Hepatocellular carcinoma (HCC) is a leading cause of cancer death worldwide [World Health Organization (WHO) 2003], and a heavy burden of HCC has been documented in sub-Saharan Africa (Bah et al. 2001; Bosch et al. 2005). Chronic infection with hepatitis B virus (HBV) is endemic in sub-Saharan Africa, and hepatitis C virus (HCV) infection is also present (McMahon 2005; The Global Burden of Hepatitis C Working Group 2004). Dietetic exposure to aflatoxin, primarily through ingestion of contaminated maize and groundnuts (peanuts), is also widespread (Turner et al. 2005; Wild et al. 1993). Largely because of the lack of clinical and research infrastructure, rigorous investigation into the etiology and characteristics of chronic liver disease in sub-Saharan Africa has been limited. Studies on HCC are relatively rare, and controlled studies on the etiology of cirrhosis have been reported even less frequently (Lesi et al. 2002).

Worldwide, cirrhosis of the liver is the 16th leading cause of death, responsible for hundreds of thousands of deaths each year (WHO 2003). Cirrhosis onset is often asymptomatic or associated with mild clinical symptoms, and individuals with subclinical cirrhosis can lead relatively normal lives for many years. Cirrhotic persons, however, are at high risk for liver decompensation and, irrespective of etiology, have a high risk for development of HCC. Diagnosis of cirrhosis, generally requiring histopathologic review of a liver biopsy specimen, is infrequently performed in many resource-constrained settings. In developed countries, cirrhosis is associated with chronic infection with HBV and HCV viruses (Corraro et al. 1998; Tsai et al. 1994, 2003), excessive use of alcohol (Corraro et al. 1998; Tsai et al. 2003), hereditary factors (Gershwin et al. 2005), obesity (Poonawala et al. 2000), smoking (Tsai et al. 2003), and occupational exposure to vinyl chloride (Mastrangelo et al. 2004), but evaluation of potential interactions between these risk factors are only beginning to be conducted (Corraro et al. 1998; Mastrangelo et al. 2004). Additionally, it is not known whether recognized etiologic factors for cirrhosis constitute an exhaustive list or whether unidentified etiologic agents remain.
Aged 44.8 ± 15.2 years (mean ± SD) and 42.5 ± 14.1 years (mean ± SD).

Ethnic group*

| Site* | Controls | Cirrhosis cases |
|-------|----------|----------------|
| RVH   | 107 (27.0) | 51 (52.6) |
| MRC   | 102 (25.7) | 21 (21.7) |
| BSG   | 188 (47.4) | 25 (25.8) |

Age group (years) and sex. Participants without suspected liver disease were administered the identical interviewer-administered questionnaire, underwent a standardized clinical examination to identify signs of underlying liver disease, and provided a blood sample. Local and international scientific and ethical review committees approved the study protocol, and informed consent was obtained from each participant before inclusion in the study.

Table 1. Characteristics of the study participants (controls, n = 397; cirrhosis cases, n = 97).

| Characteristic | Controls [no. (%)] | Cirrhosis cases [no. (%)] |
|---------------|--------------------|--------------------------|
| Sex           |                     |                          |
| Male          | 282 (71.0)         | 61 (62.9)                |
| Female        | 115 (29.0)         | 36 (37.1)                |
| Site*         |                     |                          |
| RVH           | 107 (27.0)         | 51 (52.6)                |
| MRC           | 102 (25.7)         | 21 (21.7)                |
| BSG           | 188 (47.4)         | 25 (25.8)                |
| Age group (years) |                 |                          |
| < 35          | 125 (31.5)         | 31 (32.0)                |
| 35–44         | 76 (19.1)          | 21 (21.7)                |
| 45–54         | 72 (18.1)          | 24 (24.7)                |
| 55–64         | 76 (19.1)          | 15 (15.5)                |
| ≥ 65          | 48 (12.1)          | 6 (6.2)                  |
| Recruitment timing |                 |                          |
| November–January | 98 (24.7)     | 30 (30.9)                |
| February–April   | 88 (22.2)      | 23 (23.7)                |
| May–July        | 84 (21.2)        | 26 (26.8)                |
| August–October  | 127 (32.0)       | 18 (18.6)                |
| Ethnic group*   |                     |                          |
| Mandinka       | 130 (32.8)        | 24 (24.8)                |
| Fula           | 82 (20.7)         | 31 (32.0)                |
| Wolof          | 60 (15.1)         | 23 (23.7)                |
| Other          | 125 (31.5)        | 19 (19.6)                |
| Education*     |                     |                          |
| Ever school    | 353 (88.9)        | 77 (79.4)                |
| None           | 44 (11.1)         | 20 (20.6)                |
| Earth floor in residence |       |                          |
| Yes            | 197 (49.6)        | 59 (60.8)                |
| No             | 200 (50.4)        | 38 (39.3)                |
| Regular tobacco use |                 |                          |
| Cigarettes     | 163 (41.1)        | 36 (37.1)                |
| Pipe           | 25 (6.3)          | 7 (7.2)                  |
| Chewing/snuff  | 18 (4.3)          | 8 (8.3)                  |
| Regular alcohol use |             |                          |
| 33 (8.3)       | 8 (8.3)           |                          |
| Age (years [mean ± SD]) |            |                          |
| 44.8 ± 15.2    | 42.5 ± 14.1       |                          |
| Cigarette pack-years (mean ± SD) |      |                          |
| 7.4 ± 16.2     | 5.5 ± 12.0        |                          |

