Originals

Haemostatic Changes in Diabetic Coma

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Summary. Diabetic coma is frequently associated with thromboembolic complications. A prospective study was undertaken of the haemostatic changes occurring in 15 patients (12 with ketoacidosis, three with the hyperosmolar syndrome) during diabetic coma. When compared with the results after stabilization of the diabetes, ketoacidosis was associated with significantly higher levels of factor VIII coagulant activity, factor VIII-related antigen and fibrin degradation products, a shorter partial thromboplastin time and reduced concentrations of antithrombin III. These results suggest that in uncomplicated ketoacidosis, haematological changes occur which may reflect vascular endothelial damage and intravascular fibrin deposition. Out of three deaths, two patients (both with the hyperosmolar syndrome) had evidence of disseminated intravascular coagulation. To reduce further the mortality and morbidity from diabetic coma, controlled clinical trials of anticoagulant and antiplatelet drugs may be indicated.

Key words: Diabetic coma, factor VIII, disseminated intravascular coagulation.

Many abnormalities of haemostatic function have been described in diabetes mellitus. These include increased platelet retention and aggregation [1], increased levels of coagulation factors [2] and diminished fibrinolytic activity [3]. Whether these abnormalities are related to the metabolic disturbances of diabetes is unclear.

Whatever the relationship between metabolic control and haemostatic dysfunction, it is increasingly realized that thromboembolic disease is a frequent complication of diabetic coma. Arterial thrombosis

may be responsible for up to one-third of deaths in patients with diabetic ketoacidosis [4], and is a frequent occurrence in non-ketotic hyperosmolar coma [5]. In addition, Timperley et al. have shown that localized deposition of fibrin may occur in fatal cases of ketoacidosis, particularly in the cerebral capillaries [6]. Over the past decade, a number of authors have described the association of disseminated intravascular coagulation and diabetic coma [6–8]. However, such case reports have been sporadic, and laboratory studies have generally been undertaken only after clinical signs were apparent. A prospective study was therefore designed to determine the nature of haemostatic changes in unselected cases of diabetic ketoacidosis and the non-ketotic hyperosmolar syndrome.

Subjects and Methods

Patients

Fifteen diabetic patients, admitted to the Aberdeen Teaching Hospitals with ketoacidosis or the hyperosmolar syndrome over a period of 12 months, were included in the study. Diabetic ketoacidosis was arbitrarily defined as a plasma glucose >14 mmol/l and a serum bicarbonate of 11 mmol/l or less in association with ketonuria. The non-ketotic hyperosmolar syndrome was defined as a plasma glucose >30 mmol/l with a serum osmolality of >350 mOsmol/l [9], serum bicarbonate >20 mmol/l and with no more than a trace of ketonuria.

Where possible, a second, “convalescent” sample was obtained from the patients at a subsequent outpatient visit when the diabetic state was stable (non-fasting plasma glucose <10 mmol/l and absence of ketonuria).

Methods

Venous blood was removed from the patients as soon as possible after arrival at hospital (mean 2.2 h, range 0.1–5 h) for biochemical and haematological studies. Blood for assays of the coagulation

Table 1. Clinical details of patients with diabetic coma

Patient no.	Sex	Age (years)	Duration of diabetes (years)	Previous diabetic treatment	Possible precipitating factors	Additional drugs	Depth of coma	Outcome
1	M	18	4	insulin	tonsillitis	erythromycin	3	recovery
2	F	52	new	none	breast abscess	amoxycillin, bendrofluazide K ⁺	1	recovery
3	F	65	46	insulin	unknown	—	4	death
4	F	21	10	insulin	unstable diabetes	—	3	recovery
5	M	17	new	none	unknown	—	4	recovery
6	F	15	6	insulin	unstable diabetes	—	2	recovery
7	F	13	1	insulin	dental abscess	—	2	recovery
8	M	33	13	insulin	ischio-rectal abscess	—	2	recovery
9	F	16	1	insulin	unstable diabetes	—	2	recovery
10	M	17	new	insulin	stopped insulin	—	3	recovery
11	F	33	new	none	unknown	—	2	recovery
12	M	42	16	insulin	unknown	—	3	recovery
13	M	65	new	none	unknown	—	3	recovery
14	M	73	new	none	unknown	frusemide, digoxin, inositol nicotinate	3	death
15	F	81	17	chlorpropamide	stopped sulphonylurea	—	3	death

