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Case Report (Open Access)

A CASE OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PRESENTING WITH APLASTIC ANEMIA

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ABSTRACT
Paroxysmal nocturnal hemoglobinuria(PNH) is an aquired hemolytic anemia characterized by a triad of intravascular hemolysis,pancytopenia and tendancy for thrombosis.Several episodes of intravascular hemolysis result in hemoglobinuria associated with thrombosis at unusual sites and these patients may have bone marrow failure which is seen in our case.PNH presents with variable presentation,including classical PNH and PNH with aplastic anemia. Diagnosis can be confirmed by flow cytometry. In some cases,bone marrow studies show hypercellular marrow and in other we see a severely aplastic bone marrow with clinical features of PNH.Aplastic anemia is a rare association seen in about 15-25% of the cases.The underlying pathogenesis is the presence of disease specific signature T-cell clones in aplastic anemia.Management is supportive with blood transfusion,danazol therapy and treatment of thrombosis.With evolution of treatment strategies,hemopoitic stem cell transplantation and complement inhibition with Eculizumab have been shown to be very effective. Herein we report a young male who presented with yellowish discolouration of eyes, passage of red coloured urine, passage of blood in stools, pain in abdomen associated with generalised weakness. On examination, hepatosplenomegaly was present along with pancytopenia and evidence of intravascular hemolysis.With these clinical and laboratory findings,we suspected paroxysmal nocturnal hemglobinuria which was later confirmed by flow cytometry. Histopathological examination of bone marrow was suggestive of hypoplastic marrow with reticulum cells and mast cells,phagocytes,plasma cells in opposition to a capillary and dyserythropoisis which is the gold standard for diagnosis of aplastic anemia. PNH with aplastic anemia is a uniformly fatal disease if left untreated. Thus, when a young patient presents with coomb`s negative hemolytic anemia, one must suspect PNH. Short course of androgenic synthetic steroids is helpful in the treatment to prevent further progression to fatal complications. Thus,early recognition with bone marrow failure is necessary to prevent further progression to myelodysplasia or leukemia.

Keywords: *Aplastic Anemia,Paroxysmal Nocturnal Hemoglobinuria,Pancytopenia,Danazol*

INTRODUCTION
Paroxysmal nocturnal hemoglobinuria (PNH) is an acquired hemopoietic disorder which is a rarity in occurrence. Available reports suggest that the incidence of clinically significant disease is in the range of 1 to 10 cases per million population with an incidence of about 15-25% cases developing aplastic anemia. It is chiefly a disease of adults and the peak age of onset is in third decade.PNH is caused by mutation of a PIG-A gene on X chromosome and it affects males and females equal.This disease is classified under acquired hemolytic anemia presenting with clinical features of unexplained hemolytic anemia like fatigue, jaundice and red colored urine. Thrombosis involves venous rather than arterial system.On bone marrow studies,it is observed that there is decreased cellularity with at least 2 or 3 peripheral blood cytopenias in aplastic anemia-PNH(AA-PNH),whereas anemia only and/or thrombosis at diagnosis is seen in majority

of classic PNH patients.The presence of antigen specific T cells is reflected by their contribution to the expansion of a particular variable beta chain subfamily(signature T cell clones) and also by clonal CDR3 skewing. AA-PNH may progress to myelodysplasia and leukemia if left untreated. Accurate diagnosis is important as effective therapies have become available. We present a 27 years old male with combination of symptoms and signs that made us to diagnose this rare disorder with bone marrow failure and also we have discussed the management of this patient.

CASE

A 27-year-old male presented with complaints of yellowish discolouration of eyes since 5 days; passage of red coloured urine, blood in stools, abdominal pain and generalised weakness for 2 days. There was no history of similar complaints in the family members. On examination he was afebrile, pulse rate-84/ min, blood pressure-118/76mm Hg, marked pallor and icterus (Figure 1) was present. Cardiovascular ,respiratory and CNS examination were normal. On per abdomen examination, diffuse tenderness was present with Grade 1 hepatomegaly and grade 2 splenomegaly.Initial laboratory tests (Table 1) revealed pancytopenia, raised LDH, elevated bilirubin along with raised SGOT, negative direct and indirect coombs test and raised serum creatinine. Peripheral blood smear was suggestive of anisopoikilocytosis with raised MCV. As PBS was inconclusive of the possible type of anemia,serum iron, TIBC and serum vitamin B12 levels were done, which were within normal limits. Urine analysis was suggestive of 8-10 RBCs,amorphous material,hemoglobinuria and hemosiderinuria. At this point of time,we came to a conclusion that our patient had intravascular hemolysis,with raised LDH and pancytopenia. These features made us to suspect PNH. 24 hours urine sample collection showed reddish brown colour throughout (Figure 2). Ultrasonography (USG), arteriography with venography of abdomen and pelvis was normal. Hence,two criterias were fulfilled out of the triad of PNH. Prussian blue test on urine for Hemosiderin was positive.We did Ham`s acid serum test as a screening test for PNH which came to be positive. In order to confirm the same, flow cytometry was performed using gating antibodies CD45,CD33,CD235a and GPI linked antibodies CD59,CD157 as well as fluorescent aerolysin (FLAER) which was suggestive of PNH clones identified of WBCs (Monocytes-80.5% and Granulocytes-90.3% i.e,CD157 deficiency) and RBCs (Type 3-18.1% i.e,complete CD59 deficiency and Type 2-2.4% i.e,Partial CD59 deficiency). With the clinical features and a positive flow cytometry,final diagnosis of paroxysmal nocturnal hemoglobinuria was made. Bone marrow aspiration and biopsy was done to rule out bone marrow failure. Histopathological examination was suggestive of hypoplastic marrow with reticulum cells and mast cells, phagocytes, plasma cells in opposition to a capillary and dyserythropoisis i.e, asynchrony of maturation between nucleus and cytoplasm,mitotic abnormalities,multinuclearity,nuclear lobulation,internuclear bridging and megaloblastosis, which are the gold standard criteria for aplasic anemia. Patient was started on Cap.Danazole 200 mg twice a day,Tab.Prednisolone 20 mg once a day for 4 weeks along with vit.B12 and Folic acid supplement. He was also given three units of packed cell transfusions. As a definitive therapy,patient was advised bone marrow transplantation and furthur workup regarding the same. Patient’s complete blood count improved by then and passage of normal colored urine was observed 7 days after starting the treatment (Figure 3). He was discharged with the treatment to be continued. On subsequent follow-up patient was asymtomatic and hemogram showed well preserved hemoglobin, leukocyte and platelet counts.When patient has been followed up for almost 8months, we found that he had persistent pancytopenia, without hematuria, malena, renal dysfunction or features suggestive of thrombosis.

DISCUSSION

We report a young male who presented with hematuria, jaundice, abdominal pain and malena with history of similar episodes in the past was found to have pancytopenia and evidence of intravascular hemolysis. Diagnosis as PNH was suspected by clinical features, laboratory investigations and confirmed by flow cytometry. In PNH there is complement induced lysis of RBCs due to the abnormal sensitivity of RBC cell membrane. This is due to an acquired defect in the gene for phosphatidylinositol class A (PIG A) thereby causing deficiency of glycosylphosphatidylinositol (GPI) which is sheet anchor for cell

membrane proteins (Brodsky, 2014) .CD55 and CD59, complement regulatory proteins which block intravascular and extravascular hemolysis respectively in normal human, are deficient in PNH (Pu and Brodsky, 2011). Hemolysis occurs in PNH because these patient's RBCs lack GPI anchor which is

Table 1:Outside reports

Hemoglobin	5.5 g/dL%
Total leucocyte count	3600
Platelets	1.2 lakhs/mm3
Total protein	7.0g/dl
Albumin	3.9g/dl
Globulin	3.1g/dl
Total bilirubin	6.7mg/dl
Direct	1.4mg/dl
SGOT	120 IU/L
SGPT	320 IU/L
ALP	110 IU/L
Urine analysis	
Proteins	+
RBCs	25-30
Bile salts,Bile pigments	absent
Casts	absent

Figure 1: **Demonstration of icterus**

Figure 2: 24 hours urine sample collection

Table 2:Laboratory investigations on admission

Hemoglobin	4.3g/dl
Total leukocyte count	3090
Platelets	90000/mm3
Mean corpuscular volume	104.8fl
Mean corpuscular hemoglobin concentration	31.2g/dl
Differential count	neutrophils 40%,lymphocytes50%, eosinophils4%,monocytes6%
Peripheral blood smear	anisocytosis++,predominently macrocytes(round and oval)+microcytic with hypochromic+few tear drop cells, few pencil cells,schistocytes,spherocytes Lecopenia,no hypersegmented neutrophils
Coomb`s test(direct/indirect)	Negative
Serum creatinine	2.2mg/dl
Total bilirubin	6.5mg%
Direct bilirubin	0.7mg%
SGOT	568 IU/L
SGPT	47 IU/L
Alkaline phosphatase	28 IU/L
Retic count	3.12%
Serum Lactate dehydrogenase	5092 IU/L
Serum iron	124mcg/dl (normal)
Total iron binding capacity	390mcg/dl (normal)
Serum vit.B 12 levels	normal
Prothrombin time	14.4 sec
International normalised ratio	1.2
Urine analysis	protein 2+,RBCs-8-10,granular casts ++, Amorphous material++,hemoglobinuria+, hemosiderinuria+
Pruccian blue test on urine	positive
Bone marrow biopsy	hypocellular bone marrow likely suggestive of aplatic anemia

