

Hepatitis B Surface Antigen and Hepatocellular Carcinoma in Southern Africa

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Summary. Most series in Africa show a high percentage of hepatitis B surface antigen in hepatocellular carcinoma. Two groups of cases were investigated in this study. The one was derived from the autopsy material at Baragwanath hospital from subjects who had lived in Soweto, a large Black urban town. The second group consisted of male Black mineworkers generally originating from rural areas. A combination of the aldehydefuchsin stain and immunoperoxidase technique was used. The two groups showed totally different results. The Baragwanath series consisted of 24 hepatocellular carcinomas of which only 4 (17%) were HBsAg positive. Of the 24 cases, 14 had cirrhosis of which 9 were macronodular and 5 micronodular. Ten of these cases showed heavy iron overload. The series of male Black mineworkers comprised 22 cases of which 16 (72%) were HBsAg positive. Twelve of the 22 cases showed a macronodular cirrhosis and there were no micronodular cirrhoses. Only one case showed severe iron overload. These findings delineate two different populations of hepatocellular carcinoma in Southern Africa.

Key words: Hepatitis – Surface antigen – Hepatocellular carcinoma.

Introduction

Two views of the aetiology of the high incidence of hepatocellular carcinoma in Africa have been proposed. According to some authors (Oettle, 1965; Isaacson, 1966) the hypothesis of mycotoxicosis resulting from spoilage of food by toxic moulds fits the distribution of hepatocellular carcinoma better than any other theory. The second aetiological factor that has been invoked is that of the hepatitis B virus (Vogel et al., 1970; Morrow et al., 1971; Anthony et al., 1972; Kew et al., 1974; Kew et al., 1979).

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There has, however, been an impression that the percentage of HBsAg positive hepatocellular carcinomas at Baragwanath hospital is comparatively small. This was at first thought to be due to faulty technique particularly in view of the high positivity rate in mineworkers (Cohen et al., 1978). The latter authors found orcein staining of non-tumour liver cell cytoplasm in 36% of hepatocellular carcinomas. It must however be emphasized that the material used by Cohen et al. (1978) was derived from a different population from that seen at Baragwanath hospital. The latter is essentially an urban hospital, serving a population the majority of whom have lived all their lives in Soweto, a large Black city of about 1.5 million people, situated approximately ten miles south-west of Johannesburg. The subjects investigated by Cohen et al. (1978) were rural mineworkers derived principally from Mozambique.

It was therefore considered appropriate to compare the incidence of HBsAg in hepatocellular carcinomas from these two groups of subjects using both histochemical and immunoperoxidase techniques.

Materials and Methods

Twenty four hepatocellular carcinomas from the years 1976–1978 were randomly selected from the autopsy material at the Baragwanath histopathology laboratory, the only criterion for selection being adequate amounts of non neoplastic liver tissue for examination. Formalin-fixed paraffin embedded autopsy tissue of liver and tumour was stained with orcein (Deodhar et al., 1975), and Gomori's aldehyde-fuchsin (Gomori, 1950). Immunoperoxidase was demonstrated by a modification of the techniques described by Burns (1975) and by Weisburg et al. (1978).

The second group of cases consisted of 24 autopsied male Black mineworkers from the years 1973–1975, and the material from these subjects was treated similarly. These hepatocellular carcinomas were also randomly selected, the only criterion for inclusion again being adequate quantities of non-neoplastic liver. In addition all sections were stained with haematoxylin and eosin, and Perl's Prussian-blue reaction for iron.

During the course of the investigation it was found that the aldehyde-fuchsin staining technique was more reliable than the orcein. The latter gives variable results dependent on the batch of stain.

The sections stained by aldehyde-fuchsin and immunoperoxidase were examined by independent observers (C.I. and A.C.P.).

In several instances the brownish staining of a positive immunoperoxidase was difficult to interpret in the presence of the severe iron overload so frequently found in this population (Bothwell and Isaacson, 1962).

This was overcome by preparatory staining of the haemosiderin by Perl's Prussian-blue reaction, the iron then staining purple in contrast to the brown staining of the hepatitis surface antigen.

The clinical records and serological investigations for surface antigen in all these subjects were unfortunately unavailable.

