

## Histochemical and Immunohistochemical Studies on the Relationship Between Severe Iron Accumulation and Liver Cell Injury

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**SUMMARY :** Histochemical and immunohistochemical analysis was carried out to determine the relationship between iron accumulation and hepatic cirrhosis, using 10 autopsy cases of severe iron accumulation in the hepatocytes obtained from Kenya, including cases of Bantu siderosis. These severe iron accumulation specimens were divided into two groups ; one group consists of five cases with cirrhosis with or without hepatocellular carcinoma, and the other five cases with non-cirrhosis. All cirrhosis cases included hepatitis B surface antigen. Chronic infection caused by hepatitis B virus may lead to necrosis of the liver cells, resulting in the formation of hepatic fibrosis or cirrhosis. On the other hand, none of the non-cirrhosis cases include hepatitis B virus. This finding indicates that iron accumulation does not lead to cellular injury. Therefore, it is suggested that chronic iron toxicity is not recognized in Bantu siderosis. The reached conclusion is that there is no correlation between iron accumulation and hepatic fibrosis or cirrhosis.

### INTRODUCTION

The diet of the Bantu in East Africa contains large amounts of iron most of which is derived from the soils of this area.<sup>7)</sup> This high iron intake is almost certainly the primary cause of the varying degrees of iron accumulation common in adult Bantu. There is a close correlation between severe hepatic siderosis and portal fibrosis or cirrhosis.<sup>2,3)</sup> On the other hand, in our previous study of hepatitis B surface antigen in Kenya, 29% of the liver specimens were stained positive for hepatitis B surface antigen.<sup>6)</sup>

This finding raises the question whether the cirrhosis seen in Bantu was caused by a prolonged exposure to a diet containing excess amount of iron and/or infection of hepatitis B virus. The purpose of this research is to find any relationship between iron accumulation and hepatic cirrhosis or liver cell injury.

### MATERIALS AND METHODS

The liver and spleen specimens from 182 autopsy cases at Rift Valley Provincial General Hospital in Kenya were collected. From these specimens, ten cases of severe iron accumulation

in the hepatocytes including Bantu siderosis, were selected. The ten cases were divided into two groups; one group consists of five cases

with cirrhosis with or without hepatocellular carcinoma (Table 1), and the other five cases with non-cirrhosis (Table 2).

**Table 1.** Iron accumulation in the hepatocytes associated with cirrhosis

No.	Age	Sex	Cirr	Hepa	Hepatic	Kupf	Biled	Portal	HBsAg	Spleen
1	30	Male	+	+	3	1	1	0	2	1
2	70	Male	+	+	3	1	0	0	3	1
3	60	Male	+	+	3	3	0	0	2	3
4	50	Female	+	-	3	2	0	0	2	2
5	60	Male	+	-	3	3	0	1	3	3

**Table 2.** Iron accumulation in the hepatocytes associated with non-cirrhosis

No.	Age	Sex	Cirr	Hepa	Hepatic	Kupf	Biled	Portal	HBsAg	Spleen
1	60	Male	-	-	3	3	0	0	0	3
2	65	Male	-	-	3	3	0	0	0	3
3	62	Male	-	-	3	1	0	0	0	3
4	40	Male	-	-	3	3	0	1	0	3
5	50	Male	-	-	3	2	0	1	0	3

Cirr : cirrhosis, Hepa : hepatoma, Hepatic : iron deposition in hepatocytes, Kupf : iron deposition in Kupffer cells, Biled : iron deposition in bile duct epithelia, Portal : iron deposition in portal tracts, HBsAg : hepatitis B surface antigen, Spleen : iron deposition in spleen, + : positive, - : negative, 0 : negative, 1 : slight positive, 2 : moderate positive, 3 : severe positive.

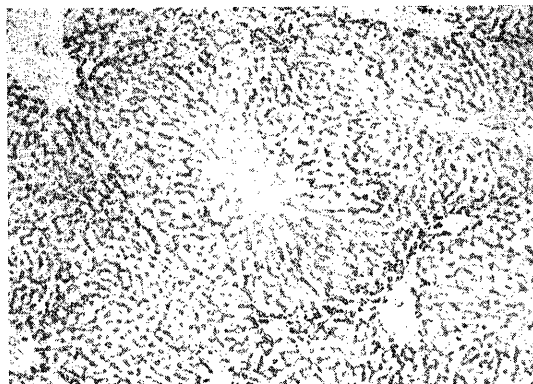
The specimens were fixed in 10% formalin, and embedded in paraffin for histochemical and immunohistochemical studies. Sections were cut at five micron and stained with hematoxylin-and-eosin, periodic acid Schiff (PAS), AFIP method for lipofuscin, Gomori's method for iron, Mallory's method for collagen fiber, and silver impregnation for reticulum fiber. These materials were stained with hepatitis B surface antigen (Dako PAP Kit : K523, Lot. 063-3) by immunoperoxidase method.

## RESULTS

The results for the degree of iron accumulation in hepatocytes, in Kupffer cells, in bile duct epithelia, in portal tracts, in spleen were shown in Table 1 and 2 with the degree of positive hepatitis B surface antigen. The severe iron accumulation cases were divided into two groups; one group consists of cirrhosis of those with cirrhosis, and the other with non-cirrhosis. There were clear differences in the histological finding between the two groups. All cases with cirrhosis involved hepatitis B surface antigen (Table 1), whereas, none of the cases with

non-cirrhosis did (Table 2).

The findings with the latter group indicate that severe iron accumulation does not lead to cellular injury and/or hepatic cirrhosis (Fig. 1). Specifically, severe iron accumulation is seen in the advance cirrhosis nodules (Fig. 2) which were infected with hepatitis B virus (Fig. 3).