Table 2. Biochemical data of patients with diabetic coma

Patient no.	Plasma glucose (mmol/l)	Serum urea (mmol/l)	Serum sodium (mmol/l)	Serum potassium (mmol/l)	Serum bicarbonate (mmol/l)	Serum osmolality (mOsmol/l)	Urinary ketones
1	32.4	9.4	128	5.4	5	309	+++
2	21.4	8.0	151	2.4	8	336	+++
3	54.9	14.7	132	7.2	5	348	+++
4	14.2	5.5	153	6.5	5	339	+++
5	78.5	38.0	146	4.4	11	417	+++
6	39.3	6.8	136	5.5	5	329	+++
7	25.0	4.0	133	3.7	5	302	+++
8	34.6	8.3	134	6.6	5	324	+++
9	17.1	8.2	142	4.6	11	318	++
10	20.9	5.6	136	3.5	5	305	+++
11	42.9	7.0	138	2.5	5	331	+++
12	69.0	39.0	143	6.5	11	407	++
13	31.1	18.2	161	4.1	24	380	—
14	56.1	35.2	145	5.7	22	393	trace
15	48.4	28.1	163	4.0	24	410	—

system was immediately placed on ice and centrifuged at $1500 \times g$ and 4°C within 30 min of venepuncture. Prothrombin and partial thromboplastin times were measured on fresh samples and the remaining plasma stored at -70°C until required.

The whole blood platelet count was measured microscopically [10]. Partial thromboplastin time was measured by the method of Langdell et al. [11] and the prothrombin time by the method of Quick [12]. Factor V and factor VIII coagulant activity (VIII: C) were assayed by one-stage techniques based on the correction of the prolonged clotting times of specific factor deficient plasmas [13, 14]. Factor VIII-related antigen (VIII R: Ag) was measured by immuno-electrophoresis using antibody to factor VIII: R: Ag produced by Behringwerke (Marburg, FRG) [15]. A normal pool of plasma obtained from 20 healthy male medical students was used as a control. Plasma antithrombin III was measured by a radial diffusion technique, using plates and standards supplied by Behringwerke. Plasma fibrinogen was measured by a modification

[16] of the method of Ratnoff and Menzie, and plasma plasminogen by the method of Alkjaersig et al. [17]. Fibrin degradation products were measured using the kit supplied by Wellcome Reagents (Beckenham, Kent, UK). Packed cell volume was estimated using a microhaematocrit centrifuge (Hawksley & Sons, Lancing, Sussex, UK). Since dehydration occurs during diabetic coma, concentrations of haemostatic factors were adjusted for changes in haematocrit [18]: Corrected concentration = measured concentration

$$\times \frac{H_1 (100 - H_2)}{H_2 (100 - H_1)}$$

Where: H_1 = haematocrit after coma
 H_2 = haematocrit during coma

This formula is based on the assumption that the red cell mass remains relatively constant during diabetic coma, and that changes in haematocrit reflect changes in plasma volume. Results are pre-

Table 3. Mean $\pm$ SEM values of haemostatic factors of 11 diabetics during ketoacidosis, ketoacidosis with the values corrected for dehydration and during convalescence

