Urinary 15-F₂t-isoprostane, aflatoxin B₁ exposure and hepatitis B virus infection and hepatocellular carcinoma in Taiwan

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Introduction

In Taiwan, primary hepatocellular carcinoma (HCC) is the leading cause of cancer death for males and the second for females. Epidemiological evidence suggests that dietary exposure to aflatoxin B₁ (AFB₁) and chronic infection with hepatitis B virus (HBV) are major risk factors for HCC (1). In a previous prospective study in Taiwan, we demonstrated that the presence of AFB₁–albumin adducts as well as urinary AFB₁ metabolites were associated with increased risk of HCC (2). A viral–chemical interaction was also observed (2). Similar results were observed in a study carried out in China (3).

Although a number of studies have demonstrated that increasing AFB₁ exposure results in increasing HCC risk, the underlying mechanisms leading to development of HCC are not fully understood. One possible mechanism of AFB₁-related hepatocarcinogenesis is the induction of oxidative DNA damage in liver tissue (4,5). Reactive oxygen species (ROS) have also been suggested to be involved in the progression of chronic liver disease and the occurrence of HCC (6). In our recent study, we found that the level of urinary 8-oxo-7,8-dihydro-2’-deoxyguanosine (8-oxodG) was associated with AFB₁ exposure in a dose-dependent manner suggesting that AFB₁-induced oxidative DNA damage may constitute an important pathway in AFB₁ hepatocarcinogenesis (7). Oxidative stress, mediated by ROS, may result in direct DNA damage as well as in lipid peroxidation (8). The measurement of F₂-isoprostanes (IsoPs), produced during peroxidation of membrane lipids by free radicals and ROS, is a specific and sensitive marker of lipid peroxidation (9). IsoPs are compounds derived from arachidonic acid via a free radical-catalyzed mechanism. IsoPs are cleaved from the sites of their origin and then circulate in plasma and are excreted in urine (10). 15-F₂t-isoprostane (15-F₂t-IsoP), one class of isoprostanes, has been recognized as a specific, chemically stable, quantitative marker of oxidative stress and can be detected in peripheral blood and in urine (9,10).

Measurement of 15-F₂t-IsoP in urine or plasma has been shown to reflect the oxidative stress of the body in patients with a variety of disease conditions (11,12). It has also been suggested that the measurement of 15-F₂t-IsoP can be used as a biomarker of exposure to relevant carcinogens and may predict cancer risk (12–14). Increasing urinary 15-F₂t-IsoP has been demonstrated to be associated with breast cancer (14). There are no human data on the effect of AFB₁ exposure and the combined effect of chronic HBV infection and AFB₁ exposure on the level of urinary 15-F₂t-IsoP. The significance of AFB₁-induced oxidative lipid damage in the carcinogenicity of AFB₁ has not been well investigated nor has any long-term follow-up study investigated the effect of oxidative lipid damage on the development of HCC. The specific aims of this study were to investigate, among subjects without HCC, whether oxidative lipid damage, as assessed by urinary excretion of 15-F₂t-IsoP, is associated with increased risk of HCC (15). It has also been suggested that the measurement of 15-F₂t-IsoP may be related to increasing level of aflatoxin exposure and are associated with an increased risk of HCC.

Materials and methods

Study cohort

Subjects are from the community-based Cancer Screen Program cohort recruited in Taiwan. This study was approved by Columbia University’s Institutional Review Board as well as the research ethics committee of the College of Public Health, National Taiwan University, Taipei, Taiwan. Written informed consent was obtained from all subjects, and strict quality controls and safeguards were used to protect confidentiality. The cohort characteristics and methods of screening and follow-up have been described in detail previously (2,7). Briefly, individuals who were between 30 and 64 years old and lived in seven townships in Taiwan, three located on Penghu Islets that has the highest HCC incidence rates in Taiwan, and the other four from Taiwan Island, were recruited between July 1990 and June 1992. A total of 12 020 males and 11 923 females were enrolled. Participants were personally interviewed based on a structured questionnaire regarding epidemiological information and disease conditions (11,12). There were 54 850 person-years of follow-up.

