Aflatoxin Exposure May Contribute to Chronic Hepatomegaly in Kenyan School Children

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BACKGROUND: Presentation with a firm type of chronic hepatomegaly of multifactorial etiology is common among school-age children in sub-Saharan Africa.

OBJECTIVE: Aflatoxin is a liver toxin and carcinogen contaminating staple maize food. In this study we examined its role in chronic hepatomegaly.

METHODS: Plasma samples collected in 2002 and again in 2004 from 218 children attending two schools in neighboring villages were assayed for aflatoxin exposure using the aflatoxin–albumin adduct (AF-alb) biomarker. Data were previously examined for associations among hepatomegaly, malaria, and schistosomiasis.

RESULTS: AF-alb levels were high in children from both schools, but the geometric mean (95% confidence interval) in year 2002 was significantly higher in Matangi [206.5 (175.5, 243.0) pg/mg albumin] than in Yumbuni [73.2 (61.6, 87.0) pg/mg; p < 0.001]. AF-alb levels also were higher in children with firm hepatomegaly [176.6 (129.6, 240.7) pg/mg] than in normal children [79.9 (49.6, 128.7) pg/mg; p = 0.029]. After adjusting for Schistosoma mansoni and Plasmodium infection, we estimated a significant 43% increase in the prevalence of hepatomegaly/hepatosplenomegaly for every natural-log-unit increase in AF-alb. In 2004, AF-alb levels were markedly higher than in 2002 [539.7 (463.3, 628.7) vs. 114.5 (99.7, 131.4) pg/mg; p < 0.001] but with no significant difference between the villages or between hepatomegaly and normal groups [539.7 (436.7, 666.9) vs. 512.6 (297.3, 883.8) pg/mg], possibly because acute exposures during an aflatoxicosis outbreak in 2004 may have masked any potential underlying relationship.

CONCLUSIONS: Exposure to aflatoxin was associated with childhood chronic hepatomegaly in 2002. These preliminary data suggest an additional health risk that may be related to aflatoxin exposure in children, a hypothesis that merits further testing.

KEY WORDS: aflatoxicosis outbreak, aflatoxin, aflatoxin albumin adducts, biomarker, child health, hepatomegaly, hepatosplenomegaly, malaria, schistosomiasis. Environ Health Perspect 120:893–896 (2012). http://dx.doi.org/10.1289/ehp.1104357 [Online 27 February 2012]
Informed consent was obtained from the parents or guardians. Ethics approval was obtained from the Kenya Medical Research Institute Ethical Review Committee.

Clinical examination. All children were examined for enlarged livers and spleens by palpation by three clinicians in 2002 and 2004, and a consensus among the clinicians had to be achieved before assigning the child to an organomegaly group, as previously described (Wilson et al. 2007b). The liver and/or spleen was considered enlarged if it was palpable > 2 cm below the costal line. The children were initially classified into five categories: no organomegaly, soft liver or spleen enlargement, firm to hard spleen enlargement (SM), firm to hard liver enlargement (HM), and both firm to hard liver and spleen enlargement (HSM).

Blood AF-alb measurements. Plasma samples collected from the 218 children examined in both 2002 and 2004 underwent AF-alb analysis in 2009. The blood AF-alb levels were determined using an enzyme-linked immunosorbent assay (ELISA) after albumin extraction from 250 μL plasma, digestion, and purification as previously described (Chapot and Wild 1991). One negative and three positive controls were analyzed alongside each batch of samples. Samples were measured using ELISA in quadruplicate on at least two occasions on separate days. The detection limit was 3 pg AF-alb per 1 mg albumin.

Statistical analyses. AF-alb data is not normally distributed and was therefore natural-log-transformed before data analysis. Geometric mean AF-alb levels and 95% confidence intervals (CIs) are presented unless otherwise stated. Student’s t-test and analysis of variance (ANOVA) were used for comparing levels between groups. Chi-square test was used for categorical variables distribution test. Pearson’s correlation coefficients were calculated for the association between continuous variables. Logistic regression models were constructed to estimate odds ratios for groupings of HM in association with aflatoxin exposure adjusted for schistosomiasis and malaria, which are known confounders or modifiers. Statistical analyses were carried out using STATA (version 9; StataCorp, College Station, TX, USA). A p-value < 0.05 was used to define statistical significance.

Results

Description of the study cohort. Table 1 summarizes the key variables of the 124 and 94 children 6–17 years of age from Yumbuni and Matangini. There were no significant differences in mean age or sex ratio between the two schools. Malaria and schistosomiasis were prevalent in this region. In 2002, the prevalence of schistosomiasis in these children was significantly higher in Matangini than in Yumbuni (70% vs. 8%, p < 0.001). There was no significant difference in the prevalence of malaria based on the presence of parasitemia (27% vs. 18%, p = 0.159) or the Pf-igG3 marker of exposure [optical density (OD) value, 0.47 vs. 0.44; p = 0.106] between children attending the two schools.

Aflatoxin exposure. Plasma AF-alb levels were not correlated with the children’s age or sex. The geometric mean AF-alb level for children in Matangini was significantly higher than that of children in Yumbuni (206.5 pg/mg (175.7, 243.0) vs. 73.2 pg/mg (61.6, 87.0) pg/mg; p = 0.001) in 2002 (Table 1). During 2004, AF-alb mean levels were significantly higher than in 2002 in both villages [combined means of 539.7 pg/mg (463.3, 628.7) in 2004 vs. 114.5 pg/mg (99.7, 131.4) in 2002; p < 0.001], but there was no longer a significant difference in the means between the two villages. AF-alb levels were not associated with malaria parasitemia or correlated with Pf-igG3 levels. Children with S. mansoni infection had more than double the AF-alb level as those without in 2002. However, the difference became nonsignificant after data were adjusted for village. There was no significant association between S. mansoni infection and AF-alb in 2004 (data not shown).

Prevalence of HM. The prevalence of HM/HSM was not significantly different between Yumbuni and Matangini in 2002 (73% vs. 76%; p = 0.686) or 2004 (74% vs. 83%; p = 0.087). There was no significant difference in HM prevalence between the subset and the wider group of the cohort (data not shown).

The number of children with HM only was 48 in year 2002, but this increased to 104 in 2004 (Table 2). Most children (88%) who...
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had HM/HSM in 2002 still had HM/HSM in 2004. Most new HM cases in 2004 had been classified as HSM cases in 2002 but had resolution of their SM. The total number of children with HM/HSM did not vary significantly between 2002 and 2004 (160 vs. 167). There was no significant relationship between the presentation of organomegaly and the age or the sex of the children in 2002 (data not shown). However, in 2004 children with HM/HSM (mean age, 11.5 years) were younger than normal children and children with SM (mean age, 13.5 years), whereas the two children with soft organomegaly were the youngest in the group (mean age, 6.5 years). More girls were in the normal group than boys in 2004 (22% vs. 12%; p = 0.041).

Aflatoxin exposure and organomegaly. In 2002, the geometric mean AF-alb level was significantly higher in children with HM than in children without firm organomegaly (Table 2)—that is, children classified as normal or with soft organomegaly (p = 0.029 and 0.004, respectively). No significant difference in geometric mean AF-alb levels was found among HM, SM, and HSM groups (176.6 pg/mg (129.6, 240.7), 102.6 pg/mg (65.4, 160.8), and 108.0 pg/mg (90.5, 129.0), respectively). For samples collected in 2004, AF-alb levels were markedly high in all groups, and no significant difference in AF-alb levels was observed between children with HM and any other groups.

After adjusting for S. mansoni infection and Pf–IgG3 quartiles, which had previously been shown to be associated with HSM in the wider cohort, there was a 43% increase in the prevalence of HM/HSM versus non-HM for each natural log unit increase in AF-alb (odds ratio = 1.43; 95% CI: 1.04, 1.97) (Table 3).

Discussion

Childhood HSM in sub-Saharan Africa is likely to be of a complex multifactorial etiology. In the present study, samples collected during a previous study among school-age children in 2002 and at follow-up in 2004 were used to examine associations between HSM and aflatoxin exposure using the AF-alb biomarker.

The AF-alb biomarker has a relatively long half-life (1–2 months) compared with other aflatoxin biomarkers. It is well accepted to be a reliable marker in aflatoxin exposure assessment and has been applied to studies of chronic diseases (Wild and Gong 2010), including carcinogenesis, and growth retardation and immune-suppression effects in young children (Gong et al. 2002, 2004; Wild and Gong 2010). The biomarker has been repeatedly applied in retrospective type studies (Wild, and Gong 2010) and there is no evidence that this biomarker is liable to degradation in stored plasma samples.

Compared with exposure in other populations in sub-Saharan Africa (Wild and Gong 2010), aflatoxin exposure was already high in 2002, especially in Matangini, with the exposure level nearly triple that of children from Yumbuni. Initial analysis in 2002 indicated that there was a significant association between increased exposure to aflatoxin and S. mansoni infection, with S. mansoni infection being more prevalent and AF-alb levels being elevated in Matangini compared with Yumbuni. The presence of permanent surface-water makes Matangini more humid than Yumbuni and hence more favorable to fungal growth and aflatoxin production compared with Yumbuni. The permanent streams and irrigation canals in Matangini also provide a habitat for Biomphalaria species, the aquatic snail that is the intermediate host of S. mansoni, whereas the lack of permanent surface-water abrogates transmission in Yumbuni.

The association between S. mansoni infection and AF-alb levels is therefore likely attributable to environmental conditions in Matangini being more favorable than in Yumbuni for both S. mansoni transmission and growth of Aspergillus species. This is substantiated by the reduced magnitude of association when comparing AF-alb levels between children with or without S. mansoni infection when adjusted for location. However, the biologically plausible hypothesis that aflatoxin exposure may induce chronic liver damage, which increases the susceptibility to schistosomiasis-associated morbidity—or vice versa, that the liver damage due to schistosomiasis infection may alter the metabolism of aflatoxin and also the synthesis of albumin, which could affect the AF-alb level—merits further analysis in studies designed to address this question.

In contrast to a previous report (Allen et al. 1992), the AF-alb level did not differ significantly between children with or without malaria parasitemia, nor was it associated with the chronic malaria marker Pf–IgG3 in the present study. Future study on the direct relationship between aflatoxin exposure and malaria is required.

The AF-alb levels were exceptionally high in 2004—in fact the highest ever reported— and the difference between villages was not observed. This exceptional level is consistent with the widely reported aflatoxicosis outbreak in 2004 in Kenya when AF-alb levels were the highest ever published (McCoy et al. 2008). The reason for the outbreak was consumption of home-grown maize that was highly contaminated with aflatoxin because of both the drought stress and being stored at warmer indoor conditions after the poor harvest, to prevent theft (Azziz-Baumgartner et al. 2005). It was also reported that the contaminated home-grown maize was sold in the local market and was bought by farmers after their home-grown supplies were exhausted, resulting in widespread distribution of the contaminated maize (Lewis et al. 2005). Village location played a less significant role in the AF-alb levels during the outbreak.

Aflatoxin is a liver toxin in several animal species (International Agency for Research on Cancer 2002). It causes liver enlargement in broiler chickens (Kumar and Balachandran 2009; Mani et al. 2000) and in animals suffering aflatoxicosis (Gonzalez-Petrya et al. 2008; Osman et al. 2004). To our knowledge there are no previous reports of chronic HM occurrence in chronically exposed humans. In 2002, the AF-alb levels were significantly higher in children with HM than in those without firm organomegaly, indicating that aflatoxin exposure may play an important role in HM occurrence. The nonsignificant difference in the AF-alb level between HSM and other groups suggests that the etiology of HSM may be even more complex than HM, so contribution by aflatoxin exposure could be diluted. For example, the effect of malaria exposure is greater on the spleen than on the liver, and vice versa for schistosomiasis, and there is evidence that both infections contribute to enlargement of both organs (Wilson et al. 2007b). After controlling for S. mansoni and Plasmodium infection, there was an association between aflatoxin and HM, with the prevalence of having HM (including HSM) being increased by 43% in association with a natural-log-unit increase in AF-alb. The mechanism needs further elucidation, although compromised liver function and the subsequent changes in liver metabolism due to chronic exposure to aflatoxin may contribute to HM development.

S. mansoni infection status and Pf–IgG3 were not significantly associated with HM/HSM in the logistic model that also included AF-alb. In the wider cohort, S. mansoni was associated with an increase in the size of the liver, suggesting this infection could exacerbate the condition, but there was no statistically significant association with prevalence of HM (Wilson et al. 2007b). Also in the wider cohort, increased exposure to Plasmodium infection did show a weak association with HM only, but the levels of the Pf–IgG3 were not elevated in HM as much as in HSM (Wilson et al. 2007b).

In a previous study in Makueni District, annual treatment of S. mansoni infections and regular mollusciding of the River Kambu (the habitat for the

Table 3. Logistic regression model for HM/HSM in 2002.

| Variable          | Odds ratio (95% CI) | p-Value |
|-------------------|---------------------|---------|
| AF-alb            | 1.43 (1.04, 1.97)   | 0.030   |
| S. mansoni infection* | 1.58 (0.73, 3.40) | 0.241   |
| Pf–IgG3           | 1.20 (0.90, 1.60)   | 0.215   |

*As determined by the presence of detectable eggs.
intermediate host) over a 3-year period did not achieve complete resolution of childhood HM (Vennervald et al. 2005). In that study the children who lived close to the river and had the highest levels of P6b-IgG3 had the poorest resolution of their HM (Booth et al. 2004a). All this evidence suggests that the HM/HSM observed in these children is of a chronic complex nature and may also be reversible in a slow process.

During the interim period between the 2002 and 2004 examinations, there was a substantial increase in HM cases, largely due to a shift from HSM to HM—in other words, a reduction in SM cases. These represent individual children whose condition changed from HSM to HM during the 2-year period; that is, the HM status persisted, showing that this was a chronic condition in these children, whereas SM was reversed. The reduction in SM cases may possibly be explained by reduced malaria transmission due to the drought that was associated with the acute aflatoxin outbreak in 2004, which would also have had a significant impact on the seasonal transmission of malaria, as evidenced by a drop in P6b-IgG3 levels (Wilson S, unpublished observations).

The aflatoxicosis outbreak in 2004 resulted in extremely high levels of AF-alb in most of the children, and there was no significant association between AF-alb and HM/HSM in 2004. It is possible that this lack of association in 2004 between HM and AF-alb may result from the high acute exposure during the aflatoxicosis outbreak masking any putative underlying relationship between aflatoxin exposure and chronic HM. It does not in itself negate the potential association between the two, but it is recognized that the association observed in 2002 may be a false positive and therefore requires investigation in other studies.

A large-scale association study is now desirable, specifically designed to address the role of aflatoxin in HM and taking account of the role of infections. Alternatively, an intervention study, if feasible, would provide the best approach to draw a conclusion about the etiological association between HM and aflatoxin exposure. Nevertheless, the present study highlights a potential new health effect of aflatoxin exposure. This further emphasizes the need for education to improve public awareness of aflatoxin-associated health risks and for the development of effective aflatoxin prevention strategies. It is worth noting that the need for intervention has already been identified in this region of Kenya and is being put into practice, which may have contributed to the reduced severity of the 2010 aflatoxicosis outbreak.

Conclusion

This study raises the possibility of an association between aflatoxin exposure and childhood HM, at least at exposure levels that do not induce acute aflatoxicosis. This finding provides the first evidence pointing to this health effect of these liver toxicants. This observation merits further study in other areas of high aflatoxin exposure, in studies designed to directly test this hypothesis.

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