Case report

Haematol J Bangladesh 2023;7(1):44-47
https://journal.hematologybd.org/index.php/haematoljbd
ISSN: 2523-1219 (print)
ISSN: 2707-1405 (online)

A Case Report: Autoimmune Haemolytic Anaemia & Paroxysmal Nocturnal Haemoglobinuria Association

Fatima- Tuz- Zohra[1], Abu Hasan Rubel[1], Mohd. Tariqul Islam[1], Md. Sirazul Islam[1]

[1]*Asgar Ali Hospital, Dhaka*

Citation: Zohra FT, Rubel AH, Islam MT, Islam MS. A Case Report: Autoimmune Haemolytic Anaemia & Paroxysmal Nocturnal Haemoglobinuria Association. Haematol J Bangladesh. 2023;7(1):44-47.

DOI: https://doi.org/10.37545/haematoljbd202391

Received: 15 February 2022

Accepted: 27 November 2022

Published: 20 March 2023

*Correspondence: Fatima- Tuz- Zohra. Specialist, Lab Medicine Department, Asgar Ali Hospital, Dhaka. Email: dr.mou.heart@gmail.com

ABSTRACT

Autoimmune haemolytic anaemia (AIHA) and paroxysmal nocturnal haemoglobinuria (PNH) are two distinct causes of haemolytic anaemia. They have different mechanisms that underpin their pathogenesis and, therefore, require different treatment strategies. The direct antiglobulin test (DAT) or Coombs' test is positive in cases of immune-mediated haemolytic anaemia and, thus, is positive in AIHA but negative in PNH. We report a case of a man presenting with a haemolytic anaemia who was found to have concomitant evidence of AIHA and PNH. The case highlights the importance of carrying out a comprehensive haemolysis work-up in patients who present with haemolytic anaemia.

Keywords: *Autoimmune haemolytic anaemia, Paroxysmal nocturnal haemoglobinuria, Glycosylphosphatidylinositol, Eculizumab, Coombs' test, Haemosiderinurea, PIGA.*

Introduction:

The hallmark of haemolytic anaemias is an increase in the rate of red blood cell (RBC) destruction. The destruction of the RBC occurs due to either an intracorpuscular defect or extracorpuscular defect.[1] Hereditary haemolytic anaemias are due to 'intracorpuscular' red cell defects whereas acquired haemolytic anaemias are usually the result of an 'extracorpuscular' defects. Paroxysmal nocturnal haemoglobinuria (PNH) is the exception because although it is an acquired disorder, PNH occurs due to an intracorpuscular red cell defect.[2] PNH is a rare, acquired genetic condition which is caused by the non-malignant clonal expansion of haemopoietic stem cells with somatic mutation of phosphatidylinositol glycan class A (PIGA), resulting in red cells being extremely sensitive to complement mediated lysis.[3] This case report presents a Coombs' positive haemolytic anaemia with PNH clone and cytopenia.

Case Report:

A 32-year-old male patient presented with a history of generalized weakness, progressive pallor & dark coloured urine over the past 2 years. He required repeated transfusions during the episode and as per

the available record a diagnosis of vit-B12 deficiency was made by the treating physician on the basis of CBC, serum vit-B12 assay & Bone Marrow Study. The patient took treatment with inj. vit-B12, improved to some extent and lost follow-up at that time.

Subsequently the patient continued his generalized weakness with yellowish discolouration of eyes and went to local doctors; he received blood transfusions for it. Another evaluation performed elsewhere later which showed extremely low Hb level, hepatomegaly with fatty change of liver on USG. When he presented to OPD of our hospital the patient's main symptoms were tiredness, passing dark coloured urine and slight yellowish discolouration of eyes. He was evaluated by CBC showing extremely low Hb level with high Reticulocyte count. Bone marrow slides (brought with him) were reviewed in our department showing marked erythroid hyperplasia with some megaloblastic features and stainable marrow iron was absent. Megaloblastic features of erythroid precursors are probably due to folate deficiency because of increased turn over related to haemolysis. However, the high reticulocyte count & the bone marrow findings considered are probably suggestive of intravascular haemolysis. Then urine haemosiderin & Coombs' tests were performed. Urine haemosiderin was strongly positive indicating haemoglobinuria hence intravascular haemolysis. Strongly positive direct Coombs' test indicates immune haemolysis. PNH was suspected of intravascular haemolysis. So, the case was diagnosed as auto immune haemolytic anaemia with co-existent PNH. Flow cytometry was performed as a confirmatory test which showed presence of PNH clone.

There was no history of fever, chest pain, repeated infection, bleeding manifestation & early satiety. There was no family history of similar disorder.

Investigations:

Investigations revealed a haemoglobin level of 3.6 g/dL, MCV 98.7 Fl, total leucocyte count 4600 cells/μL and platelet count 265 K/μL. He had reticulocyte count of 21.1%. Bone marrow examination revealed a hypercellular marrow, myeloid-to-erythroid ratio of 1:2 with some megaloblastic features and stainable marrow iron was absent, probably suggestive of intravascular haemolysis. Both Coomb's direct & indirect tests were strongly and weakly positive respectively. Urine was positive for haemosiderin. Flow cytometry was performed which showed presence of PNH clone (in 97% of granulocytes & 98% of monocytes).

