Clinicopathological Profile of Paroxysmal Nocturnal Hemoglobinuria among Omani Patients: A Case Series

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ABSTRACT

We aimed to estimate the nature and prevalence of paroxysmal nocturnal hemoglobinuria (PNH) among Omani patients. We performed a retrospective review of all patients who were tested for PNH by flow cytometry at the Sultan Qaboos University Hospital, Muscat, between 2012 and 2019. Manifestations, treatment modalities, and outcomes were assessed. A total of 10 patients were diagnosed or were on follow-up for PNH (median age 22.5 years). Clinical manifestations included fatigue (80%) and anemia (70%). Six patients had classical PNH with hemolysis, three had PNH in the context of aplastic anemia, and one patient had subclinical PNH. The median total clone size (type II + III) for neutrophils was 95.5 (range: 1.5–97) (FLAER/CD24) and for monocytes was 91.6 (range = 0.04–99) (FLAER/CD14). Four patients had clone sizes > 50% at the time of diagnosis. The median follow-up period of the patients was 62 months (range = 8–204 months). One patient suffered thrombosis. Three patients were on immunosuppressant agents, five were initiated on eculizumab, and four had a bone marrow transplant. No deaths were reported in the cohort. The estimated average incidence of PNH among Omani patients was 1.5 per 5 000 000. PNH is rare in the Omani population. The predominant presentation is hemolytic anemia.

Paroxysmal nocturnal hemoglobinuria (PNH) is a rare, acquired hematopoietic stem cell disorder that manifests with hemolytic anemia, thrombosis, and bone marrow failure.[1] It arises as a consequence of a non-malignant clonal expansion of one or more hematopoietic stem cells with an acquired somatic mutation of the x-linked phosphatidylinositol glycan (*PIG-A*) gene that encodes for the glycosylphosphatidylinositol (GPI).[2–4] Other clinical manifestations include thrombosis in unusual sites, smooth muscle dysfunction, pulmonary hypertension, and chronic renal failure.[1] The natural history of PNH is variable and ranges from indolent to life-threatening.[5–7] In a significant number of patients, PNH is associated with aplastic anemia (AA), hence is thought to be a spectrum of one disease.[8]

PNH is classified into three groups: classical PNH which includes hemolytic and thrombotic patients who have evidence of PNH clones as detectable by flow cytometry testing in the absence of bone marrow failure disorder such as AA; PNH in the context of other bone marrow disorder, such as AA and myelodysplastic syndrome; and subclinical PNH, often observed along with another bone marrow disorder such as AA in which small PNH clones (< 10% neutrophils) are detected without clinical or laboratory evidence of hemolysis or thrombosis.[9] Varying degrees of bone marrow failure underlie all patients of PNH, leading to difficulty in classifying some patients.[1]

Flow cytometry is the gold standard for PNH diagnosis due to its sensitivity, specificity, ability to detect small PNH clones among neutrophils and monocytes, determine clone sizes, as well its capability to define the type of glycosylphosphatidylinositol-anchored protein (GPI-AP) abnormality based on the degree of GPI-APs deficiency in both partial (type II) and complete (type III).[9,10] The sensitivity and accuracy of flow cytometry in detecting PNH neutrophils, and monocytes can be further enhanced

through the use of fluorescein-labeled proaerolysin (FLAER) variant (Alexa Fluor® 488 Proaerolysin, Pinewood Scientific Services, Canada).[11–14]

The Sultan Qaboos University Hospital (SQUH) is a tertiary care reference hospital. It was the first in Oman to offer flow cytometry testing for PNH, in 2002. In 2015, the SQUH laboratory introduced a FLAER-based flow cytometry protocol based on published guidelines.[15] Here we describe the clinicopathological profiles, treatment modalities, and outcomes of the patients with detectable PNH clones in our center, and estimate the prevalence of this condition in Omani population.

