Suid-Afrikaanse Tydskrif vir Geneeskunde : South African Medical Journal

Kaapstad, 8 Julie 1961 Deel 35 No 27 Volume 35 Cape Town, 8 July 1961

PAROXYSMAL NOCTURNAL HAEMOGLOBINURIA IN A SOUTH AFRICAN BANTU PATIENT

J. METZ, M.D. (RAND), R. DANDO, M.B., B.CH. (RAND), AND K. STEVENS, B.SC., M.B., B.CH. (RAND)

South African Institute for Medical Research, and Department of Medicine, Baragwanath Hospital, Johannesburg

Paroxysmal nocturnal haemoglobinuria (PNH) is a rare disease, and we are not aware of any description of proved cases in the Bantu. The clinical manifestations are not always typical, and the disease should be included in the differential diagnosis of patients with haemoglobinuria or apparent haematuria. In this paper we record the findings in a Bantu patient in whom the diagnostic criteria of PNH were fulfilled.

These criteria are: increased rate of haemolysis (as shown essentially by the demonstration of diminished red-cell life-span), haemosiderinuria, and haemolysis of red cells in acidified serum. Certain other features contribute to the diagnosis. The red cells haemolyse when incubated in serum containing high-titre cold antibodies, and haemolysis in acidified serum is enhanced by the addition of thrombin. Clotted blood from patients with PNH haemolyses rapidly when incubated at 37°C. PNH red cells are often deficient in the enzyme acetylcholinesterase. All these features have been demonstrated in the present case.

METHODS

The standard haematologic methods used in this investigation were those described by Dacie.[1]

Ham's acid-serum test: $\frac{1}{10}$ volume of a 50% suspension of washed red cells was added to normal compatible serum acidified by the addition of $\frac{1}{10}$ volume of 0·2N-HCl. The mixture was incubated at 37°C. for 2 hours, and the amount of haemolysis in the supernatant read quantitatively. Controls included normal red cells, unacidified serum, and inactivated serum.

The Crosby test was performed as originally described,[2] and the amount of haemolysis read quantitatively. Controls were set up with normal red cells.

Haemolysis by high-titre cold antibodies: 0·25 ml. volumes of a 10% suspension of test and control red cells were added to 0·25 ml. of a 1 in 25 dilution in normal serum of the serum containing high-titre cold antibodies. The mixture was left at room temperature for 2 hours, and the amount of haemolysis read quantitatively.

Heat-resistance test: Blood from the patient and controls was allowed to clot at 37°C. for 2 hours, and the amount of haemolysis in the serum was measured quantitatively.

Red-cell acetylcholinesterase was measured by the method of Michel.[3]

Neutrophil alkaline-phosphatase activity: Fresh blood smears were stained by the Gomori technique.

Red-cell survival was measured with radioactive chromium, as described by Mollison and Veall.[4] The half chromium time was estimated, and the mean cell life calculated after correction for elution ($T\frac{1}{2}$ elution 64 days).

CASE REPORT

A Bantu (Swazi) female, aged 38, was admitted to Baragwanath Hospital on 29 June 1960, complaining of the intermittent passage of blood in the urine over the previous year. She stated that the colour of the urine varied from pale red to almost black. She had recently had backache, lower abdominal pain with burning and frequency of micturition, persistent frontal headache, mild exertional dyspnoea and fatigue. The family history was non-contributory. Physical examination revealed no abnormality other than pallor of the mucous membranes and the palms of the hands.

Laboratory Findings

Blood count. On admission the haemoglobin value was 7·5 G. per 100 ml., packed-cell volume 25% and mean corpuscular haemoglobin concentration 30%, with reticulocytes 16%. Leucocytes were 2,300 per c.mm. with neutrophils 23%, monocytes 8%, lymphocytes 68%, eosinophils 0% and basophils 1%. On the stained films the red cells showed moderate anisopoikilocytosis, and the platelets were not obviously reduced in number.

Urinalysis. Microscopic examination of the centrifuged deposits from numerous specimens of urine did not show more than 5 red cells per high power field. Protein was present ++, but bilirubin was not detected, and urobilin varied from a normal amount to moderate excess. Methaemoglobin was present in almost all specimens examined. Staining of the centrifuged urinary deposit with Perl's reagent showed the presence of numerous iron granules. The amount of iron excreted in a 24-hour specimen of urine at a time when haemoglobinuria was not present, was 3·6 mg. (normal 30—65 μg.). Cystoscopy on 6 July showed puffs of reddish-brown pigment emerging from the ureteric orifices. Retrograde pyelography revealed normal calyces and pelves, and the bladder was normal.

Blood chemistry. Blood urea was 30 mg. per 100 ml. Total serum bilirubin varied from less than 1·0 mg. to 1·3 mg. per 100 ml., of which 0·2 mg. was 'direct' and 1·1 mg. 'indirect'.

Further Investigations

The patient's blood group was O, Rh positive. The direct Coombs test was negative and abnormal antibodies were not detected. Cold agglutinins were present to a titre of 1 : 32. The direct and indirect Donath-Landsteiner tests were negative. Schumm's test was positive. Electrophoresis of the haemoglobin in veronal buffer (pH 8·6) showed a single component with the mobility of haemoglobin A, and sickling could not be demonstrated. Heinz bodies were not present. The osmotic fragility of the red cells was within normal limits. The V.D.R.L. test was negative.