Table I: PNH (FLAER Method) by Flow Cytometry.

Flow Differential (%) & Population Analysis:
Granulocytes : 97% of the granulocytes lack FLAER & CD24 expression.
Monocytes: 98% of the monocytes lack FLARE & CD14 expression.
Markers Performed : CD14, CD15, CD24, CD33, CD45, FLAER (6 markers)

Treatment:

The established therapies for patients with classical PNH are allogeneic hematopoietic cell transplantation (HCT) and complement inhibition with eculizumab.[4] Patients with haemolysis are better managed with eculizumab. Financial constraints prevented us from giving the option of eculizumab, but an option of allogeneic bone marrow transplant has been offered to the patient. Now he is managed with oral prednisolone to treat autoimmune component of his haemolytic anaemia & improvement of haemoglobin was observed after one month.

Discussion:

PNH is an acquired clonal stem cell disorder characterised by chronic intravascular haemolysis that is punctuated by episodes of paroxysms which may present with macroscopic haematuria. These

paroxysms may occur spontaneously or be precipitated by events like infection. The haemolysis usually occurs at night and is manifested as frank or microscopic haematuria in the morning. The haemolysis is complement-dependent on the surface of red blood cells (RBCs) due to the absence of CD55 (decay accelerating factor) and CD59 (membrane inhibitor of reactive lysis). In a given patient, the severity of haemolysis depends on the number of RBCs which are abnormal and the population of cells in each subgroup of PNH, where the more the number of abnormal cells the more the chances of them being lysed and type III, having complete absence of GPI (glycosylphosphatidylinositol)-linked proteins and having a higher risk of getting lysed.

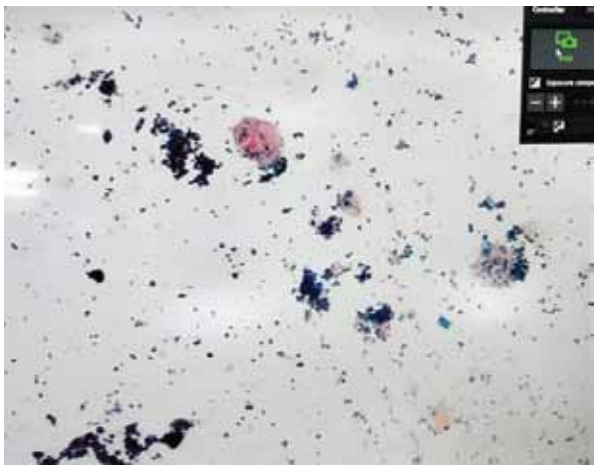

Figure 1: Perl's (Prussian Blue) staining of urinary deposits showing haemosiderin granules in urine.

The case presented a unique diagnostic challenge and hence remained undiagnosed for a period of 2 years. Although there was no splenomegaly; an elevated reticulocytes count with the positive direct Coombs' test pointed towards a haemolytic anaemia suggesting an immune-mediated extravascular haemolysis. Besides, though there was absence of frank haematuria; elevated lactate dehydrogenase and urine haemosiderin positivity with absence of stainable iron in bone marrow proved the haemolysis to be intravascular. Flow cytometry for PNH was then performed which showed presence of PNH clone (in 97% of granulocytes & 98% of monocytes). Confirmatory test for PNH is flowcytometry, though the testing of RBCs alone is a routine assay, it is not adequate for evaluation of patients with PNH, because haemolysis and transfusion may greatly underestimate the size of the PNH clone. As leucocytes have more sensitivity and PNH clone size correlates well with degree of haemolysis, many centers now test WBCs instead of RBCs with more specific antigens like CD14, CD15, CD24, CD33, CD45, FLAER instead of CD55 & CD59.[5]

PNH differs from other monoclonal haematopoietic stem cell disorders such as CML in which the fusion protein BCR-ABL is sufficient to replace the normal progenitor cells progressively and invariably by uncontrolled proliferation of a transformed clone. In PNH the peripheral blood is a mosaic of normal cells and abnormal clone through the proportion of GPI-AP- : GPI-AP+ cells varies greatly among patients. PNH is therefore, more aptly characterized as having deregulated cellular proliferation and expansion, rather than cellular transformation.

The oligoclonal nature of PNH suggests that a powerful selection process is at work in the bone marrow.[6] According to this hypothesis, stem cells with mutant PIGA have an advantage because of some pathological process that involves a GPI-AP. For example, an autoimmune process could arise in which the target antigen is a GPI-AP expressed on haematopoietic stem cells. PIGA mutant stem cells lacking GPI-AP would escape immune destruction because the target antigen is absent.

Conclusion:

In this presentation, a young male patient who presented to us with cytopenia and dark coloured urine with recent history of jaundice was found to have Coombs' positive haemolytic anaemia and haemoglobinuria. It has always been said that in a

case of confusing cases of haemolytic anaemia with cytopenia, we must suspect paroxysmal nocturnal haemoglobinuria (PNH); more so when it is coupled with urinary haemosiderinurea. Having diagnosed PNH, the management recommended is very costly and should be affordable for the patient.

Ethical Conduct of Research:

A written informed consent for publication of his clinical details was obtained from the patient.

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