Short Communication

Association of Aflatoxin B₁-Albumin Adduct Levels with Hepatitis B Surface Antigen Status among Adolescents in Taiwan


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Abstract

Chronic hepatitis B virus (HBV) infection and aflatoxin B₁ (AFB₁) exposure interact synergistically to induce hepatocellular carcinoma. One suggested mechanism for this interaction is the enhanced activation of AFB₁ in chronically HBV-infected individuals. Whereas no associations between chronic HBV infection and AFB₁-albumin adducts were observed in several studies in adults, hepatitis B surface antigen (HBsAg)-positive children were found to have elevated adducts in Gambia. To assess the association between chronic HBV infection and AFB₁-albumin adduct level in Taiwan, 200 junior high school adolescents from 20 townships were assayed for HBsAg and AFB₁-albumin adducts. The mean AFB₁-albumin adduct level was higher in HBsAg-positive compared with HBsAg-negative subjects. The association between HBsAg status and AFB₁-albumin adducts remained after multivariate adjustment. This finding additionally supports the synergistic interaction between HBV and AFB₁, but the mechanism remains to be elucidated.

Introduction

HCC³ is one of the most common cancers in the world and one of the leading malignancies in sub-Saharan Africa and Southeast Asia, including Taiwan (1, 2). Chronic HBV infection is the major cause of ≥80% of HCC (1–3). In Taiwan, HBV infection is hyperendemic with a 15–20% carrier rate of HBsAg in the general population (4). AFB₁, a potent mycotoxin, has also been well documented as a risk factor in Taiwan (5–9). It is metabolized by the CYP (10) to a reactive epoxide that can bind to DNA, forming AFB₁-guanine adducts, and to protein, forming AFB₁-albumin adducts. Albumin adducts have been used extensively to investigate exposure to AFB₁ in both epidemiological (6, 7, 11) and chemopreventive studies (12).

A synergistic interaction between AFB₁ exposure and HBV infection on HCC risk has been reported in several epidemiological studies (8, 13). Studies in several animal models including ducks and woodchucks infected with HBV as well as an HBV-transgenic mouse model also demonstrated a synergistic effect (14–16). One suggested mechanism for this effect is enhanced activation of AFB₁ in HBV-infected animals with supporting data generated in one study (17) although conflicting data have also been reported (18). In humans, several previous studies in adults failed to find an association between HBsAg status and albumin adducts (11, 19, 20), whereas one study found higher levels of AFB₁-guanine in urine of infected individuals (21). Three studies investigated this relationship in children, all in Gambia, and found higher AFB₁-albumin adducts in infected compared with noninfected children 3–8 years of age (22, 23) and 3–4 years of age (24).

To explore this association in another geographic area, we determined AFB₁-albumin adducts and HBsAg status in 200 junior high school adolescents (13–15 years of age) from 20 townships in Taiwan.

Materials and Methods

Subjects and Sample Collection. Two hundred junior high school students (50 males positive and 50 males negative for HBsAg and a similar number of females) were selected through stratified random sampling from 875 adolescents recruited between January and May 1991 for a study of geographical variation in hepatitis A and B infection in 20 townships in Taiwan (25). The junior high school attendance rate in Taiwan was as high as 95%, and this birth cohort is the last not immunized against HBV at birth. Thus, we recruited a representative sample with no bias in their HBsAg carrier rate caused by the vaccination. Information on the previous 3-day dietary consumption pattern was obtained through a standardized questionnaire by interviewers. After collection, bloods were centrifuged and separated into aliquots; serum samples were stored at −20°C until tested. Overnight urine samples were also stored at −20°C until tested.

The Measurement of HBsAg Status and AFB₁-Albumin Adduct Level and Urinary Aflatoxins. HBsAg in serum was determined by enzyme immunoassay using commercial reagents (Abbott Laboratories, North Chicago, IL) according to the manufacturer’s manuals. AFB₁-albumin adducts level was determined by competitive ELISA using a polyclonal antiserum (number 7) essentially as described previously (6, 8). Samples with <20% inhibition were considered nondetectable and as...
signed a value of 2 fmol/mg. The level of urinary aflatoxins was detected by ELISA as described previously (8).

**Statistical Analysis.** AFB1-albumin adduct levels were natural logarithm-transformed to normalize the distribution. A t test was used to evaluate the differences in mean adduct levels (ln fmol/mg) by HBsAg status, sex, urinary aflatoxins, and consumption of peanut, corn, and yam/cassava in the past 3 days, as well as by HBsAg status after stratification by gender. Both univariate and multivariate linear regression analyses were conducted to examine associations of sex and HBsAg with AFB1-albumin levels.

**Results**

The mean ages (± SD) were 14.22 ± 0.44 and 14.35 ± 0.50 years in males and females, respectively, with a range of 13–15 years in both groups. According to our definition, there were 7 (7%) male and 3 (3%) female adolescents with nondetectable adduct levels. The proportions of nondetectable adduct levels in males and females were not significantly different. Ninety-five percent (95%) of the samples had detectable levels of AFB1-albumin adducts with means ± SD of 34.50 ± 31.44 and 42.59 ± 27.47 fmol/mg for males and females, respectively. The mean value in all of the females was significantly higher than that in all of the males. The range was similar in both groups (2–138 fmol/mg in males and 2–174 fmol/mg in females).

Data were natural logarithm-transformed for statistical analysis. AFB1-albumin adduct levels (ln fmol/mg) by sex and HBsAg status are given in Table 1. HBsAg-positive males had higher adduct levels than HBsAg-negative males with mean adduct levels of 3.26 ± 1.02 and 2.83 ± 1.14, respectively (P = 0.05). The corresponding in ln fmol/mg values in females were 3.63 ± 0.62 and 3.42 ± 0.87, respectively (P = 0.16). Multivariate linear regression analysis indicated a significant association between HBsAg and AFB1-albumin adduct level (data not shown). Adolescents positive for HBsAg had a significantly higher level of AFB1-albumin adducts (P = 0.02) after adjustment for sex. The sex-adjusted mean AFB1-albumin adduct levels were 3.14 ± 0.10 (mean ± SE) and 3.46 ± 0.10 among HBsAg-negative and -positive adolescents, respectively. There was no interaction effect between HBsAg and sex on the adduct level, either comparing the sex adjusted mean to the total mean of adduct levels by HBsAg or testing the interaction term in the multivariate model.

There were no significant differences in adduct levels by sampling month. After stratifying by sampling month, adduct levels (ln fmol/mg) were 3.14 ± 0.10, 3.51 ± 0.73, 3.30 ± 1.12, and 3.10 ± 0.87 in January, March, April, and May, respectively. There were no differences in mean albumin adduct levels when subjects were classified by their urinary aflatoxins measurement as positive or negative (data not shown). Nor were there associations between albumin adduct levels and the previous 3-day consumption of peanut, corn, and yam/cassava (data not shown).

**Discussion**

AFB1-albumin adduct levels were significantly higher in female compared with male adolescents (13–15 years of age) in Taiwan. Previous studies in adults had observed that either gender was not a significant factor associated with adducts (19), or females had slightly higher adduct levels (11). The previous data on children found no difference by sex in Gambia (23). Whereas there were higher adduct levels in females compared with males in the present study, there were no gender differences in intake frequency of yams, corn, and peanuts. Thus, differences in dietary patterns probably do not account for differences in adduct levels. The mechanism responsible for the gender effect on adduct levels among adolescents remains unknown. A previous study demonstrated female-specific CYP3A expression in adult mouse liver, and the female-specific CYP3A was one of the major CYP3A forms in the female mouse liver (26). CYP3A4, a Phase 1 enzyme involved in the metabolism of AFB1, exhibits higher activity in women than in men (27). Whereas hormonally induced differences in aflatoxin metabolism may explain adduct differences between male and female adolescents, there was no female-specific effect on adducts among adults (19, 20) and children (23). Alternatively, the gender difference in the timing of horizontal sexual exposure and acquisition of HBV infection may influence gender difference in adduct formation. However, horizontal sexual exposure to HBV is uncommon and not a major route of infection in this population. In Taiwan, HBV infection occurs early, with a carrier rate in adolescents of 12–13% (28, 29). The seropositive rate of HBV infection reached 50% by the age of 14 years (28). Perinatal infection from highly infectious mothers to their infants is a major route for HBV infection in Taiwan (4). The mechanisms by which gender effects adduct formation needs additional study.

Previous epidemiological data (8, 13) suggested a synergistic multiplicative interaction effect between HBV and AFB1 on HCC risk. We observed an association between HBV and AFB1 adduct formation in this study, though the mechanism of this interaction remains uncertain. HBV infection might affect AFB1 adduct formation through alteration of AFB1 metabolism. On the other hand, the immunosuppressive actions of AFB1 might modulate HBV infection. Two mechanisms have been suggested for HBV alteration of carcinogen metabolism, a nonspecific response to liver damage or a specific effect of the virus such as activation of cellular genes by x antigen (30). There is some support for these mechanisms from animal.

