Liver in haemoglobin H disease

CHEW BENG KENG AND TAN KHENG KHOO

From the Department of Medicine, University of Singapore, and Department of Pathology, Outram Road, Singapore

SUMMARY There have been no published reports on the liver of patients with haemoglobin H disease. In 11 Chinese patients studied (six male and five female) with haemoglobin H disease hepatic function and histology were studied. Liver function was normal in all cases; however, the gamma globulin level was raised in four cases, the increase being mainly in IgG in all cases and IgA in six cases. Liver histology showed that one case had portal cirrhosis, six cases mild fibrosis, and four cases no fibrosis. Haemosiderosis, mainly in the parenchymal cells of the peripheral lobules, was mild in one case, moderate in four, and severe in the remainder of the cases.

Haemoglobin H (B₄) disease is common among Chinese and Siamese (Na-Nakorn, Wasi, and Suingdumrong, 1965). Vella (1960) also noted that in Singapore haemoglobin H is the most common haemoglobinopathy. Lie-Injo (1964), in her review on haemoglobinopathies in East Asia, observed that in such conditions medullary erythropoiesis is found in many organs, especially in the liver which is grossly disorganized. No further detail was given in support of that statement. Green, Conley, and Berthrong (1953) studied 50 patients with sickle cell anaemia and found that cholelithiasis was common; 33 of these patients had a moderately enlarged liver and four had cirrhosis which was thought not to be due to pigment deposition. Bogoch, Casselman, Margolies, and Bockus (1955) studied four patients with sickle cell anaemia and found one had hepatitis, one had haemosiderosis due to blood transfusion, and two had portal cirrhosis. Song (1957) noted that though hepatic cell damage was seen in 31 cases of sickle cell anaemia, only nine cases had cirrhosis of the liver. Bannerman, Keusch, Kreimer-Birnbaum, Vance, and Vaughan (1967) described in detail a patient with thalassaemia intermedia who developed haemochromatosis with cardiac failure and diabetes mellitus. Bowdler and Huehns (1963) described a family in which five members had thalassaemia minor; two of these had marked haemosiderin granulation in

the liver parenchymal cells. As far as we are aware no report has been made on the histological changes in the liver of patients with haemoglobin H disease. It is the purpose therefore of this paper to report on the liver function tests and the histological appearance of the liver obtained by needle biopsy in eleven cases of haemoglobin H disease.

Materials and Methods

The series consisted of 11 Chinese (six male and five female) whose ages ranged from 13 to 70 years. They all had clinical, haematological, and biochemical evidence of haemoglobin H disease.

Standard haematological studies, such as haemoglobin, white blood and differential counts, and simple coagulation studies, such as prothrombin, bleeding and clotting times, were done. Standard liver function tests such as serum bilirubin, alkaline phosphatase, thymol turbidity, serum glutamic oxalo-acetic transaminase or aspartate transaminase, and serum glutamic-pyruvic transaminase or alanine transaminase, serum iron and iron-binding capacity, and bromsulphthalein retention tests were performed by the Biochemistry Section of the Department of Pathology. Immunoglobulin levels were deter-

Fig. 1 Immunoglobulins in haemoglobin H disease.

Fig. 2 Scattergram of IgG and IgA.

mined by the methods of Chew, Yu, and Wae (1969). Haemoglobin electrophoresis was carried out by the method of Lehmann and Ager (1961). Liver biopsy was carried out with either Vim Silverman's or Menghini's needle if the prothrombin, bleeding and clotting times were normal, and the platelet counts not less than 100,000 per cubic millimetre.

Immediately after biopsy the tissue was fixed in buffered formal-saline solution. It was processed by the standard method and sections were cut at 4 μ thick and stained with haematoxylin and eosin, Perls' reaction for iron, Wilder's reticulum stain, and Masson's trichrome.

Results

HAEMATOLOGICAL FINDINGS

The haemoglobin in all these cases ranged between 7.4 and 11.7 g%. The total white cell counts were

Fig. 3 One of the several areas of micronecrosis. Note the congregation of lymphocytes and polymorphs around the necrotic liver cells. H & E $\times$ 500.

