threatening thrombotic complications develop. Anticoagulation may reduce thrombotic risk in selected patients; however, primary prophylaxis does not eliminate the risk completely, and prolonged anticoagulation may be difficult in patients with thrombocytopenia because of the associated bleeding risk.[4,7,8]

The introduction of complement inhibitors has revolutionized the management of PNH. Eculizumab, a monoclonal antibody directed against complement component C5, blocks terminal complement activation and reduces intravascular hemolysis, transfusion requirements, and thromboembolic events. Complement inhibition has substantially improved survival and quality of life and is now a cornerstone of treatment for patients with symptomatic classical PNH.[4,5,8] Anti-

coagulation may be reconsidered in selected patients after adequate control of disease activity with eculizumab, although such decisions must be individualized according to the patient's thrombotic history, platelet count, bleeding risk, and response to treatment.[9]

Despite effective control of intravascular hemolysis, some patients continue to experience anemia because of ongoing extravascular hemolysis. Eculizumab prevents formation of the membrane attack complex by blocking C5 activation but does not prevent upstream C3 deposition on erythrocytes caused by persistent CD55 deficiency. These C3-coated PNH erythrocytes may subsequently be removed by macrophages in the spleen and liver, resulting in extravascular hemolysis despite adequate terminal complement blockade.[10] Newer agents targeting proximal components of the complement cascade are being developed to address this limitation and achieve more complete control of both intravascular and extravascular hemolysis.[11]

Overall, this case emphasizes the importance of maintaining a high index of suspicion for PNH in young adults presenting with unexplained hemoglobinuria, severe anemia, and cytopenias. Prompt flow cytometric evaluation allows early diagnosis, accurate assessment of clone size, evaluation of thrombotic risk, and timely initiation of appropriate therapy, thereby improving long-term clinical outcomes.[1,5]

REFERENCES

1. Parker C, Omine M, Richards S, et al. Diagnosis and management of paroxysmal nocturnal hemoglobinuria. *Blood*. 2005;106:3699–3709. doi:10.1182/blood-2005-04-1717.
2. Takeda J, Miyata T, Kawagoe K, et al. Deficiency of the GPI anchor caused by a somatic mutation of the *PIG-A* gene in paroxysmal nocturnal hemoglobinuria. *Cell.* 1993;73:703–711. doi:10.1016/0092-8674(93)90250-T.
3. de Latour RP, Mary JY, Salanoubat C, et al. Paroxysmal nocturnal hemoglobinuria: Natural history of disease subcategories. *Blood.* 2008;112: 3099–3106. doi:10.1182/blood-2008-01-133918.
4. Hill A, Kelly RJ, Hillmen P. Thrombosis in paroxysmal nocturnal hemoglobinuria. *Blood.* 2013;121:4985–4996. doi:10.1182/blood-2012-09-311381.
5. Ziakas PD, Poulou LS, Rokas GI, Bartzoudis D, Voulgarelis M. Thrombosis in paroxysmal nocturnal hemoglobinuria: Sites, risks, outcome—an overview. *J Thromb Haemost.* 2007;5:642–645. doi:10.1111/j.1538-7836.2007.02379.x.
6. van Bijnen STA, van Heerde WL, Muus P. Mechanisms and clinical implications of thrombosis in paroxysmal nocturnal hemoglobinuria. *J Thromb Haemost.* 2012;10:1–10. doi:10.1111/j.1538-7836.2011.04562.x.
7. Hillmen P, Hall C, Marsh JC, et al. Effect of eculizumab on hemolysis and transfusion requirements in patients with paroxysmal nocturnal hemoglobinuria. *N Engl J Med.* 2004;350:552–559. doi:10.1056/ NEJMoa031688.
8. Moyo VM, Mukhina GL, Garrett ES, Brodsky RA. Natural history of paroxysmal nocturnal haemoglobinuria using modern diagnostic assays. *Br J Haematol.* 2004;126:133–138. doi:10.1111/j.1365-2141.2004.04992.x.
9. Loschi M, Porcher R, Barraco F, et al. Impact of eculizumab treatment on paroxysmal nocturnal hemoglobinuria: A treatment versus no-treatment study. *Am J Hematol.* 2016;91:366–370. doi:10.1002/ajh.24278.
10. Hall C, Richards S, Hillmen P. Primary prophylaxis with warfarin prevents thrombosis in paroxysmal nocturnal hemoglobinuria. *Blood.* 2003;102: 3587–3591. doi:10.1182/blood-2003-01-0009.
11. Hillmen P, Muus P, Dührsen U, et al. Effect of the complement inhibitor eculizumab on thromboembolism in patients with paroxysmal nocturnal hemoglobinuria. *Blood.* 2007;110:4123–4128. doi:10.1182/blood-2007-06-095646.