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**Table 1** AFB1-albumin adduct levels (ln fmol/mg) by sex and HBsAg among adolescents in Taiwan

<table>
<thead>
<tr>
<th>Variable</th>
<th>Males</th>
<th>Females</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SD)</td>
<td>P&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Mean (SD)</td>
</tr>
<tr>
<td>HBsAg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>45</td>
<td>2.83 (1.14)</td>
<td>0.10</td>
</tr>
<tr>
<td>Positive</td>
<td>49</td>
<td>3.26 (1.02)</td>
<td>0.05</td>
</tr>
<tr>
<td>All</td>
<td>94</td>
<td>3.05 (1.10)</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> P based on t test for difference between HBsAg positives and negatives.
<sup>b</sup> P based on t test for difference between males and females.
studies. In HBV transgenic mice, an increase in cytochrome P450 enzymes, which activate AFB1, was observed (31, 32). Similarly, in woodchucks infected with woodchuck hepatitis virus, increased activation of AFB1 was observed compared with uninfected animals (17). However, a study in mice showed that HBOx expression alone did not alter expression of carcinogen metabolizing enzymes (33). Nor do these data account for the consistent differences observed in humans in the relationship between AFB1-albumin adducts and HBsAg status with age, if true. The ability of the virus to alter AFB1 metabolism in children might differ from that in adults by some unknown mechanism.

Adducts were higher in both male and female HBsAg-positive adolescents compared with those HBsAg-negative (Table 1). These results are consistent with previous studies in younger Gambian children (3–8 years of age; Refs. 22, 23). A recent study also showed that the highest adducts were observed in acutely HBV-infected children, less high levels in chronic carriers, and the lowest in uninfected children (24). This is in contrast to a number of previous studies on adults in Taiwan (20), China (19), and Africa (11), which failed to find an association between HBsAg serostatus and albumin adducts. The possible effect of this age difference on the association between HBsAg and aflatoxin-albumin adducts is not clear. The HBcAg seropositive rate among HBsAg carriers is nearly 100% in infancy, decreases to around 87% in children ages 5–9 years, and to 76% in those ages 10 to 14 years (28). The annual HBcAg clearance rate increases with age among children in Taiwan (34). Among pregnant HBsAg-positive women in Taiwan, the HBcAg prevalence also decreased with age (35) to 49% in 21–25-year-olds, 37% in 26–30-year-olds, 32% in 31–35-year-olds, and 20% in 36–40-year-olds (36). The HBcAg prevalence among HBsAg-positive adolescents might be higher than that among adults possibly resulting in more active replication of HBV in adolescents than in adults. Active viral replication among carriers may result in recurrent hepatitis and higher likelihood of liver injury that might affect the enzymes metabolizing AFB1. Alternatively, children and adolescents may not have as diverse dietary or lifestyle (e.g., cigarette smoking) exposures to compounds that can induce activation enzymes as do adults. Thus, it may be more difficult to demonstrate a relationship between HBV and AFB1-adducts in adults.

Within the narrow period of sampling there was no difference in adduct levels by sampling months. We did not find associations between adduct levels and either urinary aflatoxins or the previous 3-day dietary consumption of several foodstuffs such as peanuts, corn, and yam/cassava, foods reported to be contaminated by aflatoxins (37). This lack of an association may result from the fact that the highest aflatoxins and the highest 3-day diet consumption reflect only recent exposure in contrast to albumin adducts, which reflect 2–3 months of exposure.

Exposure to aflatoxin might affect infection from HBV through its immunosuppressive actions. In male BALB/c mice, AFB1 exposure decreased peripheral leukocyte counts and natural killer cell function (38). In vitro, the degree of impairment of phagocytosis of human monocytes was dose-dependent on AFB1 (39). Mononuclear phagocytes are important both in the phagocytosis of human monocytes was dose-dependent on AFB1 (39). Mononuclear phagocytes are important both in the phagocytosis of human monocytes was dose-dependent on AFB1 (39). Mononuclear phagocytes are important both in the phagocytosis of human monocytes was dose-dependent on AFB1 (39). Mononuclear phagocytes are important both in the phagocytosis of human monocytes was dose-dependent on AFB1 (39). Mononuclear phagocytes are important both in the phagocytosis of human monocytes was dose-dependent on AFB1 (39).

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