Fig. 4 An enlarged and fibrotic portal trace; the periphery is heavily infiltrated by lymphocytes and histiocytes, the latter are filled with haemosiderin. *H & E* $\times 150$.

Fig. 5 Portal fibrosis in a portal tract. The fibrosis has also begun to extend perilobularly. Wilder's reticulin stain.

Fig. 6 Photomicrograph showing the typical cirrhotic pattern of irregular nodules in a background of fibrosis. In the latter heavy mononuclear infiltration is still in evidence. *H & E* $\times 75$.

Fig. 7 Typical regenerative cirrhotic nodule. *H & E* $\times 150$.

Fig. 8 Heavy iron pigment mainly in the liver parenchyma cells. Some pigment is present in the Kupffer cells. Perls' iron stain $\times 150$.

Fig. 10 Concentration of the iron pigment around the portal tracts and a decrease in the pigmentation approaching the central vein. Perls' iron stain $\times 75$.

Fig. 9 Heavy haemosiderosis in the portal tract. The large blobs of pigment are either in histiocytes or in the liver cells of the limiting plate. The portal tract is enlarged by fibrosis and fibrous extensions are also seen. Perls' iron stain $\times 150$.

Fig. 11 Severe haemosiderosis. There is concentration of pigment around the portal tracts and a lesser accentuation around the central veins. Perls' iron stain $\times 75$.

within normal limits (4,000-10,000) in all these cases, but in the differential counts, seven patients had relative polymorphonucleosis of more than 80%. Platelet counts were normal in all cases, as were bleeding, clotting, and prothrombin times. There was reticulocytosis ranging from 3% to 14% in every case. Erythrocyte sedimentation rates were between 2 and 7 mm/hr in seven cases, and between 13 and 22 mm/hr in the rest.

Biochemical Tests

The iron-binding capacity was normal in all cases. One patient had a low serum iron level, and in the rest the levels were normal.

Two cases had serum bilirubin levels of 1.4 and 2.3 mg%; however, only the latter case had a liver that was clinically slightly icteric.

Serum alkaline phosphatase, thymol turbidity, transaminases, serum proteins, and bromsulphthalein retention tests were normal. When serum proteins were tested by electrophoresis, the striking results were that gamma globulin was less than 1 g% in two cases; the rest of the results showed levels of more than 1 g%. Four patients had levels above the normal range of 1.7. The highest gamma globulin was 2.1. Quantitative estimation of immunoglobulins G, A, and M showed that the increase was mainly in the IgG in all cases; six patients had increased IgA and all cases had normal IgM (Figure 1). There is no correlation between IgG and IgA in this condition (Figure 2). The RA factor was negative in every case.

LIVER HISTOLOGY

There was only a negligible amount of liver cell necrosis, which occurred as tiny microscopic areas composed of, at the most, a few cells. These tiny necrotic areas were seen in five cases (Figure 3). The necrosis bore no relation either to the central vein or the portal tract.

Periportal fibrosis was on the whole mild (Figure 4). Four cases did not show any fibrosis while four others presented with mild peripheral fibrosis. In two cases the portal fibrosis had begun to produce perlobular extensions (Figure 5). Only one case was cirrhotic (Figs. 6 and 7), and was most probably a cirrhosis of the post-necrotic variety.

Haemosiderosis was in general severe, except for one mild case. All the other cases showed either moderate or severe amounts of iron pigment, which was found principally in the liver cells, while the Kupffer cells contained less iron pigments (Figure 8). The Kupffer cells were neither abnormally distorted nor increased in number. In the more severe cases, groups of histiocytes and canals of Herings were filled with haemosiderin in the enlarged portal tracts. It

Fig. 12 Heavy mononuclear infiltration of the fibrotically enlarged portal tract. Perilobular extensions are beginning to appear. The large blobs near the limiting plate are histiocytes stuffed with haemosiderin. H & E $\times 150$.

appeared that when there were pigment-laden histiocytes and hepatocytes in the portal tracts, the latter were necessarily enlarged and fibrotic. The greatest concentration of iron pigment was always around the portal tracts, and the intensity receded centrifugally from these triads. In the majority of the cases, some degree of haemosiderosis was also seen around the central vein though some cases did not show this feature. However, in the most severe case, the haemosiderin was evenly distributed throughout the liver cells (Figures 9, 10, and 11).