Ethical approval was obtained from the ethics committee at the College of Medicine and Health Sciences at the SQUH (MERC # 1770). We retrospectively reviewed all the PNH flow cytometry tests conducted at SQUH between January 2012 and December 2019. Baseline characteristics, clinical manifestations, and laboratory investigations of all patients diagnosed with detectable PNH clones were summarized. Flow cytometry results were reviewed and the type and size of PNH clones were reported. Patients with detectable clones were further classified according to the International PNH Interest Group classification.[9]

CASE REPORT

During the eight-year study period, a total of 140 patients (62% male) were screened and PNH clones were detected in ten (7%) patients. Average incidence rate was estimated at 1.9 per 5 000 000 (range = 1–3.5 / 5 000 000). There were three patients diagnosed prior to 2012 and still on follow-up. The median age at the time of PNH diagnosis was 22.5 years. The median follow-up period was 62 months (range = 8–204). The details of all the ten patients are provided in Table 1.

Case one
A 21-year-old male presented with fatigue, epistaxis, and easy bruising. The patient was found to be anemic and thrombocytopenic. Bone marrow aspirate and biopsy confirmed severe AA. Stress test for Fanconi anemia was negative. Flow cytometry testing revealed a small PNH clone [Table 1] indicative of subclinical PNH. The patient was observed and had a spontaneous recovery with disappearance of PNH clone.

Case two
A 22-year-old male presented with fatigue, jaundice, pallor, and hemoglobinuria. The patient was found to have direct antiglobulin test (DAT) negative hemolytic anemia, moderate neutropenia, and severe thrombocytopenia. Bone marrow examination confirmed AA along with erythroid hyperplasia. Flow cytometry testing revealed a large PNH clone. The patient was managed with supportive transfusion, cyclosporin, and anti-thymocyte globulin (ATG) followed by eculizumab therapy, with a good response.

Case three
A 24-year-old male presented with epistaxis, fever, and pancytopenia. Bone marrow examination confirmed AA and flow cytometry confirmed the presence of a PNH clone. The patient was managed by supportive transfusion, tacrolimus, cyclophosphamide, and ATG. Due to poor compliance to tacrolimus therapy, the patient was maintained on blood product support.

Case four
A 23-year-old female presented during pregnancy with fatigue, pallor, hemoglobinuria, abdominal pain, and esophageal spasms. She was found to have DAT-negative hemolytic anemia, cytopenia as well as portal, splenic, and inferior vena cava thrombosis. Bone marrow examination revealed a hypercellular marrow with erythroid hyperplasia. Flow cytometry testing revealed a PNH clone. The patient was initially treated with supportive transfusion, steroids, and ATG, but then was commenced on eculizumab. She then underwent bone marrow transplantation (BMT) with complete resolution of the PNH clone.

Case five
A 25-year-old male presented with fatigue and abdominal pain. Investigations revealed cytopenia, and bone marrow examination confirmed severe AA. Flow cytometry revealed a detectable PNH clone. The patient was commenced on transfusion, steroids, tacrolimus, and cyclophosphamide before he underwent BMT complicated by chronic graft versus host disease (GVHD).

Case six
A 21-year-old female presented with fatigue, anemia, and hemoglobinuria. She was found to

Table 1: Details of Omani patients with detectable paroxysmal nocturnal hemoglobinuria (PNH) clones.

Variables	Case 1	Case 2	Case 3	Case 4*	Case 5	Case 6*	Case 7	Case 8	Case 9*	Case 10
Classification	sc-PNH	PNH-BM	PNH-BM	Classical PNH	PNH-BM	Classical PNH	Classical PNH	Classical PNH	Classical PNH	Classical PNH
Age at diagnosis, years	21	22	24	23	25	21	32	49	16	20
Gender	M	M	M	F	M	F	F	M	F	M
Laboratory results at time of diagnosis										
Hb, g/dL (11.5–15.5)	13.7	7.9	8.6	6.9	7.9	7.8	7.8	9.7	6.8	9.6
WBC × 10^{12}/L (2.2–10.0)	2	1.9	3	1.5	3.4	4.2	5.8	10.2	3.3	4.4
ANC × 10^9/L (1.0–5.0)	1	0.7	0.4	0.9	0.9	1.9	2.7	6.3	1.14	2.1
PLT × 10^9/L (150–450)	129#	19	11	91	21	124	125	167	44	186
Total bilirubin, mmol/L (0–17)	11	37	7	14	7	45	37	89	NA	8
LDH, U/L (135–225)	186	587	245	1168	355	3665	4408	4595	162	1474
Haptoglobin	NA	< 0.1	2.04	< 0.1	NA	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Flow cytometry findings										
Neutrophil PNH clone size (FLAER/CD24 deficiency)	0.6 (III)	96.3 (III)	2.3 (III)	NA	1.5 (III)	NA	96.0 (II)	95.0 (II+III)	NA	97.0 (II+III)
Monocyte PNH clone size (FLAER/CD14 deficiency)	0.6 (III)	91.6 (III)	3.0 (III)	NA	2.0 (II+III)	NA	98.4 (II)	94.7 (II+III)	NA	99.0 (II+III)
RBC PNH clone size and type (CD59 deficiency)	0.5 (II), 0.3 (III)	1.3 (II), 7.1 (III)	0.1 (III)	NA	0.3 (II)	NA	6.0 (II), 50.6 (III)	4.1 (II), 33.5 (III)	NA	38.0 (III), 2.0 (II)