**Fig. 1.** Severe iron accumulation is seen in the peripheral area of liver lobules, but less in the central area. However, fibrosis is not detected in the severe iron accumulation area and portal tracts. Prussian blue reaction ( $\times 100$ ).

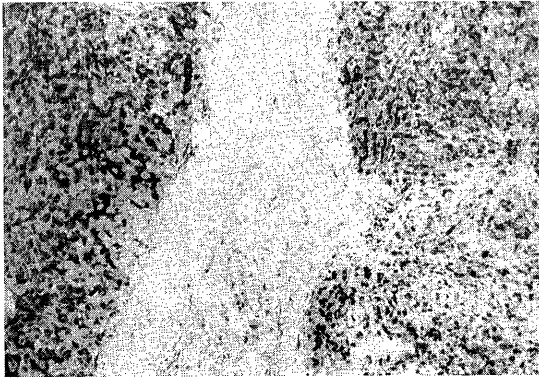


Fig. 2. Iron accumulation is seen in the advanced cirrhosis nodules. Prussian blue reaction ( $\times 100$ ).

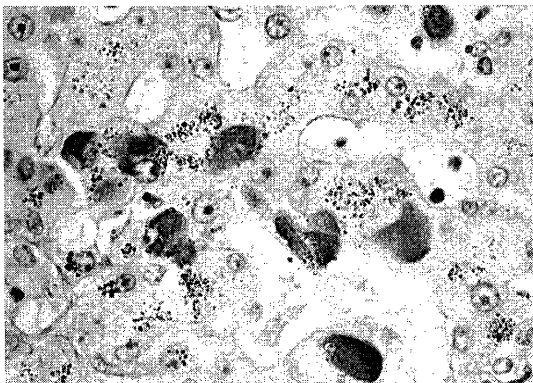


Fig. 3. Hepatitis B surface antigen and iron granules are seen in the advanced cirrhosis nodules. Immunoperoxidase for hepatitis B surface antigen ( $\times 400$ ).

Iron accumulation is seen more abundantly in the area of peripheral hepatocytes than in the center. Most of the hemosiderin granules were distributed in the hepatocytes, but a few hemosiderin granules were seen in Kupffer cells; all cytoplasm were stained a light blue color with Gomori's method for iron. Severe iron accumulation was seen in hepatocytes and Kupffer cells, though degenerative and necrotic cytoplasm were not detected. There was little, if any, iron accumulation in the nodular hyperplasia area. Iron accumulation was not seen in the tissues of hepatocellular carcinoma and metastatic neoplasm.

## DISCUSSION

In hemosiderosis seen in South African blacks, the presence of large amounts of iron in the liver is frequently associated anatomically with fibrosis, and the severity of fibrosis is roughly proportional to iron accumulation,<sup>3)</sup> in the tissue. However, animal models for iron accumulation may have failed to produce tissue damage because of the relatively short duration of iron loading, or because iron has been administered in a parenteral form that has been deposited primarily in cells of the reticuloendothelial system.<sup>1,5)</sup> In our previous study of iron accumulation,<sup>7)</sup> this finding was supported by statistical analysis that revealed no significant correlation between iron accumulation and hepatic cirrhosis (Pearson  $X^2 = 0.6734$  with  $d.f = 1$ ). In this study, severe iron accumulation was seen in the liver tissue through histochemical observation, whereas, no correlation was found between iron accumulation and hepatic fibrosis or cirrhosis. We concluded that iron accumulation does not lead to hepatic fibrosis or cirrhosis. On the other hand, all cases of cirrhosis were infected with hepatitis B virus, but none of non-cirrhosis cases was. Therefore, hepatic fibrosis and cirrhosis are greatly responsible for many of the sequelae of chronic liver diseases, *e.g.*, hepatitis B virus infection. Moreover, Africa is a high prevalence area for hepatitis B virus.<sup>4,6,8)</sup>

## REFERENCES

- 1) AWAI W: Intraperitoneal injection of a chelate iron complex salts causes diabetes in rat and rabbits. *Acta Haematol. Jpn.*, **41**: 1293-1300, 1978.
- 2) BOTHWELL TH, BRADLOW BA: Siderosis in the Bantu: A combined histopathological and chemical study. *Arch. Pathol.*, **70**: 279-292, 1960.
- 3) ISAACSON C, SEFTEL HC, KEELEY KJ, WELL TH: Siderosis in the Bantu: The relationship between iron overload and cirrhosis. *J. Lab. Clin. Med.*, **58**: 845-853, 1961.
- 4) KEW MC, GEDDES EW, MACNAB GM, BERSOHN I: Hepatitis B antigen and cirrhosis in Bantu patients with primary liver cancer. *Cancer*, **34**:

- 539-541, 1974.
- 5) MACDONALD RA, PECHET GA : Experimental hemochromatosis in rats. *Am. J. Pathol.*, **46** : 85-109, 1965.
  - 6) SENBA M, NAKAMURA T, ITAKURA H : Statistic analysis of relationship between hepatitis B surface antigen, cirrhosis, and hepatocellular carcinoma. *Trop. Med.*, **26** : 117-122, 1984.
  - 7) SENBA M, NAKAMURA T, ITAKURA H : Statistical analysis of relationship between iron accumulation and hepatitis B surface antigen. *Am. J. Clin. Pathol.*, **84** : 340-342, 1985.
  - 8) VOGEL CL, ANTHONY PP, MODY N, BARKER LF : Hepatitis-associated antigen in Ugandan patients with hepatocellular carcinoma. *Lancet*, **II** : 621-624, 1970.