On the whole there was a moderate degree of lymphocytic infiltration in the portal tracts (Figure 12). In some cases a sprinkling of polymorphonuclear leucocytes and histiocytes contributed to the infiltrates. The histiocytes were especially prominent when there was an excessive spilling over of iron pigment. The inflammatory cells tended to follow the fibrous extensions. In one or two instances, a more diffuse but mild distribution of lymphocytes and polymorphs was seen along the sinusoids. The tiny areas of necrosis were necessarily attended by polymorph-nuclear

Case Number	Haemosiderosis	Necrosis	Fibrosis
1	+	+	0
2	++	+	+
3	++	+	0
4	++	0	+
5	++	0	0
6	+++	0	0
7	+++	0	++
8	+++	0	+
9	+++	+	+
10	+++	+	++
11	+++	0	++++

Table The main histological features of the liver biopsies¹

¹0—no effect, +—mild, ++—moderate, and +++ or ++++ severe.

leucocytes. The table summarizes the histological changes seen in the 11 cases.

FOLLOW-UP AND THERAPY

The period of follow up in these cases ranged from six months to 11 years. The solitary case of cirrhosis was followed up for slightly more than five years.

Two cases did not have any blood transfusion at all. The others received blood transfusion ranging from one to 15 units. The patient with cirrhosis has received 10 units of blood since the time of diagnosis. None of them has had any iron therapy.

The patients are able to carry on with normal life. In only one case were the liver and spleen not enlarged. The other patients had hepatosplenomegaly. The liver size ranged from 1.5 to 7 cm. The spleen size ranged from 1.5 to 9 centimetres.

Only three of the patients had gallstones; of these three, one also had a non-functioning gall-bladder.

Discussion

The haematological findings in our series agree with those obtained by Na-Nakorn *et al* (1965) and Jim (1962). However, these workers do not mention the value of white cell counts or of the differential and erythrocyte sedimentation rates. In our series, we found that there is a relative polymorphonucleosis and there is also a slight increase in erythrocyte sedimentation rates. No obvious reasons could be found to account for these changes.

The clinical findings of hepatosplenomegaly and values for serum iron and iron-binding capacity agree with those of Na-Nakorn *et al* (1965) who in addition also reported that liver function tests were normal except for an elevated level of serum

bilirubin and raised transaminases during haemolytic crises. However, they did not mention the changes in globulin values. In our series we noted that the gamma globulin levels exceeded the top normal values and that the increase in the gamma globulins were mainly due to immunoglobulins G and A. These findings, as far as we are aware, have not been reported elsewhere. The reason for this change is probably due to an increased activity of lymphocytes resulting from iron deposition. The RA factor is known to be positive in viral hepatitis, cirrhosis, and primary carcinoma of the liver (Chew, 1968) but the RA factor was negative in all these cases.

Cholelithiasis was observed in three cases. Cholecystography might miss some of the cases, and Jordan (1957) showed a higher incidence of cholelithiasis in sickle cell disease when the cases were studied by cholecystogram, at laparotomy, and at necropsy.

Haemosiderin granules were moderately or severely deposited in the liver parenchymal cells, while a small number of these was found in the Kupffer cells. In cases where there was a heavy deposit of haemosiderin granules, they were also found in both the histiocytes and the canals of Herings in the enlarged portal tracts. The largest amount of iron pigment was around the portal tracts and became less centrifugally. Periportal fibrosis was on the whole mild. Four cases did not show any fibrosis while four had mild periportal fibrosis. In two cases portal fibrosis had begun to produce perilobular extensions and only in one case was there definite nodular formation. There was a moderate degree of lymphocytic infiltration in the portal tracts and liver cell necrosis was almost negligible.

As no previous report had been made on the hepatic histology in haemoglobin H disease, no comparison can be made with our series. Berry and Marshall (1967) found that in some splenectomized patients with thalassaemia major, the iron was deposited outside the parenchyma, while in splenectomized patients deposits were parenchymal. The degree of fibrosis was on the whole slight to moderate which seems to be in fair agreement with our results.