Abbreviations: AFB₁, aflatoxin B₁; AF, x-fetoprotein; BMI, body mass index; CI, confidence interval; 15-F₂t-IsoP, 15-F₂t-isoprostane; HBsAg, hepatitis B virus surface antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; IsoP, F₂-isoprostane; OR, odds ratio; 8-oxodG, 8-oxo-7,8-dihydro-2’-deoxyguanosine; ROS, reactive oxygen species.
6 months. Information about duration and intensity was also obtained. Habitual alcohol intake was defined as drinking alcohol-containing products >4 days a week for at least 6 months.

At enrollment, blood samples were tested in Taiwan for serological markers, including alanine transaminase, aspartate transaminase, g-fetoprotein (AFP), hepatitis B virus (HBV), hepatitis C virus (HCV), and anti-HCV. HBsAg was tested by radioimmunassay (Abbott Laboratories, North Chicago, IL). Anti-HCV and AFP were tested by enzyme immunoassay using commercial kits (Abbott Laboratories). Both alanine transaminase and aspartate transaminase levels were determined with a serum chemistry autoanalyzer (Hitachi Model 736; Hitachi Co., Tokyo, Japan) using commercial reagents (Biorheum, Marcy, France). Anti-HCV and AFP were assayed in all males and females who resided in Hu-Hsi and Pai-Hsa on the Penghu Islets. The other assays were carried out on samples from all participants. Any participant who had an elevated level of alanine transaminase (≥45 IU/l), aspartate transaminase (≥40 IU/l) or AFP (≥20 ng/ml) was positive for HBsAg or anti-HCV or had a family history of HCC or liver cirrhosis among first-degree relatives was referred to upper abdominal ultrasonography examination. Suspected HCC cases were referred to teaching medical centers for confirmatory diagnosis by computerized tomography, digital subtracted angiogram, aspiration cytology and pathological examination. The criteria for HCC diagnosis included the following: a histopathological examination, a positive lesion detected by at least two different imaging techniques (abdominal ultrasonography, angiogram or computed tomography) or by one imaging technique and a serum AFP level >400 ng/ml.

Results

Urinary 15-F2t-IsoP levels were analyzed using competitive enzyme-linked immunosorbent assay kits from BioAssay System (Hayward, CA), as directed by the manufacturer.

