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Classic paroxysmal nocturnal hemoglobinuria: A puzzling case of hemolytic anemia without cytopenia

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Abstract:

Paroxysmal nocturnal hemoglobinuria (PNH) is an uncommon acquired complement-mediated hemolytic anemia characterized by continuous destruction of red blood cells leading to dark red or black colored urine. A 42-year-old man has been experiencing recurring jaundice, gastrointestinal discomfort, and high-colored urine for the past 2 years. His hemogram and antiglobulin tests were normal. The hemolytic anemia workup revealed reticulocytosis, hemoglobinuria, urine hemosiderin, elevated indirect bilirubinemia, and extremely low haptoglobin. A high index of suspicion for PNH prompted the laboratory physician to do flow cytometry, which confirmed the diagnosis of classical PNH. Here, we discuss a case of PNH that remained undiagnosed for years, detailing how we successfully identified a rare and confusing case of hemolytic anemia without cytopenia.

Keywords:

Flowcytometry, intravascular hemolysis, paroxysmal nocturnal hemoglobinuria

Introduction

Paroxysmal nocturnal hemoglobinuria (PNH), caused by phosphatidylinositol gene mutation A (PIGA), is a clonal hematopoietic stem cell disorder. It results in lack of glycosylphosphatidylinositol (GPI) anchored cell surface proteins such as CD55 and CD59, which are key regulators of the complement pathway protecting host cells from hemolysis.[1] PNH often causes pancytopenia, a risk of venous thrombosis, renal insufficiency, and bone marrow failure. Flow cytometry has become the gold standard technique for the diagnosis of PNH.[2] This case was unique in that there was hemolysis without cytopenia, and the final diagnosis of PNH was obtained using flow cytometry and urine tests.

Case Report

A 42-year-old Bangla-speaking male reported to our hospital with 2 years of persistent jaundice, intermittent abdominal discomfort, and urine discoloration. He was evaluated at many different places, but they were unable to pinpoint the source of the underlying issue. Upon clinical examination, the patient was found to be icteric. His vital parameters were normal. His systemic examination was unremarkable except for a palpable spleen. The laboratory tests revealed [Table 1] a normal hemogram with elevated red blood cells (RBCs) indices, including mean corpuscular volume and mean corpuscular hemoglobin. Peripheral smear blood films revealed both normocytic and macrocytic RBCs. There were no hemoparasites found. The erythrocyte sedimentation rate and reticulocyte count were high. The direct and indirect Coombs tests were negative.

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Prothrombin time and activated partial thromboplastin time were mildly elevated. Biochemical tests revealed indirect bilirubinemia, elevated transaminases, and increased lactate dehydrogenase (LDH). Serological tests for hepatitis B and C viruses, HIV, and syphilis were negative. A complete urine test revealed proteinuria (2+) and 8–10 RBCs in the high-power field. The patient was told to collect urine in a separate sterile container for each pass. The series of containers displayed the different hues of urine, ranging from dark cola in the morning to pale yellow in the late evening [Figure 1]. The urine tests for hemoglobinuria and hemosiderinuria were positive. The hemoglobin electrophoresis and osmotic fragility tests were also normal. The glucose-6 phosphatase dehydrogenase was marginally raised, and serum haptoglobin levels were found to be very low.

Abdominal ultrasonography showed mild splenomegaly (132 mm) with normal echotexture. The echocardiography (2D) and color Doppler abdominal ultrasonography were normal. Flow cytometry was done on a peripheral blood sample. White blood cell (WBC) analysis was performed using the CD15 gating marker for neutrophils and the CD64 gating marker for monocytes. A significant PNH neutrophil clone was found using fluorescent aerolysin reagent (FLAER) and CD24, whereas a large PNH monocyte clone was detected using FLAER and CD14. Neutrophil clones (71%) and monocyte clones (77%) were identified [Figure 2].

Based on clinical, hematological, biochemical, and flow cytometry findings, a final diagnosis of PNH was established. The patient was reassured about the PNH and its potential complications, and he was encouraged to pursue allogeneic hematopoietic cell transplantation or complement inhibition with eculizumab. He was additionally referred to a hemato-oncologist and a bone marrow transplant specialist.

Discussion

In reality, PNH being neither paroxysmal nor nocturnal continues to exist and very few patients complain of about coloring of urine caused by hemoglobinuria. Unconjugated hyperbilirubinemia with recurrent jaundice and evidence of hemolysis has several possible diagnoses, with PNH being one of the rarest and characterized by a wide variety of vague symptoms.[2] Our case was the classic presentation of recurrent jaundice with altered colored urine in a 42-year-old man who had been misdiagnosed for an extended period of time. As per the International PNH register of 2012, the average age of all registered patients is 42 years, and the average duration is 4.6 years. The register includes patients aged 3–99 years.[3] The pathophysiology of PNH is linked to a gene mutation (PIGA) that stops cells from producing essential regulators of the complement cascade (CD55 and CD59) that protect host cells against hemolysis. The improper regulation of this system makes red blood cells vulnerable to complement-mediated lysis, resulting in hemoglobinuria and reddish-brown urine.[1] Hemoglobinuria is most often observed in the first urine sample collected in the morning due to a decrease in blood pH caused by hypoventilation during sleep, which facilitates hemolysis. Excess free hemoglobin in the blood caused by hemolysis, scavenges nitric oxide and increases gastrointestinal spasms, male erectile dysfunction, renal damage and thrombosis.[2]

