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Acquired dysfibrinogenaemia in liver disease

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SUMMARY Using a new and sensitive screening method, dysfibrinogenaemia (DF) was detected in 76% of patients with cirrhosis, 78% with chronic active liver disease and 86% with acute liver failure. The incidence was much lower in obstructive jaundice (8%) and miscellaneous liver disorders (4%). It is concluded that the fibrin monomer polymerisation (FMP) ratio test is a simple and sensitive test for the detection of DF, and is useful in the differential diagnosis of hepatocellular and obstructive jaundice. Hyperfibrinogenaemia, particularly in patients with obstructive jaundice, may explain the high incidence of abnormal thrombin and Reptilase clotting times despite normal FMP ratios. Dysfibrinogenaemia does not appear to be related to the degree of liver function impairment, but may be associated with regeneration of hepatic tissue.

Among the many abnormalities of blood coagulation in patients with liver disease, the finding of prolonged thrombin clotting times have been least understood. The abnormality occurs too frequently to be due to hypofibrinogenaemia or raised concentration of fibrin degradation products. There is evidence that abnormal thrombin times are associated with an increase in low molecular weight fibrinogen derivatives in some patients,[1] although other workers have found no difference in the degree of α-chain degradation between normal and abnormal fibrinogens.[2]

Recently however, the prolongation of the thrombin time in many patients has been explained by the demonstration of defective fibrin monomer polymerisation. Since the original report[3] of acquired dysfibrinogenaemia (DF) in a patient with severe hepatitis, several workers have described abnormal fibrinogen function in association with hepatic disease.[4-6] The finding of abnormal fibrin polymerisation in plasma, but not in purified fibrinogen preparations has led to the suggestion that the defect is due to a plasma inhibitor rather than a defective molecule.[7] However, under appropriate experimental conditions, the abnormality may be demonstrated using purified fibrin monomers, and is strong evidence that an abnormal fibrinogen molecule is synthesised in liver disease.[8 9]

Green *et al*[10] showed that DF was a common occurrence in patients with cirrhosis, chronic active liver disease, primary liver cell tumour and acute liver failure. These workers later provided evidence to suggest that DF is a reflection of the severity of liver function impairment,[11] although others have suggested that DF is associated with hepatocellular regeneration.[12]

The present work was performed to confirm the high incidence of acquired DF in liver disease using a new technique, and to compare the findings to conventional clotting test results.

Patients and methods

Two hundred and thirty patients with clinical evidence of liver disease were studied. The diagnosis was obtained by liver biopsy in 70 cases of cirrhosis and in all cases of chronic active liver disease and secondary carcinoma of the liver. All other patients were diagnosed on the basis of clinical and laboratory evidence. In order that the results may be directly compared to the Manchester study,[10] the patients were divided into groups according to the diagnosis. Ninety patients had cirrhosis, 18 chronic active liver disease, 48 obstructive jaundice, 29 acute liver failure and 45 miscellaneous disorders (30 secondary carcinoma, 2 Gilbert's syndrome, 8 congested liver with heart failure and 5 non-cirrhotic alcoholic liver damage). Fifty samples from healthy adult volunteers or patients referred for haemostatic evaluation with no evidence of liver disease, acted as the control group.

Thrombotest and Normotest (Nyegaard, Oslo) determinations were performed on citrated whole blood according to the maufacturer's instructions.

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Table 1 *Results of Normotests, Thrombotests, thrombin times, Reptilase times and FMP ratios in patients with normal and abnormal FMP*

		Controls	Cirrhosis			Obstructive jaundice		
			All cases	Normal FMP	Abnormal FMP	All cases	Normal FMP	Abnormal FMP
Normotest	Mean	102·1	60·5	62·2	59·9	79·3	81·5	56·0
	SD	15·2	21·2	20·0	21·7	29·3	29·0	25·8
Thrombotest	Mean	99·6	63·2	56·6	65·4	60·9	62·6	42·2
	SD	14·0	26·8	16·7	29·1	31·2	30·5	37·7
Thrombin time	Mean	19·6	29·4	23·3	31·3	25·6	24·8	34·4
	SD	1·5	6·9	3·6	6·5	4·5	3·5	5·9
Reptilase time	Mean	16·8	22·6	18·7	23·9	20·2	19·7	25·5
	SD	1·4	4·7	2·2	4·6	4·3	4·1	2·5
FMP ratio	Mean	2·4	37·1	2·2	48·3	3·0	1·7	17·3
	SD	0·25	39·2	0·3	38·9	6·0	0·4	16·4
Number		50	90	22	68	48	44	4

Thrombin times were performed by adding 0·1 ml bovine thrombin (5 units/ml) to a mixture of 0·1 ml plasma and 0·1 ml 0·15 *M* NaCl. Reptilase times were performed by adding 0·1 ml Reptilase to 0·2 ml plasma. All clotting times were recorded in duplicate on an automatic coagulometer (Burkard Scientific Ltd).