*p < 0.05 compared with controls in that location or group.
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Results

Characteristics of the study participants. After excluding liver-disease referral patients with space-occupying lesions on ultrasonography characteristic of HCC or with alternative diagnoses other than liver disease (Kirk et al. 2004), a total of 111 persons with suspected liver cirrhosis were identified. Of these, two were not tested for HBsAg. Of the remaining 109 individuals, 97 had a score of ≥ 7 on the ultrasound-based cirrhosis scale of Lin et al. (1993) and were classified as cirrhosis cases. Of 408 control participants recruited, 397 had no evidence of underlying liver disease, AFP levels in the normal range, and complete HBsAg and demographic information and were included in this analysis.

Characteristics of the 97 cirrhosis cases and 397 controls are listed in Table 1. Of the case and control participants, mostly male, they often lived in homes with dirt floors, and they were recruited throughout the year and from all adult age brackets. The mean age of cirrhotic patients was 42.5 years compared with 44.8 years for controls. Cirrhotic patients differed from control participants with regard to recruitment site and ethnicity and were less likely to have attended school.

HBV and HCV infection and cirrhosis risk. Table 2 shows markers of HBV and HCV infection among cirrhosis cases and controls, as well as unadjusted and adjusted odds ratios (ORs) for cirrhosis. HBsAg seropositivity was very common among the cirrhosis cases (58.8%) and highly associated with cirrhosis [adjusted OR = 8.0; 95% confidence interval (CI), 4.4–14.7]. Individuals who were HBeAg-positive had a 10-fold higher risk of cirrhosis than persons without chronic HBV infection. HCV infection was identified in 9.3% of cirrhotic cases and was associated with a > 3-fold higher risk of cirrhosis compared with HCV-uninfected persons.

Aflatoxin exposure and cirrhosis risk. Eighty cirrhosis cases and 327 controls provided information regarding groundnut consumption on the study questionnaire. In addition, 78 cirrhosis cases and 346 controls were evaluated for plasma 249ser TP53 mutations, which, although perhaps less sensitive, would be reflective of the biological effects of longer-term aflatoxin exposure and be less susceptible to changes with illness. The plasma 249ser TP53 mutation was detected more often among individuals with higher levels of lifetime groundnut consumption, but this association did not attain statistical significance (exact p = 0.10). Therefore, although related, the dietary and molecular markers represent different measures of aflatoxin exposure; we used separate models for analysis of each aflatoxin marker.

We evaluated interactions between aflatoxin exposure and HBV infection through participant stratification and subsequent logistic regression analysis, as well as through incorporation of first-order interaction terms into the logistic regression models. All analyses were conducted using SAS version 9.1 (SAS Institute, Inc., Cary, NC, USA).

Table 2. HBV and HCV infection and association with cirrhosis (controls, n = 397; cirrhosis cases, n = 97).

|                  | Controls (no. [%]) | Cirrhosis cases (no. [%]) | Unadjusted OR (95% CI) | Adjusted OR (95% CI) |
|------------------|--------------------|---------------------------|------------------------|----------------------|
| HBsAg status     |                    |                           |                        |                      |
| HBsAg (–)        | 336 (84.6)         | 40 (41.2)                 | Referent               | Referent             |
| HBsAg (+)        | 61 (15.4)          | 57 (58.8)                 | 7.8 (4.8–12.8)         | 8.0 (4.4–14.7)       |
| HBsAg (+)/HBeAg (+) | 2 (0.5)        | 15 (15.5)                 | 36.1 (8.1–161.0)       | 10.3 (2.0–53.9)      |
| HCV status       |                    |                           |                        |                      |
| HCV (–)          | 381 (96.0)         | 88 (90.7)                 | Referent               | Referent             |
| HCV (+)          | 16 (4.0)           | 9 (9.3)                   | 2.4 (1.0–5.7)          | 3.3 (1.2–9.5)        |

*Adjusted for age, sex, recruitment site and date, education, household floor type, tobacco, alcohol, HBV, and HCV variables.

Table 3. Exposure to aflatoxin and association with cirrhosis.

|                  | Controls (no. [%]) | Cirrhosis cases (no. [%]) | Unadjusted OR (95% CI) | Adjusted OR (95% CI) |
|------------------|--------------------|---------------------------|------------------------|----------------------|
| Lifetime groundnut intake | n = 327        | n = 80                    |                        |                      |
| Low              | 68 (21)            | 10 (13)                   | Referent               | Referent             |
| Moderate         | 189 (58)           | 43 (54)                   | 1.6 (0.7–3.3)          | 1.7 (0.7–4.2)        |
| High             | 70 (21)            | 27 (34)                   | 2.6 (1.2–5.8)          | 2.8 (1.1–7.7)        |
| 249ser TP53 mutation | n = 346         | n = 78                    |                        |                      |
| Absent           | 329 (95)           | 65 (83)                   | Referent               | Referent             |
| Present          | 17 (5)             | 13 (17)                   | 3.9 (1.8–8.4)          | 3.8 (1.5–9.6)        |

*Adjusted for age, sex, recruitment site and date, education, household floor type, alcohol, tobacco, HBV, and HCV variables.

Table 4. Joint effect of HBV infection and aflatoxin exposure and association with cirrhosis.

|                  | Controls (n = 327) | Cirrhosis cases (n = 97) | Adjusted OR (95% CI) |
|------------------|--------------------|--------------------------|----------------------|
| HBV and lifetime groundnut intake status | n = 327        | n = 80                    | Referent             |
| HBsAg (+)/High intake (+) | 218 (67)        | 25 (31)                   | 1.7 (0.7–4.1)        |
| HBsAg (+)/High intake (+) | 62 (19)         | 12 (15)                   | 8.1 (3.9–17.1)       |
| HBsAg (+)/High intake (+) | 28 (12)         | 28 (35)                   | 26.8 (8.7–82.1)      |
| HBV and 249ser TP53 status | n = 346        | n = 78                    | Referent             |
| HBsAg (+)/249ser TP53 (+) | 284 (82)        | 29 (37)                   | 4.6 (0.0–249.1)      |

*Adjusted for age, sex, recruitment site and date, education, household floor type, alcohol, tobacco, and HCV variables.
Discussion

Despite the postulated heavy burden of disease, remarkably little descriptive risk factor or natural history data have been published on chronic liver disease or cirrhosis from sub-Saharan African populations (Lesi et al. 2002; Lin et al. 2005). In this study, we found that chronic HBV infection and aflatoxin exposure, either separately or in synergy, were the etiologic agents likely responsible for most cirrhosis cases identified in this West African study population. Our results suggest that the spectrum of morbidity associated with aflatoxin exposure could include cirrhosis.

The impact of aflatoxin on human health is substantial and has been documented in both acute and chronic exposure settings (Groupman et al. 2008; Strosnider et al. 2006). Acute exposure to high levels of aflatoxin (aflatoxicosis) can result in acute toxicity, which often presents clinically as fulminant liver failure. For example, a recent outbreak of acute aflatoxicosis from contaminated maize in Kenya resulted in 125 human deaths, significant mortality among domesticated livestock, and widespread socioeconomic impact (Lewis et al. 2005). The health impacts of chronic aflatoxin exposure are equally serious. A large body of experimental, clinical, and epidemiologic evidence has defined aflatoxin as one of the most potent naturally occurring hepatocarcinogens; chronic exposure to moderate or even low levels of aflatoxin has been linked to development of HCC (IARC 1993). Chronic aflatoxin exposure has also been associated with impaired growth and perturbations in measures of immune function in young West African children (Gong et al. 2004; Turner et al. 2003). However, data regarding other potential health effects of chronic aflatoxin exposure are scarce, resulting in significant limitation of current research (Strosnider et al. 2006). Primary limitations for conducting this research include difficulties in defining clinical outcomes in often remote or resource-constrained environments and difficulty in accurately assessing aflatoxin exposure.

Although standardized clinical scoring systems, such as the Child-Pugh score, have for decades been accepted for assessing the severity of liver cirrhosis, numerous recent reports have increasingly focused on the utility of noninvasive methods and clinical prediction models to diagnose fibrosis and cirrhosis (Schneider et al. 2005; Shen et al. 2006; Yamada et al. 2006). However, most of these studies to date have been conducted among HCV-infected populations in industrialized countries. In the present study we used a validated, reproducible, and noninvasive diagnostic method to diagnose cirrhosis in a setting where an etiologic study of risk factors for biopsy-diagnosed liver cirrhosis would not have been possible. The presence of encephalopathy or marked ascites precluded biopsy in some study participants, and resources for rapidly performing prebiopsy screening laboratories were not consistently available at the three recruitment sites. Furthermore, participant acceptance of ultrasound was excellent, and potentially serious postbiopsy complications were summarily avoided. Last, given that the specificity of the ultrasound scoring system used in this study is >90% among HBV-infected subjects (Hung et al. 2003), we believe that noninvasive methods for measuring liver fibrosis and diagnosing cirrhosis hold significant promise for increasing clinical and research capabilities in African settings.