Test	Ketoacidosis (measured values)	Ketoacidosis (corrected values)	Convalescence	Normal controls ^a
Haematocrit (%)	45.6 $\pm$ 1.8 ^d		40.7 $\pm$ 1.5	
Prothrombin time (ratio)	1.00 $\pm$ 0.03		0.96 $\pm$ 0.04	
Partial thromboplastin time (ratio)	0.90 $\pm$ 0.04 ^c		0.99 $\pm$ 0.03	
Platelet count ($\times 10^9$ /l)	406 $\pm$ 43 ^c	331 $\pm$ 31	299 $\pm$ 17	
Factor V (%)	110 $\pm$ 10	90 $\pm$ 9	118 $\pm$ 15	
Factor VIII: C (%)	350 $\pm$ 36 ^b	278 $\pm$ 33 ^d	189 $\pm$ 23	
Factor VIII: Ag (%)	535 $\pm$ 156 ^b	511 $\pm$ 226 ^b	125 $\pm$ 10	
Fibrinogen (g/l)	6.35 $\pm$ 0.63	5.63 $\pm$ 1.02	5.19 $\pm$ 0.32	3.92 $\pm$ 1.35
Plasminogen (casein U/ml)	4.19 $\pm$ 0.16	3.51 $\pm$ 0.29 ^d	4.09 $\pm$ 0.17	3.56 $\pm$ 0.07
Antithrombin III (mg/dl)	32.2 $\pm$ 1.4 ^b	26.4 $\pm$ 1.0 ^b	36.3 $\pm$ 0.3	46.5 $\pm$ 1.0
Fibrin degradation products (μ g/ml)	17.7 $\pm$ 4.5 ^d	15.4 $\pm$ 4.4	8.7 $\pm$ 0.9	<5

^a Mean $\pm$ SEM values for 34 healthy non-diabetic subjects.^b $p < 0.01$, ^c $p < 0.02$, ^d $p < 0.05$ compared with convalescent values**Table 4.** Haemostatic tests of the fatal case of ketoacidosis (patient 3) and the three diabetics with the hyperosmolar syndrome (patients 13, 14, 15)

Test	Patient 3	Patient 13	Patient 14	Patient 15	Normal control ^a
Haematocrit (%)	40	40	49	39	
Prothrombin time (ratio)	1.01	1.01	1.41	0.88	
Partial thromboplastin time (ratio)	1.33	0.60	0.98	0.83	
Platelet count ($\times 10^9$ /l)	540	311	82	184	
Factor V (%)	104	102	42	104	
Factor VIII: C (%)	240	570	184	760	
Factor VIII: Ag (%)	248	280	352	1200	
Fibrinogen (g/l)	5.20	10.15	10.32	4.25	3.92 $\pm$ 1.35
Plasminogen (casein U/ml)	3.31	4.34	2.75	1.76	3.56 $\pm$ 0.07
Antithrombin III (mg/dl)	27.2	27.2	26.0	27.7	46.5 $\pm$ 1.0
Fibrin degradation products (μ g/ml)	20	10	160	80	<5

^a Mean $\pm$ SEM values for 34 healthy non-diabetic subjects

sented both as measured and corrected concentrations. Plasma glucose was measured by the glucose oxidase method and serum urea and electrolytes by autoanalyzer. Urinary ketones were measured by Ketostix (Ames). Serum osmolality was calculated using the formula of Gordon and Kabadi [9], and depth of coma on admission was assessed clinically, using a score of 1–4 (1 = normal, 2 = drowsy, but responding to verbal commands, 3 = responding only to painful stimuli, 4 = unrousable).

Statistics

Comparison between results during and after diabetic coma was made using the Wilcoxon rank sign test for paired samples.

Results

Patients

Fifteen diabetics were admitted to the study. Clinical details are shown in Table 1 and biochemical data in Table 2. Findings in the 12 patients with ketoacidosis

are discussed separately from the three patients with the hyperosmolar syndrome.

