PAROXYSMAL NOCTURNAL HAEMOGLOBINURIA IN TWO SOUTH AFRICAN BANTU PATIENTS*

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Paroxysmal nocturnal haemoglobinuria is a rare but fascinating disorder. The diagnosed incidence is about 1 : 500,000. It occurs in all races, in both sexes and usually in adult life. It is characterized by chronic haemolytic anaemia, which is worse during sleep, haemosiderinuria, and by increased sensitivity of red cells to haemolysis in acidified serum.

The disorder is neither hereditary nor familial and is not related to any known aetiological agent. Attacks are often accompanied by fever, chills and abdominal or lumbar pain. Leucopenia and thrombocytopenia are almost invariably present. Only one such patient has been described in the South African Bantu.[1] We describe here two Bantu males with paroxysmal nocturnal haemoglobinuria whose clinical presentation was not typical of the condition.

METHODS

Standard haematological tests were used as described by Dacie and Lewis.[2]

Sucrose Haemolysis Test

This test was done according to the method of Hartmann and Jenkins.[3] An isotonic solution of sucrose was prepared. The test mixture consisted of sucrose solution 0·85 ml., compatible serum 0·05 ml., and a 50% suspension of saline-washed red cells, 0·1 ml. Acidification is not required. The reaction mixture was incubated at 37°C for 30 minutes, centrifuged and observed for haemolysis.

Sugar Water Test

This is a very simplified modification of the sucrose haemolysis test used for screening purposes. One level tablespoon of commercial granulated sugar was dissolved in 3½ oz. (103 ml.) of distilled water or alternatively 9 - 10 ml. of dry sugar in 100 ml. of distilled water. One volume of oxalated blood was added to 9 volumes of sugar solution. The mixture was incubated at 37°C for 30 minutes, centrifuged and observed for haemolysis.

Crosby Test

One-tenth volume of the patient's packed red cells is added to normal serum and acidified with 0·1 N/3 HCl. Half the volume of this suspension is added to a test-tube containing 50 units of bovine thrombin. Both test-tubes are incubated at 37°C for 15 minutes. A control was set up with normal red cells.

Red cell acetylcholinesterase estimation was kindly performed by Dr Metz.

Red cell survival was performed according to the method of Mollison and Veall.[4]

CASE REPORTS

Case 1

A 28-year-old Bantu male was admitted to hospital on 22 September 1966. He complained of abdominal pain, backache, yellow discoloration of the sclera, and passage of dark urine for 4 days. He had had a similar episode about 3 - 4 months before admission; this had lasted for about 3 weeks but he had not consulted a doctor. He had not taken any drugs or received any injections for the previous 3 months and had not been in contact with any toxic substance. Family history revealed nothing of note.

The patient looked ill and toxic. He was pyrexial, clinically jaundiced, and distressed and restless because of abdominal pain and backache. The mucous membranes were pale. Apart from a 4-finger hepatomegaly, systemic examination revealed nothing of note.

His urine was dark in colour, like strong tea. An occult blood test was positive and urobilinogen was present, but bilirubin was absent and no red cells were seen on microscopy. Further haematological examination could not be carried out because the blood haemolysed immediately on venepuncture. Acute haemolytic anaemia was diagnosed and the patient was started on prednisone 60 mg. daily. One week later the acute haemolysis subsided and the following results were obtained: Haemoglobin 6·2 G/100 ml.; colour index 1, leucocytes 21,300/cu.mm. (differential count—neutrophils 63%, monocytes 4%, lymphocytes 30%); PCV 19·5%; MCHC 31·5%; ESR 115 mm. in the 1st hour (Westergren), and platelet count 181,000. His blood urea was 479 mg./100 ml.; electrolytes were normal and the reticulocyte count was 14·2%. Coombs, Schumm's and Donath-Landsteiner tests were negative, as was Ham's acidified serum test. Neutrophil alkaline phosphatase was 62 units. Warm and cold agglutinins were not present in the serum, and the Wassermann reaction was negative. Haemosiderin was not present in the urine.

He was subsequently transfused with whole blood until his haemoglobin was normal. The prednisone was gradually reduced and stopped. His blood urea returned to normal on conservative management. His recovery was uneventful and he was discharged with a diagnosis of acute haemolytic anaemia of unknown aetiology.