Statistical methods

To characterize the levels of urinary excretion of 15-F2t-IsoP and the potential factors modulating these levels in a Taiwanese population, multivariate-adjusted linear regression models were used to compute regression coefficients among cases and control, separately. Levels of urinary 15-F2t-IsoP, 8-oxodG, creatinine and urinary AFB1 metabolites were natural log transformed (ln) to normalize the distribution. Pearson partial correlation coefficient was used to determine the correlation of urinary 15-F2t-IsoP with either AFB1 metabolites or 8-oxo-dG adjusted by age, gender and urinary creatinine. To evaluate the dose–response relationship between the levels of urinary 15-F2t-IsoP and AFB1 metabolites, subjects were divided into quartiles based on the distribution of urinary AFB1 metabolites for all control subjects (<2.26, ≥2.26 to <3.64, ≥3.64 to <6.14 and ≥6.14 fmol/mmol creatinine). Then, a multivariate logistic regression model adjusted for potential confounding factors was constructed to determine whether there was a trend. Wald’s test with consecutive score 1, 2, 3 and 4 assigned to the first, second, third and fourth quartiles of urinary AFB1 metabolites was used to test for trend of adjusted odds ratios (ORs) across strata. The χ2 test was used to examine differences in the distributions of variables between cases and controls. To examine the independent and combined effects of the level of urinary 15-F2t-IsoP on HCC risk, cases and controls were compared using conditional logistic regression models. Urinary 15-F2t-IsoP was used to divide subjects into two groups: those with levels above the mean value (0.53 nmol/mmol creatinine) for all control samples versus those below the mean. To evaluate the dose–response relationship between urinary 15-F2t-IsoP and HCC risk, subjects were divided into tertiles based on control values (<0.33, ≥0.33 to <0.55 and ≥0.55 nmol/mmol creatinine). HBsAg, smoking, alcohol consumption, body mass index (BMI) and urinary 8-oxodG and AFB1 metabolite-adjusted ORs and 95% confidence intervals (CIs) were derived from conditional logistic regression models stratified on the matching factors to estimate the association between levels of urinary 15-F2t-IsoP and HCC risk. Wald’s test with consecutive scores 1, 2 and 3 assigned to the first, second and third tertiles of urinary 15-F2t-IsoP was used to test for trend of adjusted ORs across strata. To evaluate the combined effect of urinary biomarker levels and HCC risk, subjects were divided into different groups based on the levels of urinary biomarkers: those with urinary 8-oxodG levels above the mean value (24.70 nmol/mmol creatinine) for all control samples versus those below the mean and those with AFB1 metabolite levels above the mean value (5.12 fmol/mmol creatinine) for all control samples versus those below the mean. The interaction terms for urinary 8-oxodG and 15-F2t-IsoP, AFB1 metabolites and 15-F2t-IsoP were assessed as 1.37 nmol/mmol creatinine increase in urinary 15-F2t-IsoP concentration, whereas each 1 nmol/mmol creatinine of urinary 15-F2t-IsoP was associated with a 2.26 to <3.64, ≥3.64 to <6.14 and ≥6.14 fmol/mmol creatinine. Then, a multivariate logistic regression model adjusted for potential confounding factors was constructed to determine whether there was a trend. 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The levels of ln urinary 15-F2t-IsoP were correlated with ln urinary AFB1 metabolites as well as 8-oxodG, with Pearson partial correlation coefficients of 0.24 (P < 0.0001, Figure 1) and 0.12 (P < 0.0001, Figure 2), respectively. Subjects were divided into quartiles based on the distribution of urinary AFB1 metabolites in controls to evaluate the dose–response relationship with urinary 15-F2t-IsoP (Table I). When compared with control subjects in the lowest quartile of urinary AFB1 metabolites, there was an increase in detection of high level of urinary 15-F2t-IsoP.
with adjusted ORs of 0.70 (95% CI = 0.29–1.70), 3.69 (95% CI = 1.68–8.10) and 7.50 (95% CI = 3.14–16.46) for subjects in the second, third and fourth quartile, respectively, ($P_{\text{trend}} < 0.0001$).

The mean level of urinary 15-F$_2$-IsoP was statistically significantly higher in HCC cases than in controls (0.67 ± 0.34 and 0.53 ± 0.59 nmol/mmol creatinine, respectively; $P = 0.04$). The association of urinary 15-F$_2$-IsoP with HCC risk is given in Table II. After adjustment for HBsAg status, smoking, alcohol drinking, BMI and urinary AFB$_1$ metabolites and 8-oxodG, the OR for those with urinary 15-F$_2$-IsoP levels above the mean compared with those with levels below the mean was 2.53 (95% CI = 1.30–4.93). When urinary 15-F$_2$-IsoP levels were stratified into tertiles based on control values, HCC risk increased with adjusted ORs of 3.87 (95% CI = 1.32–11.38) and 6.27 (95% CI = 2.17–18.13; $P_{\text{trend}} = 0.0008$) for subjects with 15-F$_2$-IsoP in the second and third tertile, respectively, compared with those in the lowest tertile.

The combined effect of urinary 15-F$_2$-IsoP and HBsAg is given in Table III. HBsAg carriers with urinary 15-F$_2$-IsoP above the mean had a significantly increased HCC risk (OR = 19.01, 95% CI = 6.67–54.17) compared with non-carriers with urinary 15-F$_2$-IsoP below the mean ($P$ for linear trend < 0.0001). The combined effect of urinary 15-F$_2$-IsoP, 8-oxodG and AFB$_1$ is given in Table IV. In our previous study, we found that increasing level of urinary 8-oxodG was associated with a non-significant decrease in HCC risk. Thus, in the present analysis, subjects with levels of 15-F$_2$-IsoP below the mean and 8-oxodG above the mean were considered as the low-risk group. Among subjects with urinary 15-F$_2$-IsoP above the mean, the ORs (95% CIs) were 2.99 (1.00–8.98) and 2.69 (0.97–7.67; $P_{\text{trend}} = 0.008$) for those with urinary 8-oxodG above and below the mean, respectively. Among subjects with urinary 15-F$_2$-IsoP above the mean, the OR was 1.58 (95% CI = 0.58–4.31) for those with urinary AFB$_1$ above the mean compared with those with AFB$_1$ below the mean. Among subjects with urinary 15-F$_2$-IsoP above the mean, the ORs (95% CIs) were 2.76 (1.24–6.13) and 2.86 (1.26–6.49; $P_{\text{trend}} = 0.004$) for those with urinary AFB$_1$ below and above the mean, respectively.