Currently there is no gold standard methodology available to measure cumulative lifetime aflatoxin exposure. In lieu of a gold standard, we used two different approaches to assess exposure to aflatoxin during the period of time that we hypothesized would have etiologic significance for the development of cirrhosis. First, we evaluated self-reported lifetime intake of groundnuts, the primary food component contributing to aflatoxin exposure in The Gambia (Wild et al. 1993, 2000). Questionnaire-derived data have been validated for assessment of many dietary components; however, this information is obviously dependent on participant recall for its validity. Second, we evaluated a laboratory-based biomarker potentially reflecting the biological effect of aflatoxin exposure. The presence of the 249<sup>th</sup> TP53 mutation, associated with aflatoxin exposure in experimental systems (Aguilar et al. 1993; Mace et al. 1997), is commonly observed in HCC patients from high aflatoxin exposure regions (Aguilar et al. 1994) and is strongly associated with HCC in case-control and prospective epidemiologic studies (Jackson et al. 2003; Kirk et al. 2005, 2000). However, recent experimental data suggest that aflatoxin exposure alone may not be sufficient to induce 249<sup>th</sup> TP53 mutations (Tong et al. 2006) and that other cofactors such as HBV infection (Sohn et al. 2000) or host factors (Mace et al. 1997) may modulate the mutagenic capacity of aflatoxin. In addition, because detection occurs in plasma DNA, it is unclear if advanced liver disease impacts release of the mutation. Finally, levels of the 249<sup>th</sup> TP53 mutation in plasma exhibit seasonal variability (Lleonart et al. 2005), suggesting that this marker may partly reflect short-term effects of aflatoxin exposure. Longitudinal studies to determine marker stability over time and the effect of liver disease severity on detection are needed; these issues are the subject of ongoing investigations in The Gambia. Despite these limitations, we believe that the 249<sup>th</sup> TP53 mutation represents the best biomarker currently available to assess the biological effect of cumulative aflatoxin exposure.

Both measures of aflatoxin exposure evaluated as a part of this study were associated with a significant increase in the risk of cirrhosis. Our results also suggest that aflatoxin exposure and chronic HBV infection may interact synergistically to increase the risk of cirrhosis notably above and beyond that expected for two independent hepatotoxins. The mechanisms by which aflatoxin is activated and induces HCC-associated mutational changes have been well characterized (Wild and Turner 2002), but less well understood is the impact of aflatoxin on the development of cirrhosis and precarious architectural changes in the liver. Empirical evidence of AFB1-induced fibrosis and cirrhosis in humans has thus far been limited (Aguilar et al. 1994), but animal studies have found significantly more liver fibrosis among animals experimentally inoculated with aflatoxin than uninoculated control animals (Ortitatli et al. 2005; Seffner et al. 1997). In addition, although significant progress has been made with regard to understanding the mechanisms of interaction between aflatoxin and HBV in hepatocarcinogenesis (Kew 2003), it is not clear whether these proposed mechanisms will explain the interaction between aflatoxin and HBV in the etiology of cirrhosis.

As expected, we found HBsAg positivity to be a significant risk factor for cirrhosis; we have extended this work to document a further increase in risk of cirrhosis associated with HBeAg seropositivity. Similarly, we found HCV infection to be a significant risk factor for cirrhosis, although the magnitude of this risk was lower than that conferred by HBsAg positivity. Notably, the average age of HCV-related cirrhosis patients was around 15 years older than HBV-related patients, consistent with the premise of a different natural history of infection between the two viruses in West Africa (for further discussion, see Kirk et al. 2004, 2006). In contrast, we observed no significant associations between alcohol and tobacco exposure and cirrhosis (data not shown). The low overall prevalence and limited societal acceptance of alcohol consumption in this majority Muslim country may offer some explanation for these findings. Also, given that a significant association between tobacco exposure and cirrhosis has been only rarely observed to our knowledge (Tsai et al. 2003), it is possible that exposure to tobacco use conveys no increase or only a slight increase in risk of cirrhosis.

Cirrhosis of the liver is a major cause of morbidity and mortality in sub-Saharan Africa. Using a validated ultrasound scoring system to diagnose cirrhosis, we confirmed associations with chronic HBV and HCV infections and further provide evidence that aflatoxin may also be an etiologic factor for cirrhosis. As such, expanded access to
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