

# Dietary Iron Overload as a Risk Factor for Hepatocellular Carcinoma in Black Africans

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Although the iron-loading disease, hereditary hemochromatosis, has a strong causal association with hepatocellular carcinoma (HCC), the carcinogenic potential of dietary iron overload in Black Africans is not known. We investigated this potential by evaluating iron status, alcohol consumption, markers for hepatitis B (HBV) and C virus (HCV) infections, and exposure to dietary aflatoxin B<sub>1</sub> in 24 rural patients with this tumor, 48 race-, sex-, and age-matched hospital-based controls, and 75 related or unrelated close family members of the cancer patients. Iron overload was defined as a raised serum ferritin concentration in combination with a transferrin saturation  $\geq 60\%$ , and was confirmed histologically when possible. Among 24 patients and 48 hospital controls, the risk of developing HCC in the iron-loaded subjects was 10.6 (95% confidence limits of 1.5 and 76.8) relative to individuals with normal iron status, after adjusting for alcohol consumption, chronic HBV and HCV infections, and exposure to aflatoxin B<sub>1</sub>. The risk of HCC in subjects with HBV infection was 33.2 (7.2, 153.4) (odds ratio [95% confidence limits]), HCV infection 6.4 (0.3, 133.5), and alcohol consumption 2.0 (0.5, 8.2). Aflatoxin B<sub>1</sub> exposure did not appear to increase the risk of HCC. The population attributable risk of iron overload in the development of HCC was estimated to be 29%. Among 20 cancer patients and 75 family members, the risk of developing HCC with iron overload was 4.1 (0.5, 32.2). We conclude that dietary iron overload may contribute to the development of HCC in Black Africans. (HEPATOLOGY 1998; 27:1563-1566.)

Hepatocellular carcinoma (HCC) is common in southern African Blacks.<sup>1</sup> Chronic hepatitis B (HBV) and C virus

(HCV) infections<sup>2</sup> and repeated dietary exposure to the mycotoxin, aflatoxin B<sub>1</sub>,<sup>3</sup> are major risk factors for the tumor in this population, and membranous obstruction of the inferior vena cava is a minor causal association.<sup>4</sup> Although individuals suffering from hereditary hemochromatosis are at greatly increased risk for HCC,<sup>5</sup> dietary iron overload has not hitherto been considered to be an etiologic association of this tumor in Black Africans.<sup>6,7</sup> This type of iron overload is common in Sub-Saharan Africa, achieving a prevalence of more than 10% in some populations.<sup>8</sup> Excessive tissue iron is potentially toxic, mutagenic, and mitogenic, and the liver is especially vulnerable.<sup>9</sup> We have performed a case-control study to test the hypothesis that dietary iron overload is a risk factor for the development of HCC in rural southern African Blacks.

## PATIENTS AND METHODS

**Subjects Studied.** The research was approved by the Human Investigation Committee of the University of the Witwatersrand, and written informed consent was obtained from all participants. Twenty-four consecutive patients from Shongwe and Themba Hospitals in the Mpumalanga Province of South Africa in whom HCC was suspected clinically underwent percutaneous liver biopsies for confirmation of the diagnosis and assessment of iron content of nonmalignant hepatic tissue. These patients were hospitalized with symptoms of right upper abdominal pain, weight loss, weakness, or abdominal swelling, sometimes alone but usually in some combination. The median age of the patients was 41 years, and all but 4 were men (Table 1). An enlarged liver was invariably present (in one patient an arterial bruit was heard over the tumorous liver), ascites was detected in 50% of the patients, splenomegaly in 15%, and pedal edema in 8%. The veins on the anterior abdominal wall were prominent in most patients. Only 38% of the patients were jaundiced. Two hospital controls without liver disease and matched for race, age, and sex were recruited for each patient. The 48 control subjects were predominantly hospitalized for trauma or acute or chronic infections. In addition, 51 first-degree relatives, 8 second-degree relatives, and 16 unrelated family members of the index cases and living in proximity to the index cases were studied.