**Discussion**

We investigated the relative contributions of environmental determinants to urinary levels of 15-F$_2$-IsoP in a well-characterized Chinese adult population living in an area with high AFB$_1$ exposure. We found that the major factors determining urinary excretion of 15-F$_2$-IsoP in this population were levels of urinary AFB$_1$ metabolites and 8-oxodG. In addition, we observed a statistically significant increased trend in HCC risk with increasing tertiles of urinary 15-F$_2$-IsoP levels (OR = 3.87, 95% CI = 1.32–11.38 and OR = 6.27, 95% CI = 2.17–18.13, for subjects in the second and third tertile, respectively, compared with those in the lowest tertile; $P_{\text{trend}} = 0.0008$). Our results provide...
data in humans supporting the hypothesis that exposure to AFB1 contributes to increased oxidative stress and that AFB1 may play a role in HCC by enhancing ROS formation and causing oxidative DNA damage as well as lipid peroxidation.

In the present study, we failed to find an effect of smoking or alcohol intake on the urinary excretion of 15-F2t-IsoP. Alcohol intake is a well-established risk factor for HCC. Alcohol consumption may result in increased oxidative stress due to ROS generation through induction of CYP450 2E1 (8). But no effect of alcohol in the level of urinary 15-F2t-IsoP has been observed in some previous studies (12,14). Substantially higher doses of alcohol consumption were required to produce a statistically significant elevation in urinary 15-F2t-IsoP (16). While smoking has been associated with increased levels of 15-F2t-IsoP in some prior studies (12,14), our negative results may partly be due to misclassification. Information on smoking and alcohol intake in our study was based on self-report. Data on the correlation of urinary 15-F2t-IsoP with either age of subjects or BMI are conflicting. Whereas a positive association with age was observed in one small study with only 19 health controls (17), no effect of age was observed in our and other two studies with larger sample sizes (14,15). Our observation of the lack of an age effect is consistent with a hypothesis that unlike oxidative damage to DNA which is related to current with aging (15). Although obesity is associated with oxidative lipid damage is related to physiologic conditions concurrent with aging (15).

Table II. Urinary 15-F2t-IsoP and risk of HCC

| Urinary 15-F2t-IsoP (nmol/mmol creatinine) | Cases | Controls | OR (95% CI) | OR (95% CI) a |
|-------------------------------------------|-------|----------|-------------|--------------|
| Below mean (<0.53)                         | 30    | 186      | 1.0         | 1.0          |
| Above mean (≥0.53)                         | 44    | 104      | 2.51 (1.44, 4.39)** | 2.53 (1.30, 4.93)** |
| Tertile b <0.33                            | 10    | 98       | 1.0         | 1.0          |
| ≥0.33 to <0.55                             | 22    | 97       | 3.22 (1.32, 7.83)** | 3.87 (1.32, 11.38)** |
| ≥0.55                                     | 42    | 95       | 5.41 (2.32, 12.64)** | 6.27 (2.17, 18.13)** |

aAdjusted for HBsAg status, smoking, alcohol, BMI, urinary AFB1 metabolites and urinary 8-oxodG.

**P value < 0.05.

***P value < 0.005.

Table III. The combined effect of HBV infection and urinary 15-F2t-IsoP levels and risk of HCC

| HBsAg  | Urinary 15-F2t-IsoP (nmol/mmol creatinine) | Case | Control | OR (95% CI) | OR (95% CI) a |
|--------|-------------------------------------------|------|---------|-------------|--------------|
| Negative Below mean (<0.53)                | 10   | 159     | 1.0       | 1.0          |
| Negative Above mean (≥0.53)                | 22   | 85      | 3.96 (1.73, 9.07)** | 3.88 (1.61, 9.39)** |
| Positive Below mean (<0.53)                | 20   | 27      | 11.67 (4.59, 29.63)** | 13.91 (5.20, 37.25)** |
| