Two cases of the nephrotic syndrome with a reversible coagulation defect

M. R. BAKER
M.R.C.P.

J. A. DAVIES
M.D., M.R.C.P.

G. M. TATE
F.I.M.L.S.

M. R. LEE
D.M., D.Phil., F.R.C.P.

University Department of Medicine, The General Infirmary, Leeds LS1 3EX

Summary

Two cases of the nephrotic syndrome with an apparently identical defect of blood coagulation, discovered during preparation for renal biopsy are described. Plasma from both patients showed prolongation of thrombin and kaolin cephalin times which was probably due to abnormally slow polymerization of fibrin monomer. Corticosteroid therapy reversed the abnormal times in one case. One of the patients showed seasonal relapses which occurred in relation to episodes of hay fever associated with allergy to grass pollen. The other patient showed some similar features, but on renal biopsy was found to have proliferative glomerulonephritis.

radial immunodiffusion. Hepatitis B surface antigen (HB_sAg) was measured by immunoelectro-osmophoresis and turkey erythrocyte haemagglutination. Skin tests were carried out using the Bencard prick tests. HLA testing was by the NIH lymphocyte microtoxicity technique (NIAID, 1974).

Biochemistry

Standard biochemical measurements on plasma and urine were made with the Vicker's M300 multi-channel analyser. Correction of plasma calcium for plasma albumin concentration was by the method of Payne *et al.* (1973). Plasma cholesterol was measured by a modification of the method of Rappaport and Eichhorn (Annan and Isherwood, 1969).

Coagulation

Tests of coagulation function were performed according to recognized methods detailed elsewhere (Davies, Fieldhouse and McNicol, 1976). Molecular weight of fibrinogen precipitated from plasma with ammonium sulphate and subunit structure of reduced polymerized fibrin were assessed by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate (Weber and Osborn, 1969). Immunoelectrophoresis of fibrinogen was performed on agarose plates at pH 4 and pH 8.6 using commercial antisera (Hoechst Pharmaceuticals, Hounslow). Rate of release of fibrinopeptide A (FPA) was estimated after collection of native blood into a plastic syringe at room temperature and subsampling into an aprotinin-heparin-EDTA mixture at intervals of 0, 30, 60, 90, 120 and 240 sec. FPA was then assayed by the method described by Nossel *et al.* (1974) using commercially-available reagents (IMCO Corporation Limited, Grev Turegatan 8, Stockholm). FXIII was assayed by the amine incorporation technique (Lorand *et al.*, 1969) and

Introduction

The relapsing nephrotic syndrome in association with grass pollen allergy has been reported in a number of children but only 2 adults (Hardwicke *et al.*, 1959; Williamson, 1970; Wittig and Goldman, 1970; Reeves *et al.*, 1975). An association has been suggested between this disorder and HLA B12 (Thomson *et al.*, 1976). None of the reported cases has suffered from other disease processes, nor has a defect of blood coagulation been described previously. The 2 patients reported here are of interest in view of the presence of a coagulation disorder which was of an unusual type. The clotting disorder hindered the performance of renal biopsy, a necessary step in the diagnosis and management of the nephrotic syndrome. The first case is also unusual because of the rarity of the atopic nephrotic syndrome occurring in an adult.

Materials and methods

Immunology

Antinuclear factor (ANF) was measured by indirect immunofluorescence, anti-streptolysin O (ASO) by sheep cell haemolysis inhibition. Immunoglobulins and C₃ complement were estimated by

FXIII subunits were estimated by rocket electrophoresis using subunit a and b antisera (Hoechst Pharmaceuticals, Hounsflow).

Results

Case 1

A 27-year-old female Malaysian Staff Nurse, presented in September 1976. In 1962 at the age of 13 years whilst in Malaysia she had developed the nephrotic syndrome. There was no preceding history of sore throat or allergic symptoms and no associated haematuria or hypertension. She was treated with diuretics alone and underwent spontaneous remission after 4 months. Her urine remained protein-free thereafter. In 1970 she came to the U.K. She had no proteinuria at that time nor on several subsequent occasions.

In May 1975 she developed hay fever for the first time. Two weeks later she noticed ankle oedema and puffiness of the eyes. She had heavy proteinuria on self testing with Albustix. She treated herself with diuretics and her urine became protein-free about 2 months later. She remained well, without treatment and without proteinuria, until May, 1976, when she again developed hay fever followed shortly by proteinuria and oedema. The response to diuretics was incomplete and she eventually sought medical advice.

There was no previous history of allergy before 1975 and no family history of atopy or renal disease. Subsequent questioning after discovery of the defect in blood coagulation showed that she had never had any episodes of abnormal bruising, or bleeding, and that her menstrual periods had been normal in amount and duration. There was no family history of an abnormal bleeding tendency.

Clinical examination showed a moderate degree of ankle and sacral oedema and the blood pressure was 100/60 mm/Hg. Examination of all other systems was normal and at no time were there any clinical manifestations of a bleeding tendency.

