Paroxysmal Nocturnal Haemoglobinuria: A Case Series from Oman

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Abstract

Introduction

Paroxysmal nocturnal hemoglobinuria (PNH) is a rare acquired stem cell disorder that manifests by hemolytic anemia, thrombosis and cytopenia. There are no data on PNH among Omani patients.

Methods

We performed a retrospective review of all patients tested for PNH by flow cytometry at the Sultan Qaboos University Hospital between 2012 and 2019. Manifestations, treatment modalities and outcomes were assessed.

Results

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%). There were six patients who had classical PNH with evidence of hemolysis, three patient had PNH in the context of aplastic anemia, and one patient with subclinical PNH. The median reported total type II+III clone size was 95.5 (range 1.54-97) in neutrophils (FLAER/CD24) and 91.6 (range 0.036-99) in monocytes (FLAER/CD14). There were four patients who were found to have a clone size > 50% at time of

diagnosis. The median follow up of the patients were 62 months (range: 8-204). One patient suffered thrombosis. Three patients were on immunosuppressant agents, five were initiated on eculizumab and four had bone marrow transplant. No deaths were reported in the cohort. Estimated average incidence rate was 1.5 per 5,000,000.

Conclusion

PNH is rare in the Omani population with predominance presentation with hemolytic anemia.

Keywords: Flow cytometry, granulocytes, Quantification, Aplastic anemia, Paroxysmal Nocturnal Hemoglobinuria

Introduction

Paroxysmal nocturnal hemoglobinurea (PNH) is a rare, acquired hematopoietic stem cell disorder that manifests with haemolytic 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 phosphatidyl-inositol glycan (PIG-A) gene that encodes for the glycosyl phosphatidylinositol (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 an indolent disease 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 in the absence of bone marrow failure disorder, *PNH in the context of other bone marrow disorder*, such as AA and myelodysplastic syndrome and *subclinical PNH*, often observed in the setting of another bone marrow disorder such as AA in which small PNH clones are detected without clinical or laboratory evidence of hemolysis or thrombosis (9). Varying degree of bone marrow failure underlie all patients of PNH, leading to difficulty in classifying some patients (1).

Flowcytometry is the gold standard in PNH diagnosis due to its sensitivity, specifity and advantage of detecting small PNH clones, determining clone size, and in defining the type of GPI-AP abnormality based on the degree of GPI-APs deficiency; partial (type II) or complete (type III) (9,

10). The sensitivity and accuracy in detecting PNH neutrophil and monocytes has further been achieved through the use of fluorescein-tagged proaerolysin variant (FLAER, Alexa Fluor® 488 Proaerolysin, Pinewood Scientific Services, Victoria, BC, Canada) (11-14).

The Sultan Qaboos University Hospital (SQUH) is a tertiary care reference center that is the first to establish flow cytyometry testing for PNH since 2002. In 2015, the laboratory introduced a FLARE-based flowcytometry protocol based on published guidelines (15). Herein, we aim to describe the clinicopathological profile of the patients with detectable PNH clones in our center, and report treatment modalities and outcomes .

Methods

Ethical approval was obtained from the ethics committee at the College of Medicine and Health Sciences at the Sultan Qaboos University Hospital (MERC # 1770). A retrospective review of at all PNH flow cytometry tests performed at SQUH between January 2012 and December 2019 was performed. Baseline characteristics, clinical manifestations and laboratory investigations of all patients 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 IPIG classification (9).

Results

During the 8-year study period, a total of 140 samples were screened for PNH, 62% of which were males. PNH clones were detected in 7 patients (5%). Average incidence rate was estimated at 1.9 per 5,000,000 (range 1 -3.5 per 5,000,000). There were three patients who were diagnosed prior to 2012 and were on follow up. The median age at time of PNH diagnosis was 22.5 years. The median follow up period was 62 months (range: 8-204).

Case 1 is a 21 years old male who presented with fatigue, epistaxis and easy bruise-ability. The patient was found to be anemic and thrombocytopenic. Bone marrow aspirate and biopsy confirmed severe aplastic anemia. Stress test for Fanconi anemia was negative. Flow cytometry testing revealed a small PNH clone (Table) in keeping with subclinical PNH. The patient was observed and had a spontaneous recovery and disappearance of PNH clone.