Fibrin monomer polymerisation (FMP) was assessed by a modification of the technique used by Green *et al.*[10] Plasma (0·25 ml) was diluted with 1·0 ml 0·15 *M* NaCl and 0·05 ml Reptilase added. The change in optical density at 350 nm was monitored for 10 min. The test was then repeated by diluting a further 0·25 ml volume of plasma with 1·0 ml of a mixture containing equal volumes 0·15 *M* NaCl and 0·025 *M* $CaCl_2$. Reptilase (0·05 ml) was then added, and the change in optical density recorded as detailed above. The optical densities at 10 min were noted and the results expressed as the ratio A^{350} (calcium)/A^{350}(saline). Preliminary experiments (unpublished) indicated that the FMP ratio remained constant despite variations in fibrinogen concentrations between samples. Fibrinogen concentrations were therefore not adjusted before testing.

The effect of dilution on the prolonged thrombin clotting times of three patients with obstructive jaundice and hyperfibrinogeneamia was determined by serial dilution of each plasma with 0·15 *M* NaCl. Thrombin times were recorded as detailed above.

Results

Fibrin monomer polymerisation ratios of 50 normal adult controls gave results of 2·4 ± 0·25 (mean ± SD). Fibrin polymerisation was therefore considered to be abnormal when the FMP ratio exceeded 3·0.

Abnormal FMP was detected in 76% of patients with cirrhosis, 78% with chronic active liver disease, 8% with obstructive jaundice, 86% with acute liver failure and 4% with miscellaneous liver disorders. The results of Normotests, Thrombotests, thrombin times, Reptilase times and FMP ratios in patients with normal and abnormal fibrinogen function are summarised in Table 1.

Each group of patients was subdivided into those with normal and abnormal FMP ratios, and the results of each test compared using an unpaired Student's *t* test. The results are shown in Table 2. Normotest and Thrombotest values in patients with abnormal FMP ratios were not significantly different from those with normal fibrinogen function. Thrombin and Reptilase clotting times, however, were significantly more prolonged in patients with abnormal FMP ratios.

The frequencies of abnormal clotting test results are shown in Table 3. The thrombin time was most commonly abnormal, although most patients also showed prolongation of the Reptilase time. The majority of patients with abnormal thrombin and Reptilase times without demonstrable DF fell into the obstructive and miscellaneous groups.

The thrombin time was more discriminating in the detection of DF than the Reptilase time. The mean FMP ratios for increasing thrombin and Reptilase times in patients with cirrhosis are shown in Table 4.

The correlation between thrombin times, Reptilase times and FMP ratios in each group of patients with abnormal fibrinogen function is shown in Table 5. The correlation of thrombin time and Reptilase time was highly significant in all groups except obstructive jaundice. Similarly, the correlation between thrombin or Reptilase times and the FMP ratio was highly significant in all groups except obstructive jaundice.

The effects of dilution on the thrombin clotting time of three patients with obstructive jaundice and hyperfibrinogenaemia are shown in the Figure.

Chronic active liver disease			*Liver failure*			*Miscellaneous*		
All cases	*Normal FMP*	*Abnormal FMP*	*All cases*	*Normal FMP*	*Abnormal FMP*	*All cases*	*Normal FMP*	*Abnormal FMP*
69·7	72·0	69·1	53·5	45·7	54·8	87·3	88·0	72·5
21·5	10·5	24·0	30·4	5·7	32·6	22·0	22·2	3·5
70·6	60·0	73·6	55·4	43·2	57·4	79·6	79·3	85·0
24·0	19·4	24·9	30·6	6·2	32·6	21·2	21·4	21·2
26·4	21·2	27·9	29·8	22·6	31·0	23·9	23·3	37·2
4·7	2·1	4·2	7·3	2·4	7·1	5·1	3·9	11·0
21·7	18·5	22·6	22·8	17·1	23·7	19·3	18·9	28·1
4·5	4·7	4·1	5·4	2·1	5·2	4·7	4·4	7·6
31·2	2·05	39·6	29·4	2·05	33·8	3·7	1·85	42·9
37·2	0·5	38·4	28·6	0·4	28·4	11·4	0·4	49·6
18	4	14	29	4	25	45	43	2

Table 2 *Results of unpaired Student's t tests in the comparison of clotting tests on patients with normal and abnormal FMP*

Diagnosis	*n*		*Normotest*	*Thrombotest*	*Thrombin time*	*Reptilase time*	*FMP ratio*
Cirrhosis	90	*t*	0·435	1·347	5·51	4·96	5·55
		p	0·67	0·18	<0·001	<0·001	<0·001
Obstructive jaundice	48	*t*	1·695	1·26	4·92	2·75	7·13
		p	0·09	0·21	<0·001	0·08	<0·001
Chronic active liver disease	18	*t*	0·23	1·00	3·03	1·69	1·91
		p	0·81	0·33	0·008	0·11	0·07
Liver failure	29	*t*	0·55	0·85	2·3	2·67	2·2
		p	0·6	0·59	0·03	0·01	0·03
Miscellaneous	45	*t*	0·97	0·36	4·56	2·87	7·49
		p	0·66	0·72	<0·001	0·006	<0·001

Table 3 *Frequency of abnormal clotting tests in all patients (%)*

Diagnosis	*n*	*Normotest (<70%)*	*Thrombotest (<70%)*	*Thrombin time (>22s)*	*Reptilase time (>18s)*	*FMP ratio (>3·0)*
Cirrhosis	90	72	63	90	89	76
Obstructive jaundice	48	37	54	83	58	8
Chronic active liver disease	18	56	39	78	83	78
Liver failure	29	76	69	93	86	86
Miscellaneous	45	20	27	56	47	4

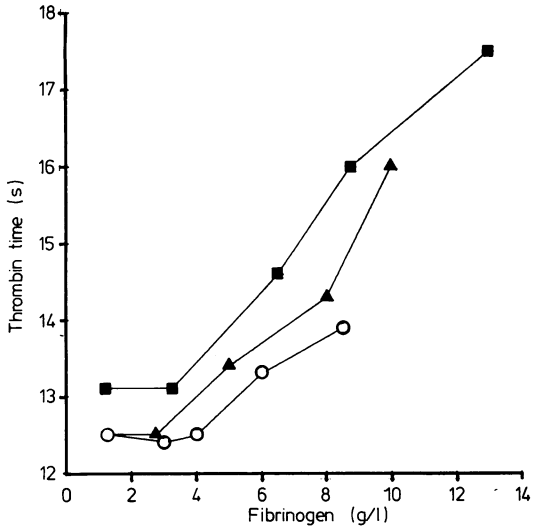
Table 4 *Discriminating power of thrombin and Reptilase times in the detection of dysfibrinogenaemia*

Thrombin time "Less than" (s)	*FMP ratio*	*n*	*Reptilase time "Less than" (s)*	*FMP ratio*	*n*
20	2·0	6	16	2·2	2
21	2·1	7	17	6·5	5
23	2·3	11	18	4·6	9
24	3·2	18	19	4·1	12
25	6·8	21	20	10·4	22
26	7·0	27	21	18·0	36
27	8·2	29	22	19·8	46
28	14·9	36	23	22·4	55
29	21·6	52	24	24·2	59
30	21·7	56	25	25·5	68
35	32·6	79	30	34·2	83

Table 5 *Correlation of thrombin times, Reptilase times and FMP ratios in patients with abnormal fibrinogen function*

Diagnosis	*n*		*Thrombin time v Reptilase time*	*Thrombin time v FMP ratio*	*Reptilase time v FMP ratio*
Cirrhosis	68	r	0·76	0·40	0·40
		p	<0·001	<0·001	<0·001
Chronic active liver disease	14	r	0·85	0·69	0·73
		p	<0·001	0·005	0·005
Obstructive jaundice	4	r	0·34	0·55	0·32
		p	0·60	0·35	0·65
Liver failure	25	r	0·91	0·70	0·67
		p	<0·001	<0·001	<0·001
Miscellaneous	2*	r	—	—	—
		p	—	—	—

* Insufficient data for statistical analysis.



The effect of dilution on the thrombin clotting time of three patients with obstructive jaundice and hyperfibrinogenaemia

Diluting the plasmas to normal fibrinogen concentrations restored the prolonged thrombin clotting times to normal values.

Discussion

The occurrence of a qualitatively abnormal fibrinogen molecule in some cases of liver disease has been recognised for some time, although it was not until the study of Green *et al*[10] that the frequency of acquired dysfibrinogenaemia (DF) was appreciated. These workers used a simple colorimetric screening test, and demonstrated abnormal FMP in 50% of patients with cirrhosis, 47% with chronic active liver disease, 66% with primary liver tumour and 100% with acute liver failure. The defect was not demonstrated in patients with obstructive jaundice or miscellaneous liver disorders. Despite this high incidence however, a number of patients exhibited prolonged thrombin clotting times although FMP was apparently normal.

In the present study, the colorimetric method of Green *et al*[10] was modified by the inclusion of an additional test in the presence of calcium ions. The results were expressed as the ratio of FMP values obtained in calcium and saline. This value was extremely constant in normal individuals, despite variation in fibrinogen concentrations, and obviated the need to adjust the fibrinogen levels before testing. The narrow normal range of this technique enabled the detection of milder degrees of DF than the original method.

Using this technique, DF was detected in 76% of patients with cirrhosis, 78% with chronic active liver disease and 86% with acute liver failure. These results suggest a higher incidence than that reported by the Manchester group,[10] probably due to the increased sensitivity of the FMP ratio method as a screening test for DF. A number of patients with normal FMP had prolonged thrombin or Reptilase times, or both, although the abnormalities were minimal in most cases. This may have been due to very mild degrees of DF not detectable by the present method, or in some cases to high fibrinogen concentrations interfering with fibrin polymerisation. The prolonged thrombin times of some patients with obstructive jaundice and hyperfibrinogenaemia were corrected by dilution to normal fibrinogen concentrations. As many patients with obstructive jaundice and secondary liver disease have hyperfibrinogenaemia, it is probable that this is the cause of the prolonged thrombin and Reptilase clotting times in patients with normal FMP.

The incidence of DF in obstructive jaundice was markedly lower than that associated with hepatocellular jaundice. This is in support of previous studies[10] which suggested that a test of FMP is a useful aid in the differential diagnosis of these two

conditions. It is of particular interest that the four patients with obstructive jaundice in whom DF was demonstrated had developed cirrhosis secondary to long-standing obstruction of the common bile duct. The FMP ratio test may therefore be of value in monitoring such patients for the development of secondary hepatocellular damage. Dysfibrinogenaemia was detected in two patients in the miscellaneous liver disease group. Both patients had secondary deposits of renal carcinoma in the liver.

The thrombin time was more discriminating than the Reptilase time in the detection of DF. This may have been because the dilution of plasma used in the thrombin time method overcame the inhibitory effect of hyperfibrinogenaemia, particularly in patients with obstructive jaundice. The Reptilase method used undiluted plasma, and prolonged clotting times may have been obtained on these samples. This is supported by the finding of longer Reptilase times in patients with obstructive jaundice and normal FMP, than in any other group with normal fibrinogen function (Table 1).

There were no significant differences between Normotest and Thrombotest results in patients with normal and abnormal FMP ratios. As Normotest results are considered to be a good test of liver function,[13] this suggests that there are no differences in the degree of liver function impairment between the two groups. This is in contrast to the findings of Green and co-workers[11] who reported greater prolongation of the prothrombin time and lower factor VII concentrations in patients with abnormal FMP. In a previous (unpublished) study, we found no significant differences in bilirubin, alkaline phosphatase, SGPT or gamma-glutamyltransferase results between patients with normal or abnormal FMP.

It has been suggested[12] that prolongation of the Reptilase time (RT) and demonstration of DF do not reflect the same phenomenon. This conclusion was based on the finding of DF without prolonged RT in some patients, and prolonged RT without DF in others. These workers found a significant correlation between the RT and serum aspartate aminotransferase activities, and postulated that the abnormal RT was due to the presence of inhibitors released from necrotic hepatocytes. However, it is difficult to compare these results to the present study as the authors did not include a thrombin clotting time as a screening test, and did not use abnormal fibrin polymerisation as their criterion for DF. We have confirmed that the RT may be normal in mild DF, and prolonged in the absence of DF as defined by an abnormal FMP ratio test. However, we have concluded that when DF and prolonged RT occur together they represent the same defect. This is supported by the finding of a significant correlation between RT and FMP ratios in patients with abnormal fibrinogen function. Mild DF may exist without prolongation of the RT however, and other factors, particularly hyperfibrinogenaemia, may prolong the RT despite normal FMP. None of our cases however had demonstrable DF without an abnormal thrombin clotting time.

Barr and co-workers[12] have suggested that DF may be the result of re-expression of fetal genes coding for fetal fibrinogen production in liver cell regeneration. There is evidence to suggest that the abnormal fibrinogens of liver disease and newborn infants are functionally identical,[14] although whether the defect is genetically determined has not been established. The sialic acid contents of both forms of abnormal fibrinogen are increased,[14 15] and raised serum and liver sialyltransferase activities have been described in association with hepatocellular regeneration.[16] This hypothesis would explain the high incidence of DF in cirrhosis where areas of hepatocellular regeneration are common, and the relatively low frequency in secondary liver disease, particularly metastatic carcinoma. In this latter condition there is usually no histological evidence of liver cell regeneration.[17]

On the basis of the present results it has been concluded that:

(i) The FMP ratio test is a simple and sensitive technique for the detection of DF, and is a useful aid in the differential diagnosis of hepatocellular and obstructive jaundice.

(ii) The incidence of DF as defined by an abnormal FMP ratio is higher than has been described previously.

(iii) Hyperfibrinogenaemia, particularly in patients with obstructive jaundice, is responsible for prolonged thrombin and Reptilase times in the absence of DF.

(iv) DF is not related to the degree of liver function impairment, but may be associated with hepatocellular regeneration. Further studies are required to establish the relation between DF and regeneration of hepatic tissue.

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