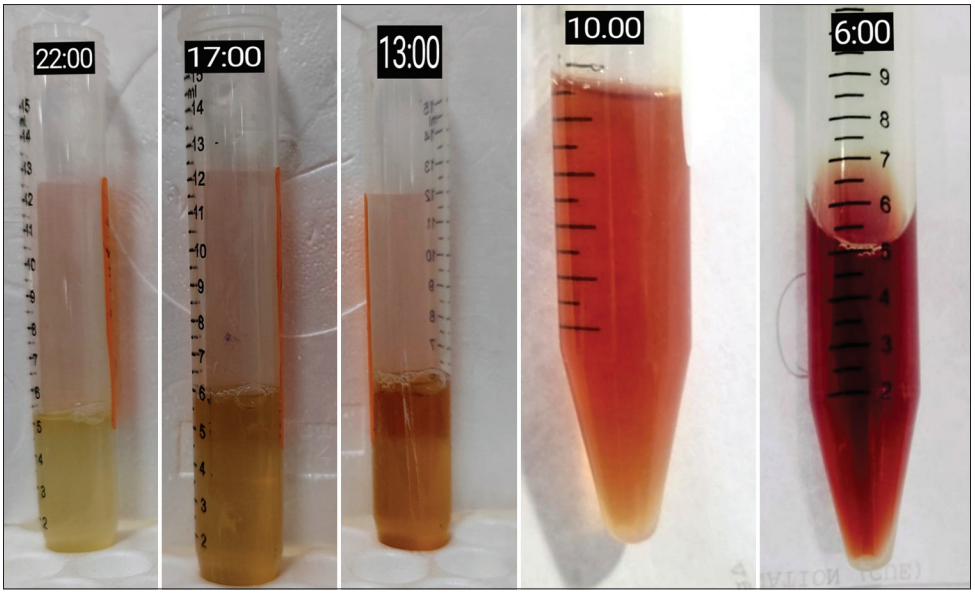

Figure 1: The series of tubes displayed the different hues of urine, ranging from dark cola in the morning (6:00) to pale yellow in the late evening (22.00)

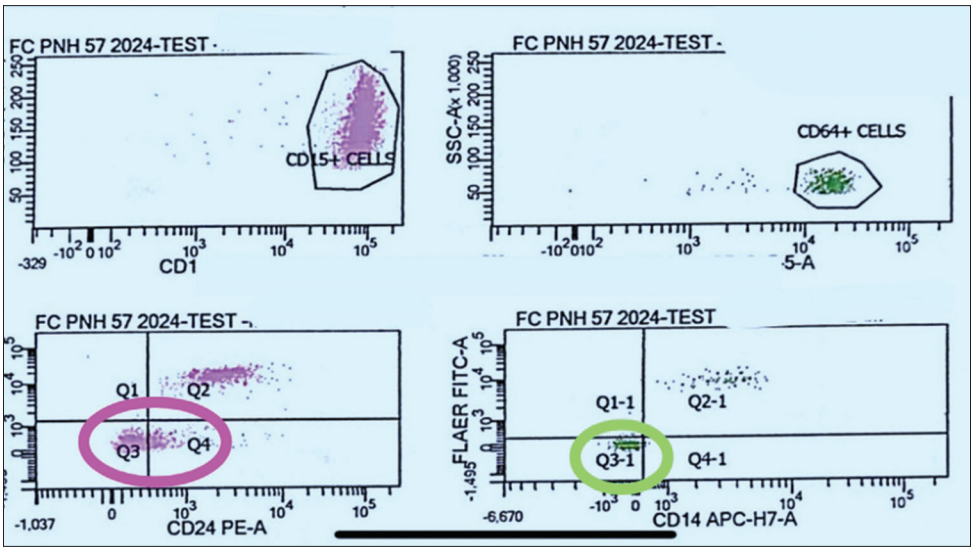

Figure 2: Illustration of the patient peripheral blood flowcytometry plots showed PNH neutrophil clone identified (pink circle) using fluorescent aerolysin reagent and CD 24 (77%). Monocyte clone (green circle) using FLAER and CD14 (71%)