**Assessment of Dietary Exposure to Iron and Alcohol.** Detailed histories were taken to quantitate the consumption of traditional, home-brewed beer, and commercial alcoholic beverages. Traditional beer is prepared in nongalvanized iron drums. It has a high iron content<sup>10</sup> and a low alcohol content. In 48 samples of this beverage collected from the Shongwe community, the mean ( $\pm$ SD) alcohol concentration was  $3.2 \pm 0.4$  g%, and the mean iron content was  $46 \pm 17$  mg/L. The other type of alcohol usually consumed was Western-style commercial beer.

**Assessment of Liver Biopsies.** Histologic sections of the liver biopsies were stained with hematoxylin and eosin, Gordon and Sweet's stain for reticulin fibers, and Prussian blue stain for iron. If the specimen contained nonmalignant liver tissue as well as HCC, the hepatocellular

Abbreviation: HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HBV, hepatitis B virus.

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Received October 24, 1997; accepted February 17, 1998.

Supported by grants from the South African Medical Research Council and the University of the Witwatersrand and the Office of Minority Health to the Cell Biology and Metabolism Branch, National Institute of Child Health and Human Development (NICHD) Contract No. I-HD 3-3196.

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0270-9139/98/2706-0014\$3.00/0

TABLE 1. Clinical Findings in Patients With Hepatocellular Carcinoma, Race-, Age- and Sex-Matched Controls, and Family Members

	Patients With HCC (n = 24)	Controls (n = 48)	P*	Family Members (n = 75)	P†
Age (yrs; median, and range)	41 (11-80)	38 (11-78)	.79	35 (12-84)	.36
Female sex (no. and %)	4 (16.7)	8 (16.7)	1.0	48 (64.0)	<.001
Alcohol consumption (no. and %)	13 (54.2)	18 (37.5)	.21	34 (42.5)	.21
Traditional beer	12 (50.0)	7 (14.6)	.0004	25 (33.3)	.15
Other alcoholic beverages	8 (33.3)	17 (35.4)	1.0	30 (40.0)	.64
Hemoglobin (g/dL; median and range)	11.5 (7.0, 20.7)	12.9 (9.4, 15.7)	.006	13.3 (7.2, 18.2)	<.001
White blood cells ( $\times 10^{-3}/\mu\text{L}$ ; median and range)	10.4 (3.0, 23.3)	6.3 (1.2, 18.7)	.012	5.3 (1.1, 12.4)	<.001
Erythrocyte sedimentation rate (mm/hr; median and range)	73 (1, 115)	47 (3, 122)‡	.050	35 (2, 105)	<.001
Transferrin saturation (%; median and range)	19 (5, 110)	26 (7, 91)	.52	30 (5, 93)	.20
Serum ferritin (ng/mL; median and range)	448 (14, 17991)	176 (1, 5765)	.024	35 (1, 2355)	<.001
Iron overload (transferrin saturation $\geq 60\%$ and serum ferritin elevated [no. and %])	5 (20.8)	3 (6.3)	.11	8 (10.7)	.20
HBsAg positive [no. and %]	16 (66.7)	5 (10.4)	<.001	13 (17.3)	<.001
Hepatitis C antibody positive (no. and %)	1 (4.2)	1 (2.1)	1.0	0 (0)	.24
Aflatoxin B <sub>1</sub> -albumin adducts (pg/mg; median and range)	7.3 (2.4, 91.2)§	21.7 (0, 45.6)	.055	8.7 (0.7, 82.1)	.64

Abbreviation: HBsAg, hepatitis B surface antigen.

\*Significance level for controls compared with HCC patients.

†Significance level for family members compared with HCC patients.

‡n = 47.