*#Post platelet transfusion in referring institution. *Diagnosis was made prior to the instate of FLAER-based flow cytometry protocol using CD55/CD59 assessment on neutrophils, monocytes, and RBCs.*
Hb: hemoglobin; Retic: reticulocyte; WBC: white blood cell count; ANC: absolute neutrophil count; PLT: platelet count; LDH: lactate dehydrogenase; RBC: red blood cell; sc-PNH: subclinical PNH; PNH-BM: PNH in the context of other bone marrow disorder.

have DAT-negative hemolytic anemia and mild thrombocytopenia. Bone marrow examination revealed a normocellular marrow with erythroid hyperplasia and increased megakaryocytes. The patient was treated with supportive transfusion and underwent BMT, complicated by chronic GVHD and residual thrombocytopenia for which she was given anti-platelet therapy (eltrombopag).

Case seven
A 32-year-old female presented with fatigue, anemia, hemoglobinuria, and abdominal pain. She was found to have DAT-negative hemolytic anemia and a large PNH clone. The patient was initiated on supportive care and eculizumab therapy, with improved symptoms. However, she continued to have mild hemolysis, managed with intermittent transfusion.

Case eight
A 49-year-old male presented with fatigue, symptomatic anemia, hemoglobinuria, and kidney failure. The patient was found to have DAT-negative hemolytic anemia. Bone marrow examination revealed marked erythroid hyperplasia. Flow cytometry revealed a large PNH clone. The patient was treated by supportive transfusion and eculizumab with improved symptoms. However, he continued to have mild hemolysis, managed with intermittent transfusion support.

Case nine
A 16-year-old female presented with fatigue, anemia, jaundice, epigastric pain, and esophageal spasms. She was found to have DAT-negative hemolytic anemia and thrombocytopenia. Bone marrow examination revealed marked erythroid hyperplasia.

The patient was initiated on supportive transfusion and underwent BMT with complete resolution of her disease.

Case ten

A 20-year-old male presented with fatigue, anemia, hemoglobinuria, and abdominal pain. He was found to have DAT-negative hemolytic anemia. Bone marrow examination revealed marked erythroid hyperplasia and flow cytometry revealed a large PNH clone. He was commenced on supportive transfusion and was initiated on eculizumab treatment with a good response.

Diagnosed patients had variable cytopenias [Table 1]. The median hemoglobin (Hb), white blood cell count, absolute neutrophil count, and platelet count at time of presentation were 7.9 g/dL (interquartile range (IQR) = 1.6), 3×10^9/L (IQR = 7.4), 1.0×10^9/L (IQR = 1.2), and 125×10^{12}/L (IQR = 108), respectively. The median reported total type II + III clone size was 95.5 (range = 1.5–97.0) in neutrophils (FLAER/CD24) and 91.6 (range = 0.04–99) in monocyte (FLAER/CD14). The median red blood cell clone size was 22.9 (range = 0.1–56.6). Four patients had PNH clone sizes > 50% at the time of diagnosis.