Figure 3: No evidence of hematuria 7 days after starting the treatment

required to attach CD55 [decay accelerating factor (DAF)] and CD59 (membrane inhibitor of reactive lysis [MIRL]) to the surface of RBC (Pu and Brodsky, 2011). This permits unregulated formation of certain complement attack complex which damages RBC membrane resulting in intravascular hemolysis. This causes reduction in hemoglobin and hemoglobinuria with resultant increase in LDH (Brodsky, 2014). The clinical features of PNH result from high levels of complement-mediated lysis of PNH clones as well as the intravascular release of hemoglobin (Brodsky, 2014). The latter is associated with kidney dysfunction and depletion of nitric oxide, which plays a role in smooth muscle function. PNH is broadly characterized by hemolytic anemia, thrombosis, and bone marrow hypocellularity. Patient symptoms may include fatigue, shortness of breath, bruising or bleeding, headaches, chest or abdominal pain, pulmonary hypertension, erectile dysfunction, and bouts of dark urine. Thrombosis is the leading cause of death in patients with PNH (Pu and Brodsky, 2011). The pathogenesis is hypothesized to be due to free hemoglobin resulting from hemolysis attracts nitric oxide which induces vasoconstriction and damages the vascular endothelium forming a nidus for thrombus formation. Also platelets release procoagulant particles during complement induced hemolysis, which facilitate thrombosis. Thromboses involve the venous rather than the arterial system (Pu and Brodsky, 2011). Minority of patients develop pancytopenia due to bone marrow disorders like aplastic anemia or primary myelofibrosis. PNH is classified into classic PNH (presence of hemolysis with no marrow abnormality), PNH with marrow disorders(aplastic anemia/myelodysplastic syndrome (MDS)/primary myelofibrosis (PMF) and subclinical PNH–without clinical evidence (Parker *et al*., 2005). Aplastic anemia arises from bone marrow failure that encompasses all 3 blood cell lineages, leading to peripheral pancytopenia and marrow hypoplasia. Most commonly presents in between the ages of 15 and 25 years, with a second, smaller peak of incidence after 60 years of age (Biswajit *et al.,* 2012). In older patients, aplastic anemia tends to be associated with more severe symptoms. The incidence of aplastic anemia is approximately 15-25% with PNH. The relationship between PNH and aplastic anemia has been proposed to arise from partially overlapping etiologies (Luzzatto, 2016). Aplastic anemia arises from a T-cell–mediated autoimmune attack against hematopoietic stem cells directed specifically at the GPI anchor, as well as other molecules.AA is thought to be an organ specific autoimmune disease,in which T cells cause damage to the hematopoitic stem cells which is also true for PNH. Thus, PNH results from the combined action of two factors: failure of normal hematopoisis and massive expansion of PNH clone.Expansion is associated with negative selection against GPI positive cells by GPI specificT cells. Thus, PNH is a prime example of a clonal disease that is not malignant.Because of the underlying autoimmunity, immunosuppressive therapy is effective for treatment of both conditions. It is important to test for PNH in patients with aplastic anemia, not only to establish the presence of PNH clones, but also because the presence of PNH cells is associated with a superior response to immunotherapy. For AA- PNH,it is important to monitor levels of PNH cells every 6 months to provide early evidence of clonal expansion. Expansion of the PNH clone may be accompanied by intravascular hemolysis. Persistent intravascular hemolysis causes anemia, hemoglobinuria, and other complications. Breakdown of the red blood cells commonly occurs during the

night, with ongoing concentration of the urine leading to dark, cola-colored urine in the morning. The diagnosis of PNH can be suspected when we come across cases of coombs negative hemolytic anemia or confusing cases of pancytopenia. The established therapies for patients with classical PNH are allogeneic hematopoietic cell transplantation (HCT) and complement inhibition with eculizumab (Parker *et al*., 2005). Patients with hemolysis are better managed with danazol and eculizumab (Hill *et al*., 2010). Patients with thrombosis are managed with therapeutic anticoagulation and eculizumab. Allogeneic HCT is advised for patients with severe cytopenias, patients with poor response to eculizumab or when not accessible to eculizumab (Parker *et al*., 2005). Supportive therapy includes red blood cell (RBC) transfusions, supplemental iron and folic acid (1 to 2 mg daily). Our patient had features of PNH with bone marrow failure i.e.pancytopenia, hemolysis and aplastic anemia. Renal dysfunction was evident from passage of cola coloured urine not only in the morning but throughout the day for several days with raised serum creatinine level.We learn that it is difficult to diagnose this disease unless we have a high index of suspicion. We present this case due to its rarity and need for early recognition of AA-PNH.

CONCLUSION

In this case report, a young male who presented to us with jaundice,abdominal pain,hematuria,pancytopenia and history of similar episodes in the past was found to have aquired hemolytic anemia with renal dysfunction and bone marrow failure. Our case report suggests that when a young patient presents with coomb`s negative hemolytic anemia, one must suspect PNH. PNH with aplastic anemia is a uniformly fatal disease if left untreated.Short course of androgenic synthetic steroids is helpful in the treatment to prevent further progression to fatal complications.

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