Patients with Ketoacidosis

Patient 3 died 3 h after admission following a cardiac arrest. The results of the haemostatic tests of the surviving 11 patients are summarized in Table 3. The normal values for fibrinogen, plasminogen, antithrombin III and fibrin degradation products shown in Tables 3 and 4 were obtained from 34 healthy non-diabetic controls (22 men, 12 women) with a mean age of 39.1 years (range 26–65 years). Compared with convalescent values, platelet count, factor VIII: C, VIII: Ag and fibrin degradation products were significantly increased. Antithrombin III concentrations were significantly lower and there was a significant shortening of the partial thromboplastin time during ketoacidosis.

When the results were corrected for the effect of dehydration, factor VIII: C and VIII R: Ag were still significantly higher and antithrombin III concentrations lower, during ketoacidosis. The differences in platelet count and fibrin degradation products were no longer significant, though seven out of the 11 patients had corrected concentrations of fibrin degradation products greater than 10 µg/ml compared with only one out of 11 convalescent samples. In addition, corrected mean plasminogen concentrations were significantly lower during ketoacidosis. The mean ($\pm$ SEM) factor VIII R: Ag/VIII: C ratio was 1.44 ± 0.27 during ketoacidosis, which was significantly higher than the convalescent value of 0.73 ± 0.08 ($p < 0.01$). There was no correlation between the levels of the various haemostatic factors and the depth of coma, the presence of overt infection, plasma glucose, serum bicarbonate and the serum osmolarity. Neither the two patients with severe hypokalaemia (patients 2 and 11) nor the two with a blood urea greater than 30 mmol/l (patients 5 and 12) had haemostatic values strikingly different from the remaining ketoacidotic patients.

Patients with the Hyperosmolar Syndrome

Out of the three diabetics with the hyperosmolar syndrome, two died (patients 14 and 15). The haemostatic results on admission are shown in Table 4, together with the fatal case with ketoacidosis (patient 3). Results are reported as uncorrected values. Patients 3 and 13 showed results similar to the surviving patients with ketoacidosis shown in Table 3. In contrast, the two fatal hyperosmolar cases (patients 14 and 15) had thrombocytopenia, low concentrations of plasminogen and raised levels of fibrin degradation products.

Patient 14 had a past history of ischaemic heart disease and intermittent claudication. On admission, he was found to have impalpable pulses from the left femoral downwards. The hyperosmolar state was rapidly corrected with fluid and insulin therapy, but perfusion of the left leg did not improve, below-knee amputation being required six days later, followed by death after a further eight days. The severe thrombocytopenia present on admission persisted throughout his final illness. Post-mortem was not performed.

Patient 15 was admitted in hyperosmolar coma. On examination she had minimal abdominal tenderness and absent bowel sounds. Death occurred 12 h after admission. At autopsy she was found to have 80 cm of infarcted small intestine and when sections were stained with Martius yellow, Crystal scarlet and soluble blue, fibrin was observed in capillaries, venules and arterioles of lungs, mesentery, renal glomeruli and cerebral cortex.

Discussion

This study shows that characteristic changes occur in the haemostatic system during diabetic ketoacidosis, the most striking abnormality being a rise in factor VIII: C and VIII R: Ag. Increased levels of the factor VIII complex are found in a variety of inflammatory, thromboembolic and neoplastic conditions [19]. On the other hand, high factor VIII: C and particularly VIII R: Ag concentrations have frequently been observed in stable diabetics [20, 21]. Recent evidence suggests that factor VIII Ristocetin cofactor activity (which tends to parallel the VIII R: Ag level) can be reduced by strict metabolic control of the diabetes [22]. The present study lends additional support to the view that poor diabetic control produces raised factor VIII levels. Since factor VIII R: Ag is synthesized by vascular endothelial cells [23], the high concentrations of this antigen found in diabetics with ketoacidosis may indicate the degree of insult sustained by the endothelium. Exposure of the vascular subendothelium is the first stage in the initiation of thrombus formation [24], so that endothelial cell damage with resulting de-endothelialization could be a major contributing factor to the high incidence of arterial thrombosis in fatal cases of diabetic ketoacidosis [4].