DISCUSSION

To the best of our knowledge, this is the first report on a cohort of Omani patients with PNH. PNH is a rare disease and can be underdiagnosed considering the heterogenicity of the disease and variable clinical presentations. We report an estimated prevalence of 1.5 per 5 million among the Omani population. The median age of diagnosed patients in the current study is similar to what has been described in the literature.[16] In line with other reports,[17] the commonest symptoms in our PNH patients are anemia and fatigue. Many clinical manifestations of PNH are explained by Hb-mediated nitric oxide scavenging.[18] The clinical manifestations due to chronic hemolysis in classical PNH appear to be more common in patients with large PNH clones (> 60% neutrophils and monocytes).[7] This typically is associated with evidence of hemolysis with elevated serum lactate dehydrogenase (LDH) and reticulocyte count.[12]

While hemolytic anemia is a common manifestation, other patients may present with bone marrow aplasia. A study on the Saudi population showed 4% detectable PNH clones in tested patients, albeit 73% of them presented with AA.[19] In our cohort, more than half of the diagnosed patients had classical PNH with manifestations of DAT-negative hemolytic anemia and large PNH clones (> 60% neutrophils and monocytes). One of these patients presented with hemolytic anemia, aplastic marrow, and a large PNH clone, suggesting an overlap presentation. This has been observed in other series supporting the heterogeneity in the clinical phenotype of these patients.[20] In this cohort, one patient had a large PNH clone and an aggressive disease with hemolytic anemia and thrombosis involving portal, splenic, and superior mesenchymal veins. Previous data showed that the risk of thrombosis is greatest where PNH clones manifest > 50% in the neutrophils.[7] The mechanism through which hemolysis leads to thrombosis is multifactorial, and involves the toxic effect of circulating free Hb, heme, and iron. Therefore, elevated serum LDH levels and hemoglobinuria are risk factors.[17] Although venous thrombosis is more common, with hepatic vein thrombosis being the commonest, thrombosis can occur at any site.[1] The wide variations in the type and severity of symptoms in PNH patients can complicate the diagnosis which can be delayed or missed.[9]

Three patients in our cohort had small PNH clones (< 10% granulocytes and monocytes) in the context of underlying bone marrow aplasia. One of them had a clone size of < 1% at the time of diagnosis in keeping with subclinical PNH. These observations are in-line with published data showing that the clone sizes in patients with PNH in the context of AA are lower than in patients with classical PNH.[12] It has also been reported that up to 40% of PNH evolve from AA, and the subclassification in some patients might be challenging.[12] PNH clone size in the context of other primary bone marrow failure disorders is highly variable and the smallest clones are seen in patients with sub-clinical PNH.[12] These patients typically present with moderate to severe pancytopenia with low reticulocyte count, along with a normal or mildly elevated LDH level. It is recommended that patients with detectable clones should have their PNH clone size monitored at regular intervals, preferably every 6–12 months as some patients will experience further expansion of the clones and progress to classical PNH.[4]

For patients with classical PNH, complement inhibition with eculizumab and allogenic BMT is the only proven effective therapies. Our cohort had five patients who were treated with eculizumab. Eculizumab therapy reduces intravascular hemolysis leading to Hb stabilization, lower serum LDH levels, reduced fatigue, less transfusion requirement, and improvement in the patients' quality of life.[5] Most patients on eculizumab, however continue to experience mild to moderate extravascular hemolysis, mediated via C3d deposition on CD55 deficient PNH red cells, but not the normal red cells, leading to premature spleen removal and selective destruction.[12] The only curative therapy for PNH is BMT. In the current cohort, this was received by four patients, three of whom had classical PNH while one had subclinical PNH in the context of underlying bone marrow aplasia. BMT is recommended in patients with life-threatening cytopenias, disabling hemolysis, or thrombosis that is not controlled with eculizumab.

CONCLUSION

This retrospective study that reviewed patient records over an eight-year period is the first to report the prevalence of PNH among Omani patients. PNH is rare among Omanis and has variable presentations. Hemolytic PNH is the commonest followed by PNH in the context of bone marrow aplasia. In line with other publications, patients with larger PNH clones are associated with classical PNH symptoms and increased risk of thrombosis, even in patients with bone marrow failure, whereas smaller PNH clones are associated with bone marrow aplasia.

Disclosure
The authors declared no conflicts of interest.

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