Case 2 is a 22 years old male who presented with fatigue, jaundice, pallor and hemoglobinuria. The patient was found to have DAT negative hemolytic anemia. In addition, the patient had moderate neutropenia and severe thrombocytopenia. Bone marrow examination confirmed aplastic anemia along with erythroid hyperplasia. Flow cytometry testing revealed a large PNH clone. The patient was managed with supportive transfusion, cytclosporin and anti-thymocyte globulin (ATG) before he was commenced on Eculizumab therapy with good response.

Case 3 is a 24 years old male who presented with epistaxis, fever and pancytopenia. Bone marrow examination confirmed aplastic anemia and flow cytometry confirmed presence of a PNH clone. The patient was managed by supportive transfusion, tacrolimus, cyclophosphamide and ATG. The patient had poor compliance to tacrolimis therapy and was maintained on blood product support.

Case 4 is a 23 years old female who presented during pregnancy with fatigue, pallor, hemoglobinuria, abdominal pain and oesophageal spasms. She was found to have DAT-negative hemolytic anemia, cytopenia and 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 Ecluzimab. She then underwent a bone marrow transplantation with complete resolution of PNH clone.

Case 5 is a 25 years old male who presented with fatigue and abdominal pain. Investigations revealed cytopenia and bone marrow examination confirmed severe aplastic anemia. Flowcytometry revealed detectable PNH clone. The patient was commenced on transfusion, steroids, tacrolimus and cyclophosphamide before he underwent bone marrow transplantation complicated by chronic graft versus host disease (GVHD).

Case 6 is a 21 years old female presented with fatigue, anemia, hemolglobinurea and was found to have DAT negative hemolytic anemia. The patient also had a mild thrombocytopenia. Bone marrow examination revealed a normocellular marrow with erythroid hyperplasia and increased megakaryocytes. Patient was treated with supportive transfusion and underwent bone marrow

transplantation, complicated by chronic GVHD and residual thrombocytopenia for which she was give anti-platelet therapy (Eltrombopag).

Case 7 is a a 32 years old female presented with fatigue, anemia, hemoglobinura and abdominal pain. The patient was found to have DAT negative hemolytic anemia and a large PNH clones. The patient was initiated on supportive care and Ecluzimab therapy, with improved symptoms. However, she continued to have mild hemolysis, managed with intermittent transfusion.

Case 8 is a 49 years old male who 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 and flow cytometry revealed a large PNH clone. The patient was treated by supportive transfusion and Eculizumab with improved symptoms. However she continued to have mild hemolysis managed with intermittent transfusion support.

Case 9 is a 16 years old female who presented with fatigue, anemia, jaundice and 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 a bone marrow transplantation with complete resolution of her disease.

Case 10 is a 20 years old male who 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 flowcytometry revealed a large PNH clone. He was commenced on supportive transfusion and was initiated on Eculizumab treatment with good response.

Diagnosed patients had variable cytopenias (Table). The median haemoglobin, White blood cell count, absolute neutrophil count and platelet count at time of presentation were 7.9 g/dl (Interquartile range [IQR] =1.55) , $3x10^9$/L (IQR= 7.4), $1.0x10^9$/L (IQR =1.15) and $125x10^{12}$/L (IQR =108) respectively. The median reported total type II+III clone size was 95.5 (range 1.54-97) in neutrophil (FLAER/CD24) and 91.6 (range 0.036-99) in monocyte (FLAER/CD14). The

median red blood cell (RBC) clone size was 22.9 (range 0.1-56.6). There were four patients who were found to have PNH clone size > 10%, all of which were with clone size > 50% at time of diagnosis.

Discussion

To the best of our knowledge, this is the first cohort of Omani PNH patients reported. 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, the commonest signs and symptoms reported in our PNH patients are fatigue and anemia (17). Many of the clinical manifestations of PNH are explained by hemoglobin-mediated nitric oxide (NO) 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 & monocytes) (7). This typically is associated with evidence of haemolysis with elevated serum LDH and reticulocyte count (12).