The quantitative Ham's acid-serum test was positive, 23% haemolysis of patient's cells occurring in acidified normal serum. Haemolysis did not occur in unacidified or inactivated serum. Haemolysis by high-titre cold antibodies was 31%, there being no haemolysis of control cells. The Crosby test was positive, with a 50% increment in the amount of haemolysis in acidified serum with added thrombin, as compared with acidified serum without thrombin. Haemolysis of clotted blood left at 37°C. for 2 hours (heat-resistance test) was 12·5%, with no haemolysis of control blood. Red-cell acetylcholinesterase was 0·22 Δ pH per hour (normal range 0·65—0·95 Δ pH per hour). Alkaline-phosphatase activity could not be demonstrated in the patient's neutrophils, but was present in control smears.

A red-cell survival study (Fig. 1) showed a half chromium time ($T\frac{1}{2}{}^{51}Cr$) of 8 days (normal 30 ± 2 days) with mean cell life 28 days

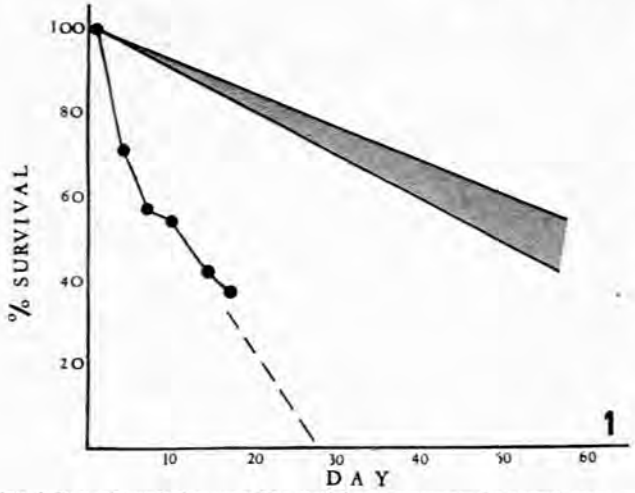

Fig. 1. Red-cell survival pattern. The shaded area represents the normal range.

(normal 100—130 days). Red-cell destruction was thus taking place at approximately 4 times the normal rate.

Course and Treatment

On 13 July, 2½ litres of blood were transfused. The haemoglobin value after transfusion was 11·3 G. per 100 ml., with 1,400 leucocytes per c.mm. (neutrophils 17%). Between 13 and 19 July the urine remained clear, and the patient was discharged from hospital. One week later she returned, complaining of the passage of dark-red urine. Physical examination showed pallor and mild jaundice. The haemoglobin value was now 6·3 G. per 100 ml., with reticulocytes 29% and leucocytes 2,100 per c.mm. (neutrophils 18%). Platelets were 50,000 per c.mm. Specimens of urine passed at intervals during the day varied in colour from dark red to almost black. Morning specimens were consistently blackish and those in the late afternoon varied from light to dark red.

On 2 September, a further blood transfusion was given, and the patient was subsequently discharged from hospital.

DISCUSSION

The findings in this patient can be described as classical of PNH. The haemoglobinuria was paroxysmal, and was more pronounced in the early morning specimens. The blood picture showed a haemolytic anaemia with reticulocytosis, intravascular haemolysis, and mild hyperbilirubinaemia, and the red-cell life-span was markedly diminished. There was leucopenia with persistent neutropenia, and thrombocytopenia, typical features of PNH. The various haemolytic tests were strongly positive. The red cells were deficient in the enzyme acetylcholinesterase, and deficiency to this degree is apparently specific for the disease.[5-7] Alkaline-phosphatase activity could not be demonstrated in the neutrophils, lack of this activity having being described in PNH by Beck and Valentine.[8] There was very marked haemosiderinuria, confirmed by chemical analysis.

PNH would be expected to occur in the Bantu, for the disease is well described in the American Negro. Crosby[9] referred to Negro patients, and 3 of the 6 patients described by Hartman and Auditore[10] were Negroes.

SUMMARY

A case of paroxysmal nocturnal haemoglobinuria in a Bantu female is described.

We wish to thank the Director, South African Institute for Medical Research, for facilities to carry out this study; Prof. J. V. Dacie for supplying the serum containing high-titre cold antibodies; Dr. V. H. Wilson for his help; Mr. D. Hart and Miss V. Brandt for technical assistance; and the Superintendent, Baragwanath Hospital, for permission to publish the case.

REFERENCES

1. Dacie, J. V. (1956): *Practical Haematology*. London: Churchill.
2. Crosby, W. H. (1950): Blood, **5**, 843.
3. Michel, H. (1949): J. Lab. Clin. Med., **34**, 1564.
4. Mollison, P. L. and Veall, N. (1955): Brit. J. Haemat., **1**, 62.
5. de Sandre, G., Ghiotto, G. and Mastella, G. (1956): Acta. med. patav., **16** 310.
6. Auditore, J. V. and Hartman, R. C. (1959): Amer. J. Med., **27**, 401.
7. Metz, J., Bradlow, B. A., Lewis, S. M. and Dacie, J. V. (1960): Brit. J. Haemat., **6**, 372.
8. Beck, W. S. and Valentine, W. N. (1951): J. Lab. Clin. Med., **38**, 245.
9. Crosby, W. H. (1953): Blood, **8**, 769.
10. Hartman, R. C. and Auditore, J. V. (1959): Amer. J. Med., **27**, 389.