§n = 22.

||n = 45.

lar iron was graded on Prussian blue stained sections with a scale of 0 to 4+ using Scheuer's method.<sup>11</sup>

**Examination of Blood Samples.** Morning venous blood samples were collected from all fasting study subjects. Full blood counts were determined by an automated method. Serum iron concentrations and total iron binding capacity were measured by methods modified from those recommended by the International Committee for Standardization in Hematology.<sup>12,13</sup> Transferrin saturation was calculated as the serum iron level divided by the total iron binding capacity, multiplied by 100. Enzyme-linked immunosorbent assays were used to measure serum ferritin concentrations (Ramco, Houston, TX), hepatitis B and C virus markers (Abbott Laboratories, North Chicago, IL), and serum aflatoxin B<sub>1</sub>-albumin adducts.<sup>14</sup>

**Definition of Iron Overload.** Iron overload was defined as a markedly raised serum transferrin saturation combined with an elevated serum ferritin concentration.<sup>15</sup> We used a cut-off level for transferrin saturation of  $\geq 60\%$ , which is widely used in the diagnosis of hereditary hemochromatosis<sup>16</sup> and is more than 4 SD greater than the mean transferrin saturation described for African Americans.<sup>17</sup> We followed the example of an assessment of the iron nutritional status of the US population and regarded a raised serum ferritin concentration to be greater than 150 g/L in women less than 44 years of age; greater than 200 g/L in men less than 44 and women 45 to 65 years; greater than 300 g/L in men 45 to 64 and women over 64 years of age; and greater than 400 g/L in men over 64 years of age.<sup>17</sup> The combination of a raised transferrin saturation and a raised serum ferritin concentration in this definition excludes assigning as iron-loaded, subjects with an elevated serum ferritin level solely because of the inflammatory response that occurs in the presence of infection, malignancy, immunologic diseases, or trauma, because these conditions are associated with a marked decrease in the serum iron and transferrin saturation.<sup>18</sup>

**Statistical Analysis.** Comparisons were made between subjects with HCC and either controls or family members by means of the Mann Whitney U test for continuous variables and Fisher's Exact test for proportions. Logistic regression was used to examine the relationship of iron status, a history of alcohol consumption, HBV and HCV markers, and the concentration of albumin-aflatoxin adducts to HCC in the patients and control subjects. An identical logistic regression model was used for patients and family members, except that age and sex were added to the model. The population attributable risk of iron overload for HCC was estimated.<sup>19</sup>

## RESULTS

The characteristics of 24 patients with HCC, 48 race-, age-, and sex-matched, hospitalized control subjects and 75 family members are shown in Table 1. Five patients with HCC were considered to be iron overloaded; in 3 this diagnosis was confirmed by finding 4+ hepatocellular iron in nonmalignant tissue of the liver biopsy specimen, whereas in 2, no nonmalignant tissue was available for assessment.

Among 24 patients and 48 hospital controls, the risk of developing HCC in the iron-loaded subjects was 10.6 (95% confidence limits of 1.5 and 76.8) relative to individuals with normal iron status, after adjusting for alcohol consumption, chronic HBV and HCV infections, and exposure to aflatoxin B<sub>1</sub>. The risk of HCC in subjects with HBV infection was 33.2 (7.2, 153.4) (odds ratio [95% confidence limits]), with HCV infection 6.4 (0.3, 133.5), and with alcohol consumption 2.0 (0.5, 8.2). Aflatoxin B<sub>1</sub> exposure did not appear to increase the risk of HCC in this study. The population attributable risk of iron overload to the development of HCC was estimated to be 29%, and of HBV infection, 69%. Among the 24 patients and 75 family members, the risk for developing HCC with iron overload was 4.1 (0.5, 32.2).