Similar to the findings in our study, a study on the Saudi population showed 4% detectable PNH clones in tested patients, albeit 73% of which presented with aplastic anemia (19). Majority of our patients had hemolytic PNH. That said, it is possible that there are other patients that are under diagnosed considering the heterogenicity of the disease and variable clinical presentation. Considering that the number, severity and type of symptoms vary widely, this can complicate the diagnosis which can be delayed or missed (9).More than half of the diagnosed patients were in the classical PNH group with manifestations of DAT-negative hemolytic anemia, and large PNH clones (> 60% neutrophils & monocytes). One of these patients is a patient who presented with hemolytic anemia, an aplastic marrow and a large PNH clone suggesting an overlap presentation. This observation has been observed in other series supporting the heterogeneity in the clinical phenotype of these patients (20). In this cohort, we report one patient with large PNH clone who had an aggressive disease presenting with hemolytic anemia and thrombosis including portal, splenic and superior mesenchemal veins. Previous data showed that risk of thrombosis is greatest with the presence of PNH clones >50% in the neutrophils (7). The mechanism through which hemolysis leads to thrombosis is multifactorial, and involve 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 in any site (1).

Our cohort included three patients with small PNH clones (< 10% granulocytes & monocytes) in the context of underlying bone marrow aplasia. One of these had a clone size that is < 1% at time of diagnosis in keeping with subclinical PNH. These observations are in-line with published data showing that the clone size 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 disorder is highly variable and smallest clones are seen in patients with sub-clinical PNH (12). These patients typically present with moderate to severe pancytopenia with low reticulocyte count, 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 to 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 (Soliris ®; Alexion Pharmaceuticals, Inc., Cheshire, CT, USA) and allogenic bone marrow transplant are the only proven effective therapies. Our cohort had five patients who were treated with eculizumab. This results in reduced intravascular hemolysis leading to Hb stabilization, decreased serum LDH levels, reduced fatigue, transfusion requirements 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 bone marrow transplantation; which was performed in four patients, three of which had classical PNH while one had subclinical PNH in the context of underlying bone marrow aplasia.. Bone marrow transplant is recommended in patients with life-threatening cytopenias, disabling hemolysis or thrombosis that is not controlled with eculizumab.

Conclusion

In conclusion, this retrospective study over 8 year period represent the first reported cohort of PNH patients among Omani patients. 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 being 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.

Statement of Ethics: Ethical approval was obtained from the ethics committee at the College of Medicine and Health Sciences at the Sultan Qaboos University Hospital (MERC # 1770).

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Authors' contributions

AZR initiated the idea of the study, obtained ethical approvals and wrote the manuscript. YK and AZR collected the clinical details and flow cytometry findings on the patients. JQ performed the flow cytometric analysis. AZR, NF, SM and JQ were involved in the development of the PNH flow cytometry protocols and reporting the flow cytometry tests. MK, MH and SK are patients' care givers. All authors approved the manuscript before submission.

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Table 1: Demographics and symptomatology of Omani patients with detectable PNH clones

	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 investigation 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	6.76	9.6
WBC $x10^{12}$/L (2.2-10.0)	2	1.9	3	1.5	3.4	4.2	5.8	10.2	3.3	4.4
ANC $x10^{9}$/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 $x10^{9}$/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	<0.06	NA	<0.06	<0.1	<0.1	<0.06	<0.1
Flow cytometry findings										
Neutrophil PNH clone size (FLAER/CD24 deficiency)	0.63 (III)	96.33 (III)	2.26 (III)	NA	1.54 (III)	NA	95.99 (II)	95 (II+III)	NA	97 (II+III)
Monocyte PNH clone size (FLAER/CD14 deficiency)	0.63 (III)	91.58 (III)	2.96 (III)	NA	1.98 (II+III)	NA	98.43 (II)	94.72 (II+III)	NA	99 (II+III)
RBC PNH clone size & type (CD59 deficiency)	0.46 (II), 0.30 (III)	1.32 (II), 7.08 (III)	0.1 (III)	NA	0.3 (II)	NA	6.03 (II), 50.59 (III)	4.08 (II), 33.46 (III)	NA	38% (III), 2% (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, PNH : paroxysmal nocturnal hemoglobinuria, sc-PNH: subclinical PNH, PNH-BM: PNH in the context of other bone marrow disorder