On the basis of the clinical features and the investigations described below, a diagnosis of seasonal nephrotic syndrome was made (see Table 1). This was not confirmed by renal biopsy because of the coagulation defect discovered on routine screening. She was treated with bed rest, a thiazide diuretic, and protection from outdoor dusts and allergens. Within one month, oedema and proteinuria had cleared and diuretic treatment was stopped. By mid-October 1976 tests of coagulation function showed prothrombin time of 12/12 sec, thrombin time 21/15 sec, kaolin cephalin time (KCT) 32/32 sec. By the beginning of November she had become symptom-free, there was no proteinuria and plasma albumin had risen to 35 g/l. Prothrombin time, KCT, and thrombin time were normal. Repeat tests of immunological function showed IgE 2800 i.u./ml,

IgG 171 i.u./ml, and C₃ complement 82 mg/100 ml. The eosinophil count was $1.5 \times 10^9/l$.

Case 2

A 28-year-old male Nigerian student presented in December 1976 with a one-week history of swelling of the face and ankles. A similar episode, lasting a few weeks, had occurred while he was in Nigeria in June 1976. His previous history included an attack of malaria for the first time in August 1975, which had been treated with chloroquine. A week before admission he had received ampicillin and probenecid for an attack of gonorrhoea. Personal and family history showed no features suggestive of atopy or of a tendency to bleed.

There was considerable pitting oedema of both legs and sacrum and clinical evidence of bilateral pleural effusions. His arterial pressure was 115/60 mmHg lying. Clinical examination was otherwise normal, and there was no evidence of bleeding or bruising. The clinical features, and the investigations described below (Table 1) indicated a diagnosis of nephrotic syndrome. Routine coagulation screening before renal biopsy indicated a coagulation disorder. An attempt was made to correct the clotting abnormality by infusion of fresh frozen plasma but this had only a minimal effect on the abnormal coagulation tests. Consequently treatment was begun with prednisolone 60 mg daily, resulting in a rapid reduction in proteinuria, which had completely cleared 11 days after the start of treatment. The simultaneous improvement in the coagulation disorder allowed renal biopsy to be performed safely 4 weeks after the start of steroid therapy. In remission, immunological studies showed IgE 800 i.u./ml; IgG 124 i.u./ml; C₃ complement, 152 mg/100 ml; and an eosinophil count of $0.1 \times 10^9/l$.

Investigations

The results of investigations in both patients were surprisingly similar. The significantly abnormal values are shown in Table 1. Normal or negative values were obtained in both patients for the following investigations: plasma concentrations of urea; electrolytes; creatinine; alkaline phosphatase; aspartate aminotransferase; bilirubin; calcium corrected for albumin; phosphate; creatinine clearance; haemoglobin; white cell count; platelet count; anti-nuclear factor; anti-streptolysin O titre; HB_sAg; MSU; urine culture for acid-fast bacillus (AFB); screening for parasites; ECG; IVP. Chest X-ray showed small bilateral pleural effusions in Case 2, but was normal in Case 1.

Tests of coagulation function, apart from those shown in Table 1, showed normal values for both patients: whole blood clotting time; bleeding time;

platelet adhesiveness; platelet aggregation to ADP, adrenaline, and collagen; clot stability; euglobulin lysis time; serum fibrin/fibrinogen-related antigen; prothrombin time; anti-thrombin (by 2 methods); factor V; factor VIII C; factor VIII R; AG; factor IX; molecular weight of fibrinogen on polyacrylamide gel electrophoresis; factor XIII; factor XIII subunit concentration and subunit composition of polymerized fibrin.

TABLE 1. Abnormal results obtained from investigations of biochemical, immunological and coagulation functions

Investigation	Case 1	Case 2	Concurrent control value or normal range
Plasma albumin (g/l)	27.9	22.4	37-59
Plasma globulin (g/l)	17.4	25.3	24-37
Urine protein (g/24 hr)	8.8	14.7	<0.2
Selectivity index for proteinuria	0.14	0.2	-
Plasma cholesterol (mmol/l)	13.0	13.5	<8
Eosinophil count ($\times 10^9/l$)	1.6	1.0	<0.5
C ₃ complement (mg/100 ml)	92	165	104-161
IgG (i.u./ml)	66	54	128-199
IgE (i.u./ml)	3000	1000	<250
Plasma fibrinogen (g/l)	4.1	4.2	1.5-4.5
Thrombin time (sec.)	32	31	18; 13
Kaolin cephalin time (sec.)	50	43	34; 32
Reptilase time (sec.)	29	34.1	17; 16.7
Ancrod time (sec.)	not done	68.7	20

Results of mixing and dilution experiments on patients' plasma were similar for both patients. Mixture of pooled normal plasma and patients' plasma used in the KCT assay before and after incubation at 37°C did not indicate the presence of inhibitor activity in patient plasma. The results of a representative dilution experiment suggest that patient plasma does not contain anti-thrombin activity. Immunoelectrophoresis of plasma fibrinogen suggested that for both patients there might be a slight increase in cathodal mobility of fibrinogen, compared to that in normal pooled plasma (Fig. 1). The rate of release of fibrinopeptide A from fibrinogen was faster in both patients than in a control subject and this is shown for Case 2 in Fig. 2.