Results

Table 1 demonstrates the findings in both series. There were 24 cases from Baragwanath hospital, 16 males and 8 females. Three subjects (12.5%) showed positive staining with aldehyde-fuchsin, and immunoperoxidase added one further case giving a total of 4 positives out of 24 (17%). Two of these showed macronodular cirrhosis and in two cirrhosis was absent. The ages of these cases varied from 22 to 79 years with an average of 57 years. Fourteen cases had cirrhosis,

Table 1. Hepatitis B surface antigen in hepatocellular carcinoma

	Black mineworkers	Baragwanath blacks
No. of cases	22 – all males	24 – 16 males 8 females
AF+	11 – 50%	3 – 12.5%
IP+	14 – 64%	4 – 17%
AF+ and IP+	16 – 72%	4 – 17%
AF+ and IP–	2 –	0 –
AF– and IP+	5 –	1 –
Cirrhosis	12 – all macro	14 – 9 macro 5 micro
Heavy Fe overload	1 –	10 –

AF=Aldehydefuchsin, IP=Immunoperoxidase, Macro=Macronodular cirrhoses, Micro=Micronodular cirrhoses

Table 2. Countries of origin of black mineworkers with hepatocellular carcinoma

	No.	HAA positive
Mozambique	10	8
Malawi	3	3
Transkei	1	0
Botswana	1	0
Unknown	7	5
Total	22	

of which 9 were of the macronodular and 5 of the micronodular type. Cirrhosis was absent in 9 cases, and in one case the appearances were equivocal. Heavy iron deposits were present in 10 cases, and mild deposits in 3 cases. Of those with heavy iron deposits, 5 had micronodular cirrhosis. Heavy deposits of iron were present in 4 subjects without cirrhosis. Mild fatty change was found in four subjects, three females and one male, and in only one case – a female – was cirrhosis present and this was of the iron-overloaded micronodular type. One case with mild fatty change also showed alcoholic hyalin. In no case of macronodular cirrhosis were significant iron deposits found.

The series of Black mineworkers showed a totally different picture. There were 22 cases and as expected, all were males. Sixteen cases (72%) were positive for hepatitis B surface antigen. As can be seen in Table 1, 11 (50%) were positive by aldehydefuchsin staining alone and immunoperoxidase added a further 5 cases. Cirrhosis was present in 12 cases, all of the macronodular type. Surface antigen was found in 9 of the 12 cirrhotics and in 7 non-cirrhotics. Iron overload was a feature in one case only. Table 2 demonstrates the countries of origin of the Black mineworkers.

Discussion

Several studies in Africa have shown an association between hepatocellular carcinoma and hepatitis B virus. In South Africa, Kew et al. (1974) found hepatitis B antigen in the serum of 40% of Black patients with hepatocellular carcinoma but in only 7% of apparently healthy rural Blacks. They suggested that the persistence of hepatitis B virus may be the cause or result of primary

liver cancer, or that possibly some immunologic defect is responsible for both. If the former, the virus must be directly oncogenic, in addition to having the potential to induce neoplasia indirectly via chronic parenchymal liver disease. Additional evidence supporting an aetiological role for HBV has been the successful production of HBsAg by a hepatocellular carcinoma in tissue culture (Macnab et al., 1976). In the series of Kew et al. (1974) macronodular cirrhosis was present in 60% of the cases and HBsAg was not found significantly more often in these patients than in those without cirrhosis.

The second factor that has been incriminated in the aetiology of hepatocellular carcinoma in Africa is the mycotoxin aflatoxin. Van Rensburg et al. (1974) working in Mozambique, in an area with the highest known liver cancer incidence in the world, found that the diet contains the highest known aflatoxin intake, the average adult consuming 15.6 µg of aflatoxin per day. The incidence rate of liver cell carcinoma in this area is 25.4 per 100,000 per year.

Following on the observation of Peers and Linsell (1973) who demonstrated a significant association between the incidence of primary liver cancer and ingested aflatoxin in a study in the Murango district of Kenya, Bagshawe et al. (1975) studied the incidence of hepatitis B antigen in the same district and showed no significant difference between the low altitude area with a relatively high incidence of primary liver cancer, and the high altitude area with a low incidence of the tumour. They therefore concluded, that current evidence is more in favour of aflatoxin playing an important role in the aetiology of primary liver cancer, with hepatitis B virus playing an ancillary role.

With these conflicting views, the findings in the current study are of significance. The hepatocellular carcinomas observed in the predominantly urban Black subjects from Baragwanath hospital show a comparatively low incidence of HBsAg positive staining (17%), whereas those from the rural Black mineworkers show a 72% positivity rate. These are two contrasting Southern African populations, both showing a high incidence of hepatocellular carcinoma, yet with grossly differing incidences of HBsAg positivity. While acknowledging the incompleteness of this study because of the absence of serological investigations, the significance of these findings cannot be dismissed, as in most other studies serological results have paralleled histological findings (Omata et al., 1979).

Do these findings downgrade the role of HBV in the aetiology of hepatocellular carcinoma? Johnson et al. (1978) stated that if the hepatitis B virus were an important factor in the progression of cirrhosis to hepatocellular carcinoma, it would be expected that HBsAg would be found significantly more frequently in those with cirrhosis and tumour than in those with cirrhosis alone. Their results did not support this, and were more consistent with the view that the hepatitis B virus is one of the many factors associated with the development of cirrhosis, and that progression to malignant change depends on the sex and age of the patient. Recently Lutwick (1979) has postulated a new role for aflatoxin in the production of hepatocellular carcinoma, suggesting that it suppresses cell-mediated immunity which would then allow the hepatitis B virus to maintain itself more easily in the liver, to produce more chronic infection and cirrhosis, and in the long term to lead to a high incidence of hepatocellular carcinoma.

A further finding of significance in this study is the comparatively high percentage of subjects with iron overload and micronodular cirrhosis in the group from Baragwanath hospital. Where cirrhosis was present in the mine workers, it was always of the macronodular type, and in only one instance was there significant iron overload. The Baragwanath findings differ from previous studies at the same hospital which demonstrated that, when hepatocellular carcinoma develops in a liver with cirrhosis, the cirrhosis is almost invariably of the macronodular type with no evidence of iron overload (Higginson, 1957; Steiner, 1960; Isaacson et al., 1961). Iron overload is common in this population and results from the ingestion of large amounts of iron in fermented alcoholic drinks which are prepared in iron containers (Bothwell and Bradlow, 1960; Bothwell and Isaacson, 1962). The increase in hepatocellular carcinoma in livers the site of cirrhosis with iron overload may be a reflection of the recent liberalization of the liquor laws and an increased consumption of the "White man's liquor".

There has in recent years been a considerable change in the picture of the Black alcoholic's liver (Isaacson, 1978). In previous years, it had been shown that the iron-overloaded cirrhosis associated with the consumption of traditional homebrewed beverages rarely gave rise to hepatocellular carcinoma (Higginson, 1957).

However, histological assessment of the non-neoplastic liver showed only mild fatty change in four subjects – three females and one male – and in only one were structures present suggestive of alcoholic hyalin. None of the other stigmata of alcoholism such as alcoholic hepatitis or perivenular sclerosis were seen. It is possible that the ten subjects with hepatocellular carcinoma and severe iron overload had supplemented their intake of traditional beers with "Western" type liquor, in which case, if the quantities of the latter were small, it would be difficult to demonstrate any significant histological changes. However, three of the four cases with mild fatty change showed no significant iron overload. The role of Western type alcohol is therefore in doubt, and if of importance must be effected by comparatively small quantities. In contrast, in Western societies, hepatocellular carcinoma not infrequently develops in alcoholic cirrhosis (Lee, 1966; Johnson et al., 1978).

A noteworthy finding in the Baragwanath series is the increased percentage of women with hepatocellular carcinoma (33% of all cases) as compared with previous studies in Africa where the percentage of women rarely exceeded 18 (Higginson and Oettle, 1957; Davies, 1957). The cause of this rise in incidence in women is obscure but the possibility that the increased use of contraceptive steroids may be a factor should be considered (Christophersen and Mays, 1977), although in this series only one of the eight female subjects was in the childbearing age.

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