About 4 months later he was readmitted, again with a 4-day history of abdominal pain and backache, with the passage of dark urine. He also complained of tiredness and dimness of vision. On examination he was very pale and he had retinal and subhyaloid haemorrhages in both fundi. His haemoglobin was 4 G/100 ml. He was transfused with whole blood and discharged on prednisone. He remained well until May 1967, when he was again transfused because of anaemia. In October 1967 it was decided to review the diagnosis. His vision had improved and was completely normal at the time. He was moderately anaemic and his platelet count varied between 30,000 and 78,000/cu.mm., despite full doses of prednisone (60 mg./day). The white cell count varied between 3,000 and 13,000/cu.mm. The reticulocyte count was 9·8%. The bone marrow showed a hyperactive normoblastic erythropoiesis; megakaryocytes were present in normal numbers and at the granular stage of maturation; platelet genesis was depressed. Bone-marrow iron content was increased

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and there was some evidence of hypochromic maturation. Red cell fragility was slightly decreased. Haemoglobin electrophoresis showed a single component with the mobility of haemoglobin A. Haemoglobin F and H were not detected on suitably stained smears. Serum haptoglobulin was low. LE cells were not detected on several occasions. The red cell survival time was normal. Ham's acidified serum test was now positive. Haemosiderin (Perl's test) was detected in the urine for the first time, and a positive Crosby test suggested a diagnosis of paroxysmal nocturnal haemoglobinuria. The neutrophil alkaline phosphatase was now 6 units (normal range 15 - 100 units) and acetylcholinesterase △ pH/hour 0·52 (normal average 0·7). The repeat red cell survival time was 24 days (normal $T\frac{1}{2}^{51}C$ = 25 days).

The patient has subsequently been admitted on 2 occasions—once because of anaemia, when he was transfused with washed red cells. On another occasion he was admitted with severe pains in the calves. There was no evidence of deep vein thrombosis. The pain lasted for a few days and then subsided spontaneously. This was not associated with exacerbation of haemolysis.

Case 2

The second patient, a Bantu male aged 24 years, was first seen at Livingstone Hospital in 1964. The history then was one of shortness of breath, palpitations and bleeding from the gums. There were no other significant features on interrogation.

The patient was not acutely distressed, but the mucous membranes were pale. There was no jaundice or purpura. The cardiovascular and respiratory systems were normal. No hepatomegaly or splenomegaly was detected.

Special investigations showed the following: haemoglobin 3·6 G/100 ml.; colour index 1·3; leucocytes 4,400/cu.mm. (differential count—neutrophils 18%, monocytes 1%, lymphocytes 81%); PCV 11·5%; MCHC 31·5%; ESR 160 mm./hour (Westergren). The platelet count varied between 15,000 and 35,000/cu.mm. The bone marrow was of good cellularity, myelopoiesis was normal, megakaryocytes were almost absent and erythropoiesis showed mild megaloblastic change. LE cells were not detected on several occasions. The reticulocyte count was 6·7%. Secondary thrombocytopenia was diagnosed. About 2 months later it was found that there was a progressive diminution of the cellularity of the bone marrow. The hypoplasia was affecting the platelets and the white cell series, but not the red cells. About 5 months later all 3 elements of the bone marrow were affected—platelet genesis most severely. During this period the patient had been treated with prednisone but in spite of this had to be repeatedly transfused.

Hypoplastic anaemia was diagnosed and the patient was admitted on numerous occasions for transfusion, until October 1967 when the diagnosis was reviewed. He complained of dyspnoea on exertion, general weakness, headache, and dimness of vision for 2 weeks. His urine was dark in colour, especially after hard physical labour, running and exercise. This had been present since 1964 though not complained of previously. While he was in the ward he passed dark urine, port-wine in colour, in the morning. Thrombocytopenia and leucopenia were still present and in view of these features paroxysmal nocturnal haemoglobinuria was suspected. Ham's acidified serum test and Crosby test were positive. Schumm's test was positive during the time that the urine was very dark in colour, and methaemoglobinuria and haemosiderinuria were present during these episodes of haemolysis.

Red cell survival time was slightly shortened to 20·5 days. Haemosiderinuria persisted during his stay in hospital. The bone-marrow examination was repeated and showed good cellularity and a normal number of megakaryocytes with normal platelet genesis. Myelopoiesis was active, with no evidence of maturation arrest; erythropoiesis was hyperactive.

Neutrophil alkaline phosphatase was 3 units and red cell acetylcholinesterase △ pH/hour 0·35. The following tests were normal: haemoglobin electrophoresis, cold and warm agglutinins, Donath-Landsteiner, Coombs, red cell osmotic fragility, and glucose-6-phosphate dehydrogenase.

DISCUSSION

Paroxysmal nocturnal haemoglobinuria (PNH) is an acquired disease of unknown aetiology, characterized by chronic intravascular haemolysis, a tendency to thrombosis, leucopenia and thrombocytopenia. Classically, haemolysis is worse at night and this manifests itself with haemoglobinuria in the morning but not during the day. In some patients haemoglobinuria is infrequent or may never occur at all.[5]

In other patients the nocturnal rhythm is not obvious and episodes of haemoglobinuria may last continuously for days. The condition may present with abdominal pain, nausea and vomiting, resembling a surgical problem rather than a haematological one. If other signs of haemolysis are not obvious or the anaemia is hypochromic (because of chronic loss of iron in the urine), or the bone marrow is hypoplastic, the diagnosis may be missed because one does not think of it. It is for these reasons that Dacie insists that PNH be considered in any unexplained anaemia, with or without reticulocytosis, particularly if accompanied by leucopenia, thrombocytopenia or abdominal pain. Since the classical laboratory tests may be negative in the initial stages of the disease, frequent assessment may be necessary. This was demonstrated in our first patient who developed a positive acidified serum test and haemosiderinuria approximately 10 months after the onset of his first episode of haemolysis. Hinz[6] suggests that this delay tends to occur if the major manifestations of the disease are those of marrow hypoplasia.