Kent and Popper (1968) stated that siderosis occurs due to blood transfusion, haemolysis, or preceding hepatitis. A function of combined reticulo-endothelial and parenchymal siderosis in equal degree is suggestive of haemochromatosis associated with anaemia. The presence of portal fibrosis and septal formation points to an acquired type of anaemia, especially thalassaemia. Only one case with cirrhosis in the present series seems to fit in with the description by Kent and Popper; the other cases, however, seem to be different.

Hepatic lesions in sickle cell anaemia, as described by Song (1957), differ from those in our series. Song found that hepatic cell damage was noted in every case and cirrhosis was present in nine cases. The liver showed broad bands of

loose fibrillar material punctuating atrophic lobules with foci of recent necrosis and a small amount of iron pigment in the hepatic necrotic cells. The Kupffer cells and scar tissue had no pigment. These lesions were believed to be due to impairment of blood flow resulting in an anoxic necrosis of hepatic cells.

Controversy still rages on as to whether iron can cause cirrhosis of the liver. According to MacDonald (1966), cirrhosis cannot be produced in rats by feeding them only iron; however, if they were given a diet deficient in lipotropic factors together with iron, they developed cirrhosis of the liver. As only one case out of eleven in our series developed cirrhosis it is difficult to say whether this cirrhotic condition arose from iron deposition. Further, as this patient also had several blood transfusions, viral hepatitis could not therefore be excluded. It is also possible that the period of follow up is not long enough to allow a definite conclusion as to what the end results of iron pigment deposition in the liver following haemolysis will be.

References

- Bannerman, R. M., Keusch, G., Kreimer-Birnbaum, M., Vance, V. K., and Vaughan, S. (1967). Thalassemia intermedia, with iron overload, cardiac failure, diabetes mellitus, hypopituitarism and porphyrinuria. *Amer. J. Med.*, 42, 476-486.
- Berry, C. L., and Marshall, W. C. (1967). Iron distribution in the liver of patients with thalassaemia major. *Lancet*, 1, 1031-1033.
- Bogoch, A., Casselman, W. G. B., Margolies, M. P., and Bockus, H. L. (1955). Liver disease in sickle cell anemia. *Amer. J. Med.*, 19, 583-609.
- Bowdler, A. J., and Huehns, E. R. (1963). Thalassaemia minor complicated by excessive iron storage. *Brit. J. Haemat.*, 9, 13-24.
- Chew, B. K., and Yeo, K. L. (1966). Latex fixation tests in liver disease. *Singapore*, 7, 38-41.
- Chew, B. K., Yu, M., and Wae, R. (1969). Immunoglobulin level in four ethnic groups in Singapore. *Far East med. J.*, 5, 98-100.
- Green, T. W., Conley, C. L., and Berthrong, M. (1953). The liver in sickle cell anaemia. *Bull. Johns Hopk. Hosp.*, 92, 99-127.
- Jim, R. T. S. (1962). Haemoglobin H disease in Hawaii. *Acta Haemat (Basel)*, 27, 274-277.
- Jordan, R. A. (1957). Cholelithiasis in sickle cell disease. *Gastroenterology*, 33, 952-958.
- Kent, G., and Popper, H. (1968). Liver biopsy in diagnosis of hemochromatosis. *Amer. J. Med.*, 44, 837-841.
- Lehmann, H., and Ager, J. A. M. (1961). The laboratory detection of abnormal haemoglobins. *Ass. clin. Path. Broadsheets*, 33.
- Lie-Injo Luan Eng (1964). Haemoglobinopathies in East Asia. *Ann. hum. Genet.*, 28, 101-111.
- MacDonald, R. A. (1966). Idiopathic hemochromatosis: acquired or inherited? In *Controversy in Internal Medicine*, edited by F. J. Ingelfinger, A. S. Reiman, and M. Finland, pp. 271-284. Saunders, Philadelphia.
- Na-Nakorn, S., Wasi, P., and Suingdumrong, A. (1965). Hemoglobin H disease in Thailand. *Israel J. med. Sci.*, 1, 762-764.
- Song, Y. S. (1957). Hepatic lesions in sickle cell anemia. *Amer. J. Path.*, 33, 331-351.
- Vella, F. (1960). Abnormal haemoglobin variants in 10,441 Chinese subjects. *Acta Haemat. (Basel)*, 23, 393-397.