A few further investigations were performed. *Case 1*: skin tests to grass pollen, B2++, B3++, B5++, house dust +; histocompatibility testing - HLA: A10, A11, B5, BW15. *Case 2*: malaria, fluor-

escent antibody test 1/320; serological tests for syphilis negative. In this patient renal biopsy was performed 4 weeks after starting steroid treatment. The main histological feature of the biopsy was a mild focal proliferation of mesangial cells, the appearances being those of generalized mesangial cell proliferative glomerulonephritis. Immunofluorescent studies showed finely granular capillary basement membrane deposits of IgM, involving segments of capillary loops throughout the tuft. Staining for IgA, IgG, C₃ and C1q components of complement and for fibrin, was negative.

The response of urinary protein and tests of coagulation function to treatment with steroids are shown for this patient in Fig. 3.

FIG. 1. Immunoelectrophoresis of plasma from patients and pooled normal plasma at pH 8.6. Staining shows the precipitin arcs formed against anti-human fibrinogen antiserum (Hoechst Pharmaceuticals). Fibrinogen in plasma of both patients shows a slight increase in cathodal mobility.

Discussion

The second case showed high blood eosinophil count, high serum IgE concentration, low serum IgG concentration and normal glomerular filtration rate, features described in patients with atopic nephrotic syndrome (Reeves *et al.*, 1975), although there was no history of atopy in this patient. He also showed laboratory evidence of a coagulation defect strikingly similar to that found in the first

patient. In view of the similarity of the 2 cases and the selectivity of the proteinuria, it was decided to treat him with corticosteroids. The subsequent remission of the coagulation disorder, simultaneously with the improvement in renal function, allowed renal biopsy to be performed. The pathological diagnosis obtained of proliferative glomerulonephritis was unexpected, in view of the otherwise close resemblance to the first case and the rapid response to corticosteroid therapy.

The abnormality of coagulation function was apparently the same in both patients and unusual in type. It was characterized by prolongation of thrombin, kaolin cephalin and reptilase times in the presence of normal concentrations of fibrinogen.

FIG. 2. Rate of release of fibrinopeptide A (FPA) in blood allowed to stand at 22°C in a plastic syringe and sub-sampled at timed intervals, comparing a patient (Case 2) with a normal control subject. FPA was assayed as described above. ●—● control, ■—■ case 2.

The 2 most likely explanations of the defect were either that the patients had circulating anti-thrombin activity in plasma or that fibrin production was abnormal. It is most unlikely that the defect was produced by anti-thrombin activity since none was indicated by the results of mixing and dilution experiments, anti-thrombin concentration assayed by 2 different methods was normal, reptilase and anacrod times were prolonged, and FPA was released from fibrinogen at a normal or accelerated rate during blood clotting. Attempts were therefore made to demonstrate an abnormality of fibrinogen-fibrin conversion. After precipitation from plasma with ammonium sulphate, fibrinogen from both patients appeared to have normal molecular weight as assessed by polyacrylamide gel electrophoresis. There was possibly a small change in electrophoretic mobility of their fibrinogen demonstrated by immunoelectrophoresis. Factor XIII concentration was normal and supported polymerization of exogenous fibrin. Fully polymerized clots had normal stability in urea-acetic acid. Unfortunately, better

characterization of the defect was prevented because the coagulation abnormality rapidly disappeared with treatment of the nephrotic syndrome.

One plausible explanation of the defect is that fibrin monomer polymerized abnormally slowly in both patients. This is supported by the similarity between results of coagulation function tests in these patients and a group with liver disease (Lane *et al.*, 1977). In the patients reported by Lane and his colleagues, prolongation of thrombin and partial thromboplastin times was shown to be due to defective polymerization of fibrin monomer.

FIG. 3. Case 2. Response of proteinuria, thrombin time (TT) and kaolin-cephalin time (KCT) to treatment with prednisolone.

While abnormal bleeding is a well recognized feature of renal failure (Kazatchkine *et al.*, 1976) a coagulation defect in the nephrotic syndrome has been described only rarely. Reduced concentrations of factor IX (Rahman, Zanger and Natelson, 1975) and factor VII (Epstein *et al.*, 1976) have been reported. The suggested mechanism for the coagulation disorder in these patients was loss of factor IX and VII in the urine, accompanying the heavy albuminuria. A study of coagulation function in 45 patients with nephrotic syndrome (Kanfer *et al.*, 1970) indicated that increased procoagulant activity of plasma was much commoner than defective haemostatic function. However, prolongation of the thrombin time was a frequent finding. This was thought to be due to increased anti-thrombin activity because there was a close correlation with raised concentrations of α -2-macroglobulin in plasma. The prolonged thrombin time in these

patients was also unaffected by remission of the disease or administration of corticosteroid therapy. The defect of coagulation described in the present 2 patients is not explicable on the basis of these earlier reports.

The authors' findings and those previously reported underline the importance of careful screening of the coagulation system in cases of the nephrotic syndrome. Injudicious biopsy in patients similar to those described might easily result in serious haemorrhage from the kidney.

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