Absence of TP53 Codon 249 Mutations in Young Guinean Children with High Aflatoxin Exposure

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Abstract

Infection with hepatitis viruses and chronic exposure to high levels of dietary aflatoxins are the major etiologic agents for hepatocellular carcinoma in west Africa. A challenge for the prevention of hepatocellular carcinoma in this region is that both hepatitis B virus and aflatoxin exposures start early in life; indeed, aflatoxin exposures can start in utero and continue unabated throughout childhood. A mutation in the TP53 tumor suppressor gene at codon 249 (TP53 Ser249 mutation) has been reported previously for hepatocellular carcinoma tumors and matched plasma DNA samples in individuals from areas with high aflatoxin exposure. We examined whether the TP53 Ser249 mutation could be observed in DNA found in plasma of young children (ages 2-5 years) from Guinea, west Africa, a region of high aflatoxin exposure. Plasma aflatoxin-albumin adducts were present in 119 of 124 (96%) of the children, geometric mean of positives 9.9 pg/mg albumin (95% confidence interval, 8.8-11.0 pg/mg). This is the level and prevalence of exposure observed previously in adults. Following PCR amplification of plasma-derived DNA and detection using mass spectrometry, none of the samples were found to contain the TP53 Ser249 mutation. Because ~50% of the hepatocellular carcinomas in adults in west Africa have this specific TP53 Ser249 mutation, a lack of detection in samples from children ages <5 years may indicate that a window of opportunity for intervention exists that could be exploited to lower hepatocellular carcinoma risk.

Introduction

Hepatocellular carcinoma is a major cause of morbidity and mortality in many regions, including sub-Saharan Africa, with upwards of 600,000 new cases each year worldwide (1, 2). The age-standardized rate of hepatocellular carcinoma for males in Guinea is 37.6 per 100,000 and represents the most common cancer in males, a situation mirrored in several sub-Saharan countries (3). Infection with hepatitis viruses and chronic exposure to high levels of dietary aflatoxins are the major etiologic agents for hepatocellular carcinoma in sub-Saharan Africa (3). A challenge for the prevention of hepatocellular carcinoma in these high-risk regions is that both hepatitis B virus (HBV) and aflatoxin exposures start early in life. Previous data from studies in west Africa have shown that aflatoxin exposures can start in utero (4) and continue unabated throughout childhood (5-8), with levels increasing significantly on the introduction of adult foods used for weaning (9, 10). Thus, the pattern of chronic exposure is established early in life and this may promote the high risk for hepatocellular carcinoma development. Further, because aflatoxin-albumin adducts have been detected in both cord blood and blood samples of children, this shows that children have the requisite metabolic capacity to convert aflatoxin B1 to its toxicologically potent metabolite, aflatoxin B1-8,9-epoxide. Because aflatoxin-albumin adducts are correlated with the promutagenic aflatoxin-DNA adduct, their presence early in life suggests that exposure during this period to aflatoxin could result in mutations that trigger the cascade of events leading to hepatocellular carcinoma (11). Aflatoxin-albumin is easily measured, provides an integration of aflatoxin exposure over 2 to 3 months, and acts as a valuable surrogate marker for aflatoxin-DNA adducts (11). Because hepatocellular carcinoma in west Africa occurs as early as adolescence and incidence peaks at age ~35 to 55 years (3), exposures early in life to both HBV and aflatoxins could be crucial for these at-risk individuals.

Mutations in the TP53 tumor suppressor gene are found in most human cancers and distinct mutational spectra have been observed for different cancer types (12). In the TP53 gene, a high prevalence of an AGG to AGT (arginine-to-serine) transversion at codon 249 (TP53 Ser249 mutation) is reported for hepatocellular carcinoma tumors from areas with high aflatoxin exposure (11). This genetic alteration in tumor-derived DNA has recently been detected in plasma or serum DNA from adult hepatocellular carcinoma patients (13). In sub-Saharan Africa, the presence of this mutation before hepatocellular carcinoma onset (e.g., in patients with cirrhosis and patients without clinically diagnosed liver disease) may indicate that the mutation is a marker of chronic exposure to aflatoxin (14). Therefore, in this study, we aimed to assess whether the TP53 Ser249 mutation could be observed in DNA found in plasma of young children (ages 2-5 years) in a region of high aflatoxin exposure.

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Materials and Methods

The study was conducted in the Kindia prefecture of lower Guinea. Five villages were included and the number of available children ages 2 to 5 years was determined (Bangouya, n = 261; Damouya, n = 322; Koliady, n = 390; Minyaya, n = 204; Ségouya, n = 254). From these villages, we recruited 150 children (15 male, 15 female from each village); all families in these villages are reliant on groundnuts as a dietary staple and thus have probable high aflatoxin exposure. Blood samples were taken between March and May 2002; one child failed to provide a sample. This period is around that of the highest aflatoxin exposure, because it is the time when the groundnuts have been in storage for the longest period after harvest but are still readily available. Plasma samples were separated and stored at −20°C. Plasma DNA was extracted using Qiagen kits (Valencia, CA) according to the manufacturer’s instructions. The presence of a mutation at codon 249 of the TP53 gene was determined by short oligonucleotide mass analysis as described previously (13, 15). A previously characterized wild-type (negative control) and a mutant (positive control) sequence of DNA were analyzed in parallel with each set of DNA samples. A sample was considered positive when both sense and antisense fragments were present. The levels of aflatoxin-albumin were determined by ELISA with a limit of detection of 3 pg aflatoxin-lysine equivalent/mg albumin (7). The presence of HBV DNA in plasma was assessed by PCR (16). The study was approved by the Comité National d’Ethique pour la Recherche en Santé in Guinea. The purpose of the study was explained to community leaders in the villages and then to potential study participants in their own language. The mothers of all children gave informed consent.

Results

The aflatoxin exposure status of the children was assessed using aflatoxin-albumin adducts. The adducts were measured in 124 children of whom 119 (96%) had detectable levels [geometric mean of positives 9.9 pg/mg albumin (95% confidence interval, 8.8-11.0 pg/mg)]. Insufficient sample was obtained for analysis from 25 children. There were no differences in adduct levels between males and females. These data are comparable with those we have reported previously in adults from Guinea (Fig. 1).

Because hepatocellular carcinoma risk is increased in those individuals persistently infected by HBV, we examined the prevalence of HBV DNA in the plasma samples by PCR (16). Plasma DNA was successfully extracted from 149 children, with 11 of 149 (7.4%) positive for HBV DNA. The aflatoxin and HBV data confirm the occurrence of the two major risk factors for hepatocellular carcinoma in this population.

The extracted plasma DNA was then examined for the occurrence of the aflatoxin-specific TP53 Ser249 mutation. Following PCR amplification and detection using short oligonucleotide mass analysis, neither the sense nor the antisense fragment for the TP53 Ser249 mutation was observed for any sample. Wild-type control DNA gave positive tandem mass spectrometry spectrum for forward and reverse fragments of the wild-type TP53 sequence. The positive control for the TP53 Ser249 contains mutant and wild-type sequences, and as expected, the short oligonucleotide mass analysis was positive for both forward and reverse and wild-type and mutant fragments (Fig. 2). Insufficient plasma was available from one sample to conduct this analysis.

Discussion

In west Africa, the onset of hepatocellular carcinoma is relatively early in adult life, occurring in some adolescents, and with a peak incidence between age 35 and 55 years (3). This suggests that exposure to risk factors in early childhood is likely to be important. It is known that chronic HBV infection is established in the first 5 years of life (17). In addition, aflatoxin exposure occurs during the perinatal period, including transplacentally (4), and continues at a high level on weaning (9). Our hypothesis was that TP53 Ser249 mutations could be established early in life and that, as a consequence, these could be detected in plasma of children. In utero exposure could lead to mutations during development and one possibility we have considered was that such a mutation could lead to a chimeric liver with a clonally expanded hepatocyte carrying the TP53 Ser249 mutation. In this study, we confirmed that children were exposed to aflatoxin using aflatoxin-albumin adducts as a biomarker. These data agree with aflatoxin-albumin levels observed previously in adults in Guinea (refs. 11, 18; Fig. 1) and children in other parts of west Africa where adducts were present in >95% of children (7, 9).

It was expected that the mutation would be present, if at all, at a low copy number; thus, the highly sensitive short oligonucleotide mass analysis technique (13) was employed (detection limit: 1 mutant strand in 250 wild-type strands). Despite the high level of aflatoxin exposure and the use of short oligonucleotide mass analysis to detect the mutation, none of the children had detectable plasma DNA containing the TP53 Ser249 mutation. It is possible that, despite aflatoxin exposure, the level and duration of that exposure in the age range examined was as yet insufficient to have established any TP53 Ser249 mutations. Alternatively, the copy numbers of the mutation within the liver may have been too low. If hepatocytes with a TP53 mutation acquire a survival advantage and avoid apoptosis, then mutant DNA is less likely to be released into the circulation until further clonal expansion associated with the cancer process. We measured both aflatoxin-albumin and the mutation at the period of highest aflatoxin exposure in this population. It might be argued that a later time point would be more likely to detect the mutations consequent to exposure and DNA adduct formation. However, aflatoxin exposure in this population is continuous and mutations are likely to accumulate from prior exposure so that we do not think the timing of our analysis is an explanation for the negative mutation data. It is plausible however that the processes of inflammation, cell death, and regenerative proliferation associated with HBV infection may be required to increase the amount of any existing mutant DNA released. In this study, only 11 children were HBV DNA positive (HBV DNA is a marker of active viral replication),
although none of these were positive for the TP53 Ser249 mutation. Our previous study in 3- to 4-year-old Gambian children observed that markers of liver damage were elevated with HBV infection, particularly acute infections (7). A larger study of children of different ages specifically with chronic active HBV infection, elevated markers of liver damage, and high levels of aflatoxin exposure may be more informative in this respect. These data would help clarify the natural history of TP53 Ser249 mutations in the development of hepatocellular carcinoma.

Alternatively, if our observation is representative of an absence of TP53 mutations in the liver, then this time period provides a window in which aflatoxin intervention strategies in childhood based on the successful approach shown previously in Guinea, involving improved drying and storage of groundnuts (19), may delay or reduce the incidence of hepatocellular carcinoma in later life.

References

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