SHORT COMMUNICATION

# Clinico-Haematological Features of Paroxysmal Nocturnal Haemoglobinuria

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## Abstract

The aim of this study was to determine the frequency of various clinico-haematological features in patients suffering from paroxysmal nocturnal haemoglobinuria (PNH). It was an observational study carried out from October 2008 - January 2016. All the patients of PNH, diagnosed on the basis of clinical and laboratory findings and confirmed by CD55 and CD59 deficiency on red cells by means of flow cytometry, were included in the study. A total of 22 patients were diagnosed which included 18 (81.8%) males and 4 (18.1%) females. Median age was 27 years. Pallor, fever, fatigability and haemoglobinuria were the most common clinical features. Pancytopenia was seen in 13 (59.09%) and hypocellular marrow was found in 14 (63.6%) patients. One patient presented with Budd Chiari syndrome.

**Key Words:** *Paroxysmal nocturnal haemoglobinuria (PNH). Haemoglobinuria. Bone marrow aplasia. Haemolysis.*

Paroxysmal nocturnal haemoglobinuria (PNH) is an acquired clonal disorder of haematopoietic stem cell (HSC), resulting from somatic mutation in the PIGA gene located on the X-chromosome.[1] This gene is responsible for the synthesis of the glycosylphosphatidylinositol (GPI) moiety that anchors certain proteins to cell membrane. Mutation renders haematopoietic stem cells and their progeny deficient in all the GPI-anchored proteins. The complement-mediated intravascular haemolysis and the resulting haemoglobinuria, which are characteristic clinical features of PNH, are due to the deficiency of the GPI-anchored complement regulatory proteins, CD55 or decay accelerating factor (DAF) and CD59 or membrane inhibitor of reactive lysis (MIRL).[1]

Cardinal features of PNH include haemolytic anaemia, haemoglobinuria, thrombotic tendency and bone marrow (BM) failure.[1] Inclusion of flow cytometry for detection of PNH clone in patients with aplastic anaemia (AA) as part of routine work-up has blurred the traditional clinical definition of PNH. PNH clone has been detected in up to 50% of patients of AA with the help of flow cytometry. Many of these patients have very small size clones and they do not have haemoglobinuria.[2] In order to overcome the ambiguity, 'International PNH Interest Group' proposed a classification consisting of three categories: (1) classical PNH; (2) PNH in the context of AA or myelodysplastic syndrome, and (3) subclinical PNH.[1]

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*Received: May 06, 2016; Accepted: December 23, 2016.*

The aim of this study was to determine the frequency of various clinico-haematological features in patients diagnosed as PNH by means of flow cytometry in a tertiary healthcare setting.

This descriptive study was carried out at Army Medical College, Armed Forces Institute of Pathology, and Armed Forces Bone Marrow Transplant Centre, Rawalpindi from October 2008 to January 2016. Clinical and laboratory record of all the patients diagnosed as suffering from PNH during the period of study was retrieved. The diagnosis was made on the basis of history, clinical features, examination of peripheral blood, raised reticulocyte count, elevated lactate dehydrogenase levels, bone marrow examination, urine examination for haemosiderin, and flow cytometry of red blood cells for CD55 and CD59. Patients with history of recent blood transfusions were excluded.

The patients subjected to flow cytometry presented with haemoglobinuria, AA or unexplained thrombosis. CD55 and CD59 assay was performed using fresh peripheral blood red cells. Fifteen µl of blood diluted in buffered saline was used in each tube. Acquisition was set at 50,000 events for each tube. Percentages and frequencies were calculated for various qualitative variables. Mean and standard deviations were calculated for different quantitative variables.

A total of 22 patients with a median age of 27 years were diagnosed as suffering from PNH. Male to female ratio was 4.5. Frequencies and percentages of various clinical and laboratory features are shown in Table I. Mean and SD of the quantitative variables of PNH patients are summarised in Table II. Comorbidities were seen in 4, including 2 patients each with diabetes mellitus and essential hypertension.