The International PNH Interest Group divided PNH into three groups: classic PNH (the presence of hemolysis but cytopenia), PNH with marrow diseases (aplastic anemia/myelodysplastic syndrome/primary myelofibrosis), and subclinical PNH with the absence of clinical evidence.[4] In PNH, anemia has varied degrees of severity, hence, reticulocytes may vary, and peripheral blood films are not diagnostic. The presence of hemoglobinuria and hemosiderin is a useful clue for intravascular hemolysis. Serum LDH levels are significantly high, and low serum iron is prevalent due to persistent iron loss from hemoglobinuria.[2] Our case was classified under the classic PNH category due to evidence of intravascular hemolysis, such as high reticulocytosis, high LDH, and altered color urine. Differential diagnoses for acute hemolysis include megaloblastic anemia with hemolytic element, malaria, immune and nonimmune hemolytic anemias, hemoglobinopathies, heriditary anemias, medication or toxin-induced anemias, and bone marrow abnormalities. A delay in diagnosing PNH results in persistent chronic hemolysis, leading to kidney failure, vasospasm, and thrombosis.[5,6] Mitchell *et al*. conducted a survey of 163 cases with PNH in 2017 and found that the majority were diagnosed within the 1st year of symptom onset (38% in 1 year, 24% between 1 and 2 years) with the remaining 37% diagnosed more than 2 years later.[7]

Diagnostic flow cytometry method involves using monoclonal antibodies and a reagent called FLAER to attaches to GPI-anchored protein directly, specifically their glycan component. This test evaluates GPI-anchored proteins, including CD55 and CD59, with good sensitivity and specificity. The PNH granulocyte percentage is often considered the best way to assess the PNH clone size since white cells, unlike red cells, are least affected by hemolysis and transfusion.[8] Thrombosis, particularly venous thrombosis (intra-abdominal and cerebral), is the major cause of substantial morbidity and mortality in this disease. Imaging studies, such as echocardiography to measure pulmonary hypertension and Doppler abdominal ultrasound to assess intraabdominal flow, play an important role in such cases.[9] Our case eventually was identified using peripheral blood flow cytometry tests, and his imaging study revealed no signs of thrombosis.

Current therapy for PNH with severe hemolysis primarily consists of medicines that target alternative complement pathways, such as eculizumab and ravulizumab. Eculizumab, a humanized monoclonal antibody against complement protein C5, is administered every 2 weeks. If PNH is accompanied by aplastic anemia or myelodysplasia, allogeneic hematopoietic stem cell transplantation is the chosen treatment. Blood transfusions, iron supplements, and steroids were used as supportive forms. The primary issue with both treatment approaches is high expense, which reduces patients' quality of life.[10]

Conclusions

To conclude, PNH is a rare and often overlooked type of hemolytic anemia. It is essential to consider the possibility of PNH, particularly in cases of coombs negative hemolytic anemia. Flow cytometry has been recognized as definite method for diagnosing PNH and also helps in initiating early prophylaxis for its associated complication. Allogeneic hematopoietic stem cell transplantation and complement inhibitors have proven efficacy in treatment, however, high

Table 1: Laboratory data

Lab parameters	Test results	Biological reference interval
Hemogram		
Hemoglobin	12.2	12–15 g %
Packed cell volume	39	36%–46%
MCV	105	83–101 fL
MCH	33	37–32 pg
MCHC	31.6	31.5–34.5 g%
White blood cells	7040	4000–11,000/mm^3
Platelets	1.5	1.5–4.1 lakhs/mm^3
ESR	110	0–15 mm 1 h
Reticulocyte count	22	0.2%–2.5%
PT/INR	14.9/1.1	12.6–14.6 s/<1.3
APTT	33.3	36.7–32.0 s
Liver function tests		
Total bilirubin	3.4	0.2–1.3 mg/dL
Conjugated	0.3	0–0.3 mg/dL
Unconjugated	3.1	0–1.0 mg/dL
Alkaline phosphatase	79	38–126 U/L
SGOT	208	14–60 U/L
SGPT	53	0–35 U/L
Total proteins	7.4	6.3–8.5 g/dL
Albumin	4.5	3.5–5.0 g/dL
Globulin	2.9	2.3–3.5 g/dL
Serum LDH	4663	120–246 U/L
Serum creatinine	0.6	0.7–1.2 mg/dL
Random plasma glucose	122	70–150 mg/dL
Serum TSH	2.1	0.27–4.2 uIU/mL
Serum iron	127	37–170 μg/dL
TIBC	274	265–497 μg/dL
Trasferrin saturation	46.3	13%–45%
G6PD assay	19.0	4.6–13.5 U/g Hb
Serum haptoglobin	02	30–200 mg/dL

PT=Prothrombin time, APTT=Activated partial thromboplastin time, MCV=Mean corpuscular volume, MCH=Mean corpuscular hemoglobin, SGOT=Serum glutamic-oxaloacetic transaminase, SGPT=Serum glutamic pyruvic transaminase, LDH=Lactate dehydrogenase, TSH=Thyroid-stimulating hormone, G6PD=Glucose-6 phosphatase dehydrogenase, ESR=Erythrocyte sedimentation rate, MCHC=MCH concentration, TIBC=Total iron-binding capacity, INR=International normalized ratio

costs and limited accessibility contribute to increased morbidity and mortality, especially in developing countries.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patient has given his consent for his images and other clinical information to be reported in the journal. The patient understands that his name and initials will not be published and due efforts will be made to conceal his identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

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