The cause of raised factor VIII: C activity during ketoacidosis is not known, but could be a non-specific response to acute stress [19]. Though raised levels of individual coagulation factors do not necessarily lead to an increased tendency to thrombosis [25], the finding of a significantly shortened partial thromboplastin time during ketoacidosis suggests that there is an increased activity of the intrinsic coagulation system, which might be expected to favour intravascular clotting. A slight, but significant, rise in the ratio of factor VIII R: Ag/VIII: C was observed during ketoacidosis. Denson has shown that a proportionate decrease in factor VIII: C in relation to VIII R: Ag can indicate intravascular coagulation [26]. However, since factor VIII: C activity is relatively labile, the possibility that coagulant activity was lost during blood collection or storage of the plasma cannot be excluded.

A fall in the concentration of antithrombin III appears to be an early and sensitive indicator of intravascular coagulation [27]. In the present study, a significant fall in antithrombin III together with a modest rise in fibrin degradation products provides evidence that even in relatively uncomplicated cases of ketoacidosis, a degree of fibrin generation occurs, and could contribute to the cerebral dysfunction often seen in this condition [6]. No correlation was found between the magnitude of the individual

haemostatic factor abnormalities and clinical and biochemical variables such as presence of overt infection, depth of coma, extent of hyperglycaemia, osmolality or uraemia. Since levels of both haemostatic and metabolic factors can change rapidly, future studies with more frequent blood sampling during recovery from ketoacidosis are required for clearer understanding of the relationships between clinical, metabolic and haemostatic parameters.

Two of the three patients with the hyperosmolar syndrome died during their admission to hospital. Both deaths occurred in elderly patients and illustrated the considerably higher mortality rate due to the hyperosmolar syndrome compared with that from ketoacidosis [9]. Both patients had laboratory, and in one case, pathological evidence of disseminated intravascular coagulation. The majority of patients with disseminated intravascular coagulation have reduced levels of fibrinogen and plasminogen, thrombocytopenia and diminished factor V and VIII:C with high levels of fibrin degradation products [27, 28]. These haematological findings do not clearly distinguish disseminated intravascular coagulation from massive thrombosis at a single site, as was found in patient 14. Nevertheless, the persistent thrombocytopenia found in this patient is suggestive of platelet consumption in sites additional to the occluded femoral artery.

As shown in the present study, coagulation factor levels are frequently raised during uncomplicated diabetic coma, making any changes induced by disseminated intravascular coagulation difficult to interpret. Thus in previous reports of disseminated intravascular coagulation associated with diabetic coma, fibrinogen and factors V and VIII:C were high, low or normal [6–8], in agreement with the two cases in the present series. Thrombocytopenia and raised fibrin degradation products appear to be more consistent findings. In addition, a low plasma plasminogen concentration may point to the diagnosis.

The aetiology of disseminated intravascular coagulation during diabetic coma is likely to be multiple. Infection [27, 28] and hypovolaemia [28] are known precipitating factors. Metabolic abnormalities may also play a part since disseminated intravascular coagulation is more readily induced in alloxan-treated compared with control rats [29]. The use of sensitive radioimmunoassays such as those for fibrinopeptide A, beta-thromboglobulin and platelet factor 4 may provide information on the relative contributions of the coagulation system, platelets and endothelium to the pathogenesis of intravascular coagulation during diabetic coma.

In summary, this study shows that during diabetic ketoacidosis, haemostatic changes are found which

may reflect endothelial damage and also suggest intravascular fibrin deposition. In two fatal cases of the hyperosmolar syndrome, evidence of disseminated intravascular coagulation was found. In view of the high incidence of thromboembolic complications associated with diabetic ketoacidosis and the hyperosmolar syndrome, and the disturbances of the haemostatic system reported in this paper, controlled clinical trials of anticoagulant and antiplatelet drugs would appear to be justified in the management of these two conditions.

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