A simple screening test has been described by Hartmann and Jenkins,[3] the 'sugar water test'. Up to the present this test has proved to be specific for the condition. The only possible false positive may be found in the acquired immune haemolytic anaemias with potent haemolysins. This test is so simple and requires so little apparatus that it should be done in any anaemia of unknown aetiology, hypoplastic anaemias, thrombocytopenias and certain iron-deficiency anaemias where the cause of blood loss has not been determined.

The Nature of the Red Cell Defect

It is not possible to define the exact fundamental lesion in PNH. Electron-microscopic abnormalities have been defined by some and refuted by others.

In our second patient the delay in diagnosis was about 3 years. Hypoplasia may also occur after overt haemolysis.

Haemoglobinaemia and Haemoglobinuria

Normally haemoglobin in the plasma combines with haptoglobulins and only about 5 mg./100 ml. of haemoglobin is free in the plasma. The renal threshold for haemoglobin is 130 mg./100 ml. and thus haemoglobin does not pass into the urine. In PNH the renal threshold for haemoglobin is 50 - 100 mg./100 ml. Loss of iron in the urine alone may amount to 3 - 5 mg./day compared with the normal loss of 1 mg. in urine, faeces and sweat combined. If there is haemoglobinuria as well, 50 mg. of iron is lost for every 15 G of haemoglobin destroyed. Iron is therefore depleted in the spleen and liver but not in the kidney. The more frequent use of transfusion with washed red cells may explain the low incidence of iron-deficiency anaemia in PNH patients today. In spite of the severe siderosis, impairment of renal function is not seen.

Laboratory Diagnosis

Acidified serum test (*Ham's test*). Dacie states that this test is always positive in PNH. Hofmeyr *et al.*[12] reported a patient with haemolytic anaemia highly suggestive of PNH but with a negative test. However, it is known that this test may remain negative for many months and then become a positive. It would be interesting to know if this will happen in the case cited above. There are no false positives in the acidified serum test. The purpose of acidifying the serum is to adjust the pH to an optimum level for the action of the serum factors. PNH red cells are not more sensitive than normal cells to the effect of acid *per se,* hence the term acidified serum test is preferred to acid haemolysis.[13]

Thrombin test of Crosby. According to Dacie, the thrombin test is only positive if the acidified serum test is also positive. The basis of the test has been discussed.

The cold antibody haemolysis test (high titre cold antibody). It is now clear that PNH erythrocytes will undergo lysis when exposed to potentially lytic antibodies, primarily because they are very much more sensitive than normal cells to the haemolytic effect of complement. The test has been modified recently[14] and it seems that it is a little more sensitive and reliable than the acidified serum test. Both tests should therefore be done in a patient suspected of having PNH.

Prognosis and Course

Patients may live for many years. The disease may revert to a subclinical state, but the acidified serum test remains positive. Few patients have a complete remission, and others die as a result of complications. In one series of 21 patients the average duration of life was 6·6 years but only 4 patients died because of the PNH itself. Eleven died following splenectomy and 6 from an unrelated cause.[15] Dacie quotes the duration of illness to vary from 3 to 22 years. The prognosis depends on the severity of the disease, i.e. the degree of marrow hypoplasia, thrombocytopenia, leucopenia, and severity of haemolysis.

Treatment

There is no cure for PNH. Treatment consists of repeated blood transfusion with washed red cells because of the danger of precipitating a crisis with whole blood. It is interesting to note that both our patients were given whole blood repeatedly with no ill-effects before the diagnosis was made. Since then washed red cells only have been given. Steroids are contraindicated because they increase the tendency to thrombosis. Haemoglobinuria has been noted to follow 6 - 8 days after iron therapy. Iron apparently stimulates the marrow to produce new sensitive cells. Hartmann and Jenkins[3] have combined iron therapy with androgens. They reported good response in 4 out of their 6 cases. The anaemia in these patients was partially or completely relieved for long periods and haemolysis was distinctly less. They suggest that, in addition to the stimulation of the bone marrow, androgens also diminished the tendency to haemolysis. In practice iron does not produce haemolysis in all cases. Heparin also tends to aggravate haemolysis and should not be used in the treatment of thrombotic episodes. Dicoumarol is preferred.

SUMMARY

Two South African Bantu males with paroxysmal nocturnal haemoglobinuria are presented. The importance of considering the diagnosis as a possibility in any unexplained anaemia is stressed. A simple screening test, the 'sugar water test', is now available. Recent literature on the aetiology is briefly reviewed. Diagnosis, complications, prognosis and treatment are discussed.

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