PNH is a rare haemolytic disease characterised by the increased susceptibility of red blood cells to complement

**Table I:** Frequency and percentage of various clinical and laboratory features (n=22).

| Clinical features | Frequency and percentage |
|---|---|
| Male | 18 (81.8%) |
| Female | 4 (18.1%) |
| Fever | 18 (81.8%) |
| Pallor | 22 (100%) |
| Jaundice | 6 (27.2%) |
| Fatigability | 22 (100%) |
| Haemoglobinuria | 22 (100%) |
| Bleeding (Petechiae and purpura) | 3 (13.6%) |
| Pain abdomen | 2 (9.09%) |
| Thrombotic manifestations | 1 (4.5%) |
| Anaemia | 22 (100%) |
| Thrombocytopenia | 15 (68.2%) |
| Leucopenia | 9 (40.9%) |
| Pancytopenia | 13 (59.09%) |
| Hypocellular marrow | 14 (63.6%) |

**Table II:** Range, mean ±SD of the various haematological variables of PNH patients. Median with inter-quartile range (IQR) of variables with non-parametric distribution is also given (n=22).

| | Range | Mean ±SD / Median (IQR) |
|---|---|---|
| Haemoglobin (g/dl) | 2.5-12.5 | 7.47 +3.44 |
| WBC count ($x10^9$/l) | 1.4-9.5 | 5.34 +1.99 |
| Reticulocytes (%) | 0.2-12 | 6.06 +3.26 |
| Platelets ($x10^9$/l) | 15-363 | 126.6 (130) |
| CD55/CD59 deficient red cells (%) | 6-73 | 40 (139) |

mediated lysis. In the United Kingdom, its incidence has been estimated to be 0.13 cases/100,000.[3] However, international studies suggest that there might be an increased incidence of PNH in Asian countries, primarily due to high incidence of marrow aplasia.[4]

Most of our patients were male. In contrast to Western studies, Asian studies have shown male preponderance.[5,6] The median age of our patients was 27 years. This is less than the median ages reported in various European studies, i.e. 42, 38, 33 years narrated in British, Spanish and French studies, respectively.[6] This study also included 2 patients less than 15 years of age. PNH is considered rare in paediatric population.

Haemoglobinuria was seen in all of our patients. This corresponds with the fact that all our patients had appreciable proportion of CD55/CD59 deficient red cells. It is sometimes tricky to elicit the history of haemoglobinuria because dark colour urine is often mistaken for either choluria or haematuria. Moreover, the urine analysis strips generally do not differentiate between haemoglobinuria and haematuria.

Patients in this study, presented mostly with general symptoms of anaemia viz. pallor and easy fatigability. Jaundice was seen in 6 patients. Jaundice was not marked because haemolysis is mostly intravascular. Abdominal pain was seen in only 2 of our patients. It is attributed to haemolysis with release of free haemoglobin, which causes depletion of nitric oxide (NO). Endothelial cells are responsible for the synthesis of NO, which is important for smooth muscle tone. Depletion of NO causes deregulation of muscle tone and abdominal pain.[1]

Thrombotic complications were seen in only one of our patient. Thrombosis occurs more commonly as a complicating event than as a presenting feature. Other studies have also shown decreased incidence of thrombosis in Asians as compared to Europeans.[7] Fourteen (63.6%) of our 22 patients had various degree of hypocellularity on BM examination. Co-existence of the AA and PNH is not unusual. Patients of PNH with hypocellular marrow have more marked pancytopenia, smaller PNH clones, and lower reticulocyte counts.[1,6] An earlier study carried out in our institute showed the presence of a small PNH clone in one out of 20 patients of AA.[8]

Although flow cytometric screening for PNH is advised to all the patients with AA and unexplained thrombosis in our clinical setting, not all the patients comply due to the high cost of the test. These patients are lost to follow-up and may make the data less representative.

This study shows that most of the patients of PNH are adult males who present with the disease in the third decade of life. Symptoms of anaemia and haemoglobinuria are the most common clinical presentations. Cytopenias of different severity are seen in all the patients. Association of PNH with marrow hypoplasia is not unusual and seen in 2/3 of the patients. Thrombotic manifestations were rare in patients of PNH in this study.

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