Liver function and the diagnostic significance of biochemical changes in the blood of African children with sickle cell disease

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SUMMARY The blood biochemistry of 84 young homozygous sickle cell patients aged 1 to 11 years was analysed for evidence of liver disease. A comparison of the values found in their blood with those seen in normal children from an identical age group, assessed at the same time, shows no convincing evidence of liver cell damage except in 12% of cases. The normal transaminase observed in many of the patients assessed, together with the high alkaline phosphatase activity which seemed to be out of proportion to plasma bilirubin, is a picture compatible with localised obstructive lesions of the liver or bone lesions, both of which are common in sickle cell disease. This biochemical pattern suggests that the conjugated bilirubin, which dominates the picture in 40% of patients who have 'haemolytic jaundice', is due largely, not to liver cell damage, but to a combination of two factors, namely, intrahepatic cholestasis and the presence of actively functioning liver cells. Adequate albumin synthesis found in these patients, together with normal thymol reactions, provides further evidence of the absence of severe liver cell damage.

Although much is known about the macroscopic and microscopic appearances of the liver in sickle cell disease,¹⁻⁴ still very little is known about the biochemical consequences of this disease at the cellular level. The data gathered (Table 1), nearly all of which were based on case studies of adult patients, can hardly be regarded as adequate, especially considering the fact that most of those who have this disease are children, since few survive into adult life. Besides, it is possible that what may be happening in the liver of the adult patient, as well as the postmortem changes described by previous authors, may be the terminal development, the result of a

continuous assault of this disease on this vital organ, which may not necessarily be found among the younger patients in the early phase of the disease. The assessment of this group on a wider scale was therefore thought to be an important exercise which could yield additional and valuable information.

Material

A total of 84 young homozygous sicklers aged 1-11 years were examined. For the purpose of clinical assessment they were divided into two further age groups: 1-4 years and 5-11 years. A reference control group of healthy children of the latter age group was also investigated.

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Table 1 *Results of biochemical analysis of blood from patients with homozygous sickle cell disease in previous investigations*

Reference	No. of patients	Jaundice %	High alk phosphatase %	High SGOT %	Mean serum protein (g/l)			A/G ratio
					Total	Albumin	Globulin	
1	1	(++)	(Normal)	—	88	42	46	1.12/1
2	4	75	75	—	77	40	38	1.05/1
3	5	100	100	(Markedly elevated)	92	41	51	0.80/1
4	12	100	82	82%	72	42	30	1.4/1

The patients were first seen in the paediatric outpatients clinic. The diagnosis of the disease was made on clinical grounds, and confirmed by electrophoresis in the laboratory, after preliminary investigations had revealed extremely low haemoglobin and haematocrit values. There was no history or evidence of intrinsic liver disease in the patients. The control group was also screened for evidence of sickle cell disease.

Methods

Blood was collected from each child for the following determinations: serum bilirubin (total and direct reacting bilirubin), serum alkaline phosphatase, serum alanine aminotransferase, total serum protein, albumin, globulin, and thymol turbidity. Urine collection from the young patients proved difficult and had to be abandoned owing to reasons beyond one's control. Suitable laboratory control samples were included in each batch of tests to ensure accuracy, and only unhaemolysed specimens were analysed.

HAEMOGLOBIN ELECTROPHORESIS

This was performed on cellulose acetate strips according to Dacie and Lewis.⁵

BILIRUBIN

The estimation was done according to the method of Malloy and Evelyn.⁶

SERUM ENZYMES

The serum alkaline phosphatase was determined according to the modified version of Kind and King.⁷

The alanine aminotransferase was determined according to the colorimetric method of Reitman and Frankel.⁸

SERUM PROTEIN

The Biuret method used for protein estimation was according to Wooton.⁸

Results

The details of these are shown in Table 2.

SERUM BILIRUBIN

Total bilirubin

Raised bilirubin levels were seen in only 68% of the 84 sickle cell patients investigated. The concentration varied from patient to patient but the highest bilirubin level seen was 239 $\mu\text{mol/l}$ (14 mg/100 ml). The overall mean and range values were very low because a good proportion of the patients had normal bilirubin values (Table 2). The changes seen in the mean total bilirubin in both groups of sickle cell patients were highly significant compared with those of the normal group (Table 2):

Hb SS (1-4 years) $t = 3.11$, $df = 42$, $P < 0.0025$;

Hb SS (5-11 years) $t = 5.51$, $df = 43$, $P < 0.0005$.

Conjugated bilirubin

Conjugated bilirubin dominated the picture in 40% of the patients. Compared with the values in normal children, the mean value was also significantly higher (Table 2): Hb SS (1-4 years) $t = 2.28$, $df = 41$, $P < 0.025$; Hb SS (5-11 years) $t = 6.02$, $df = 42$, $P < 0.005$.

Table 2 Serum bilirubin, alkaline phosphatase, alanine aminotransferase, serum protein, and thymol turbidity: mean and range values of sickle cell patients compared with values from normal children of equivalent age

Group (No. of patients)	Age (yr)	Bilirubin ($\mu\text{mol/l}$)		Alanine aminotransferase (IU/l)	Alkaline phosphatase (IU/l)	Serum protein (g/l)				Thymol turbidity
		Total	Conj.			Total	Albumin	Globulin	A/G ratio	
Sickle cell anaemia (42)	1-4									
Mean		29.2	13.6	17	96	74	45.3	29	1.61/1	2.0
SD		2.3	1.7	13.8	41	6	4.6	5.5	0.37	1.2
Range		3.4-109.4	1.7-42.8	3-44	14-178	62-86	36-55	18-40	0.87-2.3/1	0.4-5
Sickle cell anaemia (42)	5-11									
Mean		35.2	16.0	14	100	80	43.5	35	1.28/1	1.6
SD		1.7	0.79	9.6	37	9	4.3	8.1	0.32	1.0
Range		6.8-95.8	5.1-42.8	4-28	26-174	62-94	35-52	19-40	0.80-1.92/1	0.3-8
Normal children (42)	5-11									
Mean		10.3	3.4	13.8	50	74	43.7	30	1.52/1	2.1
SD		0.25	0.10	3.3	30	5.8	3.4	5.8	0.39	1.2
Range		4.3-17.1	1.71-8.6	7-20	20-110	63-85	37-51	19-41	0.90-2.3/1	0.7-4.5

Conversion: SI to traditional units—Bilirubin 1 $\mu\text{mol/l} \approx 0.058$ mg/100 ml. Standard deviation for bilirubin calculated from traditional unit values.

ALANINE AMINOTRANSFERASE

Only isolated cases, 12%, among the sickle cell patients were found with high levels ranging from 25 to 70 IU (Table 2; Fig. 1). There was a higher proportion of children with extreme values in the younger group with the disease. Compared with the normal children of identical age, there was no significant change in the mean value of this enzyme (Table 2): Hb SS (1-4 years) $t = 1.46$, $df = 46$, $P < 0.1$; Hb SS (5-11 years) $t = 0.13$, $df = 51$, $P < 0.45$.

Fig. 1 Alanine aminotransferase activity in serum of children with sickle cell disease and of normal children of equivalent age.

ALKALINE PHOSPHATASE

The difference between the mean values of the serum enzyme activity of the sickle cell patients and the normal children was highly significant: Hb SS (1-4 years) $t = 5.87$, $df = 77$, $P < 0.0005$; Hb SS (5-11 years) $t = 6.80$, $df = 80$, $P < 0.0005$.

ALBUMIN

No significant changes in the mean values were seen in the two abnormal groups compared with the

normal children: Hb SS (1-4 years) $t = 1.8$, $df = 77$, $P < 0.05$; Hb SS (5-11 years) $t = 0.24$, $df = 80$, $P < 0.45$.

Discussion

In discussing the efficiency of liver function in sickle cell Africans, one must not fail to recognise the fact that the biochemical pattern of liver function in blood, as seen in the African, differs in certain respects from that accepted as typical for Caucasians.⁹

The alanine aminotransferase (SGPT) levels seen among the sicklers in this investigation (Table 2; Fig. 1) show no convincing evidence of significant liver cell damage as a result of the disease, except in a small number of cases. Normal African children appear to have higher values of this enzyme than their adult counterparts who were also assessed in whom the mean and range values were 8 IU/l and 2-14 IU/l respectively. Compared with their normal counterparts, the abnormal children generally had higher levels of this enzyme (probably due to cholestasis), but the changes seen cannot be considered significant. The higher incidence of liver cell damage found in the younger group (Table 2; Fig. 1) seems to suggest that age may well be an important determinant of liver efficiency in this disease. At the other end of the spectrum, it is also likely that the cumulative effect of a continuous assault on the liver by repeated crises may cause irreparable damage and lead to gross changes, with their biochemical effects, such as were described by previous workers on adult patients. Bogoch *et al.*,² for example, found one instance of haemochromatosis and two of portal cirrhosis in the four adult patients they studied.