## DISCUSSION

This study shows that Black Africans with dietary iron overload are at increased risk of developing HCC. Two earlier observations had hinted that dietary iron overload may contribute to hepatocellular carcinogenesis. In a study of serum and tumor ferritins in HCC in Black Africans, the nontumorous liver tissue of 6 of 17 patients contained chemical iron in the range expected in patients with advanced hereditary hemochromatosis, and heavy iron deposits were evident in the hepatocytes on histological examination.<sup>20</sup> A recent proportional mortality analysis of the 1920s autopsy series, in which Strachan first described iron overload in Africans, showed an association between high hepatic iron levels and death from HCC.<sup>21</sup>

Because of the limited information obtained in this study

concerning the presence of coexisting cirrhosis, we are unable to state whether iron loading is an independent cause of the tumor or acts as an indirect carcinogen by inducing chronic necroinflammatory hepatic disease. The controls did not have liver biopsies performed, and it was not possible to reliably diagnose cirrhosis on the biopsies used to confirm the presence of HCC. This limitation is analogous to that which pertains to the attempt to elucidate the nature of the association between iron overload in hereditary hemochromatosis and HCC. As many as 30% of patients with hereditary hemochromatosis develop this tumor, and the lifetime relative risk has been calculated to be over 200.<sup>5</sup> Until recently, all reported cases of HCC complicating this disease have arisen in cirrhotic livers, and it was assumed that excess tissue iron was only indirectly carcinogenic. However, a number of patients have now been described in whom HCC developed in the absence of cirrhosis,<sup>22</sup> implying that iron may also be directly carcinogenic.

Experimental evidence of the mutagenicity and mitogenicity of iron in tissue supports this conclusion.<sup>23-25</sup> The precise way or ways in which increased tissue iron may contribute directly to hepatocarcinogenesis are, however, yet to be determined. One possible mechanism is that free cellular iron may induce mutations by generating reactive oxygen species.<sup>23-25</sup> A number of DNA changes are produced by reactive oxygen species. These include base changes, some of which have been shown to be mutagenic,<sup>26</sup> breakage of DNA strands,<sup>27</sup> and effects on apoptosis.<sup>25</sup> In addition, reactive oxygen species may induce lipid peroxidation of polyunsaturated fatty acid moieties, the products of which may be genotoxic.<sup>25,28</sup> Further evidence supporting a role for iron in carcinogenesis is the observation that tumor cells are better able to grow and survive *in vitro* in the presence of high levels of extracellular iron.<sup>29</sup> The clinical observation that excess hepatic iron may facilitate persistence of chronic hepatitis B and C virus infections, both of which are major risk factors for the development of HCC, suggests an indirect way in which iron may contribute to the genesis of this tumor.<sup>30</sup> Whether the mutation in a novel major histocompatibility complex class I-like gene recently described in the majority of patients with hereditary hemochromatosis<sup>31</sup> has a direct bearing on the high risk of HCC in this disease remains to be determined. Similarly, the emerging evidence that dietary iron overload in Black Africans may have a genetic component<sup>32</sup> raises the possibility that the latter may also contribute to hepatocarcinogenesis.

In a recent study, chronic HBV infection alone accounted for an estimated 43% of HCC in southern African Blacks. Chronic HCV infection alone accounted for 5%, and the two infections together, 20%.<sup>2</sup> Exposure to dietary aflatoxin B<sub>1</sub> in southern African Blacks varies according to geographical location. An overall population attributable risk has not been estimated. Nevertheless, in regions of heavy exposure to this mycotoxin, evidence for a causal association exists, and at least one possible mechanism for a promoter function has been described, namely, mutational inactivation of the p53 tumor suppressor gene.<sup>3,33,34</sup> Membranous obstruction of the inferior vena cava is a risk factor with a small population attributable risk.<sup>4</sup> Thus, unidentified risk factors that could act as initiators or promoters of hepatocarcinogenesis in Black Africans remain. Dietary iron overload is common in sub-Saharan Africa, and hepatic iron concentrations rival those occurring in hereditary hemochromatosis. Moreover,

iron loading of hepatocytes is common to the two conditions.<sup>8</sup> Our estimate of a population attributable risk of 29% for dietary iron overload suggests a significant role for this disorder in the pathogenesis of HCC in Black Africans and the need for further investigations.

**Acknowledgment:** The authors thank Professor B. I. Schoub of the National Institute of Virology, Johannesburg, for the hepatitis virus serology.

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