

*Case report*

# Paroxysmal nocturnal haemoglobinuria and diabetes mellitus

W J Andrews, A G Magee, P V Gardiner, I Fleming, T C M Morris

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Paroxysmal nocturnal haemoglobinuria is an acquired disorder of the red cell membrane rendering the cell especially liable to lysis by activated complement. There may be a single or several clones of sensitive red cells and the disorder represents a defect at the pluripotential stem cell stage. This is supported by the findings of leucopenia and thrombocytopenia and the possible progression to pancytopenia and acute leukaemia.

## CASE REPORT

A 69-year-old non insulin dependent diabetic was first diagnosed at age 57. Her sister was also diabetic and a brother had died from leukaemia. She had initially been treated with oral hypoglycaemic agents, but because of persistent hyperglycaemia had been changed to insulin at age 63. She remained well until shortly before the present episode, when she developed increasing fatigue, lost 7 lb weight and had a poor appetite with intermittent abdominal pain. She was pale, haemoglobin concentration 7.9 g/dl with normocytic normochromic indices, 1.5% reticulocytes and white cells  $3.0 \times 10^3/\text{mm}$ . Platelet count was normal, and ESR 23 mm/hour. Urinalysis for blood, and six faecal occult blood examinations were negative. Serum iron was 6  $\mu\text{mol/l}$  (normal 9–29), ferritin 16  $\mu\text{g/l}$  (normal 18–50), and total iron binding capacity 71  $\mu\text{mol/l}$  (normal 43–73). Serum B<sub>12</sub>, folate and total bilirubin concentrations were normal. Barium enema and intravenous pyelography were normal; gastroscopy revealed chronic gastritis. The plasma haptoglobin concentration was low (0.05 g/dl, normal 0.20–1.44) and plasma haemoglobin was increased to 0.25 g/dl (normal 0–0.04), suggesting haemolysis. The direct Coombs' test was negative. Bone marrow biopsy showed depressed myelopoiesis, a reduced number of megakaryocytes, and marked erythroid hyperplasia with dyserythropoietic features. Paroxysmal nocturnal haemoglobinuria was confirmed by a positive Ham's test, and a sucrose

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Whiteabbey Hospital, Newtownabbey BT37 9RH.

W J Andrews, MD, MRCP, Consultant Physician.

A G Magee, MB, BCh, MRCP, Registrar.

P V Gardiner, MB, BCh, Senior House Officer.

Belfast City Hospital, Belfast BT9 7BL.

T C M Morris, FRCPath, Consultant Haematologist.

I Fleming, FIMLS, Senior Medical Laboratory Scientific Officer.

Correspondence to Dr Andrews.

lysis test. Haematological measurements had been normal on several previous occasions, but she had noticed occasional episodes of dark urine on walking and following exertion. Her haemoglobin concentration is now maintained between 8.0 and 9.0 g/dl by transfusion of washed red cells.

Because of the occurrence of her symptomatic haemoglobinuria during a period of poor diabetic control, Ham's tests and sucrose lysis tests were performed on red blood cells from a normal subject, cells from this diabetic patient, and cells from a non-diabetic patient with known paroxysmal nocturnal haemoglobinuria, using different concentrations of glucose, (Tables I and II). There was no additive effect of hyperglycaemia on haemolysis before or after acidification in either patient.

TABLE I

*Ham's test. Red cells are exposed at 37°C to normal serum, which is then acidified (pH 6.5–7.0) for lysis. The inactivated samples were placed at 56°C for 10–30 minutes to inactivate complement.*

*Red cells from a normal subject, from this patient and from a non-diabetic patient with paroxysmal nocturnal haemoglobinuria are compared. Each was also exposed to three concentrations of glucose.*

| Red blood cells tested   | Haemolysis            |               |           |                         |              |              |                     |              |              |
|--|-----------------------|---------------|-----------|-------------------------|--------------|--------------|---------------------|--------------|--------------|
|  | Acidified inactivated | Not acidified | Acidified | Not acidified + glucose |              |              | Acidified + glucose |              |              |
|  |                       |               |           | 10<br>mmol/l            | 20<br>mmol/l | 40<br>mmol/l | 10<br>mmol/l        | 20<br>mmol/l | 40<br>mmol/l |
| Normal subject   | 0.5%                  | 0.5%          | 0.5%      | 0%                      | 0%           | 0%           | 0%                  | 0%           | 0%           |
| This patient   | 1.8%                  | 4.8%          | 24.5%     | 5.9%                    | 5.9%         | 5.9%         | 26.3%               | 24.5%        | 25.1%        |
| Non-diabetic patient with paroxysmal nocturnal haemoglobinuria | 0.0%                  | 3.0%          | 15.4%     | 3.6%                    | 3.6%         | 3.6%         | 13.8%               | 14.3%        | 13.8%        |

TABLE II

*Sucrose lysis test. Percentage haemolysis in a solution comprising 0.1 ml of a 50% suspension of washed red blood cells, 0.5 ml, ABO compatible serum and 0.9% sucrose, 0.9% saline, and 0.9% glucose respectively. Incubated at 37°C for 30 minutes and centrifuged.*

| Red blood cells tested   | Haemolysis |         |         |
|--|------------|---------|---------|
|  | Saline     | Sucrose | Glucose |
| Normal subject   | 0.07%      | 0.46%   | 0.75%   |
| This patient   | 0.09%      | 40.04%  | 41.94%  |
| Non-diabetic patient with paroxysmal nocturnal haemoglobinuria | 0.09%      | 16.09%  | 17.78%  |

## COMMENT

The basic defect in paroxysmal nocturnal haemoglobinuria remains unknown. The clinical features derive from red cell membrane abnormalities resulting in lysis of the membrane by complement. Complement may be activated either by classical or by the alternative pathways, as shown by the sucrose lysis test and Ham's test respectively. *In vivo* lysis tends to be precipitated by the acidification of the blood which occurs during sleep and on exercise. The results of the Ham's test performed in the presence of increasing quantities of glucose, and the sucrose lysis test in which sucrose was substituted for glucose do not suggest that increasing glucose concentration was a factor in precipitation of haemolysis. In both tests, except on exposure to saline, the diabetic red blood cells showed a greater percentage of haemolysis than cells from the non-diabetic patient with paroxysmal nocturnal haemoglobinuria. This is a reflection of the number of complement sensitive cells produced by the abnormal clone in each individual and where the patient has received treatment, is adversely related to the number of transfused cells present. It is normal procedure to wash cells prior to transfusion to reduce the amount of transfused complement thus avoiding a precipitation of a further haemolysis.

This patient did not develop symptoms of paroxysmal nocturnal haemoglobinuria until three years after the introduction of insulin therapy for her diabetic control. Previous surgery did not precipitate symptoms, but the retrospective evidence of low leucocyte counts suggests that the defect may have been present at that earlier time, although the haemoglobin was not significantly reduced. Her platelet count remains at the lower limit of normal which reflects the variability of the disease and the degree of stem cell disorder. Co-existence of paroxysmal nocturnal haemoglobinuria and diabetes mellitus has not previously been reported, but the *in vitro* experiments suggest that elevated glucose levels do not aggravate the tendency to haemolysis.

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