Overt jaundice does not seem to be a constant feature of sickling, as only 68% of the children investigated showed biochemical evidence of jaundice (Table 3 group A). This finding supports the view of Bogoch *et al.*,² who found jaundice in 75% of the limited number of cases they studied. The degree of jaundice seen can be described as only moderate, the vast majority having a bilirubin level below 51 $\mu\text{mol/l}$ (3 mg/100 ml). Of these, some had a biochemical picture typically seen in haemolytic jaundice (Table 3 group B). In two-fifths of the patients a preponderance of conjugated bilirubin dominated the picture, masking the haemolytic component. Similar increases in this fraction of bilirubin have also been described previously in sickle cell patients by Owen *et al.*³ as well as Rosenblate *et al.*⁴

There is overwhelming evidence of increased alkaline phosphatase activity in the blood (Table 2;

Table 3 Pattern of biochemical changes in blood of 30 children with homozygous sickle cell disease

Group		Patient	Bilirubin (μmol/l)		SGPT (iu/l)	Alk.phos. (iu/l)	Biochemical features
			Total	Conjugated			
A	SS patients with no biochemical evidence of jaundice	EN	17	5	12	31	Normal bilirubin
		EO	14	10	9	95	„ aminotrans-
		NC	14	7	5	90	ferase
		NI	12	9	15	71	„ alk.phos-
		OI	10	7	4	27	phatase
		OS	7	3	6	34	
B	SS patients with uncomplicated haemolytic jaundice	IC	240	34	10	71	Relatively high total bilirubin. Preponderance of unconjugated bilirubin. Normal aminotransferase
		EV	104	44	17	56	
		OO	90	29	9	18	
		MU	78	29	15	24	
		CC	75	7	14	63	
		OC	65	14	8	83	Normal alk.phosphatase
C	SS patients with jaundice and biochemical evidence of liver cell damage	OE	108	80	63	192	High aminotrans-
		OO	51	43	41	174	ferase
		ON	31	24	41	71	Moderate or high total bilirubin
		NM	26	15	34	158	Preponderance of conjugated bilirubin
		ON	32	24	25	71	High or normal alk. phosphatase
		MA	31	21	24	182	High alk.phosphatase
D	SS patients with high alkaline phosphatase as the dominant feature	NN	29	21	12	261	Moderately raised or normal bilirubin
		OE	21	17	15	240	Bilirubin mostly in conjugated form
		NC	56	36	10	215	Normal aminotrans-
		IE	56	34	12	190	ferase
		IC	87	68	10	190	
		UK	22	5	10	182	
		EV	12	3	10	180	
		EO	12	5	6	174	
		IO	27	7	25	174	
		AS	12	5	6	165	
		EM	19	14	15	162	
		OC	10	7	8	160	
		Normal values [mean and range]			10[4-17]	3[2-9]	14[7-20]

Conversion: SI to traditional units—Bilirubin $1 \mu\text{mol/l} \approx 0.058 \text{ mg/100 ml}$.

Fig. 2), but this did not show any consistent pattern, indicating that this rise may have a multiple aetiology. Only a small proportion of those studied (12%) had biochemical evidence of hepatocellular damage (Table 3 group C; Fig. 1), a figure very close to that observed by Diggs.¹⁰ In the rest (Table 3 group D), the rise in alkaline phosphatase, which appeared to be the dominant feature, seemed to be out of proportion to plasma bilirubin levels. This biochemical relationship is compatible with localised biliary obstructive lesions in the liver or bone lesions, both of which are known to be common in sickle cell disease.^{2,11} The liver specific 5-nucleotidase or γ -glutamyltransferase could not be estimated to differentiate this latter group from the former, owing to reasons beyond one's control. The association of conjugated bilirubin with high alkaline phosphatase in some of these patients (Table 3 group D), however, is significant, suggesting that intrahepatic obstructive pathology is present, at least in association with bone lesions. It would therefore not be entirely wrong, in the absence of any evidence of liver cell dysfunction, to draw the conclusion that intrahepatic cholestasis, rather than

liver cell dysfunction, is largely responsible for the increase in conjugated bilirubin seen in a large proportion of these patients who have haemolytic disease.

Adequate albumin seemed to be produced, in spite of the low albumin/globulin ratio observed in some patients. A mean albumin level of 44 g/l found in these patients suggests that there is no hypalbuminaemia and therefore no liver cell dysfunction (Table 1). The hyperglobulinaemia, which characterises the serum protein pattern of the patients with sickle anaemia, formed one of the themes of one of my earlier papers.¹² The comment must, however, be made that albumin/globulin relationships are of little value in assessing hepatic function in sickle cell patients, since a moderately low albumin/globulin ratio is accepted as normal for the African.¹³ In contrast, there is evidence⁹ that protein flocculation tests are more sensitive for distinguishing Africans who have high globulin due to liver disease from healthy Africans with a low albumin/globulin ratio. But that, too, has proved negative in over 80% of the cases assessed here (Table 2) and is further evidence that there is no severe hepatocellular damage

Fig. 2 Alkaline phosphatase activity in serum of children with sickle cell disease and of normal children of equivalent age.

in the young patients with this disease investigated in this study.

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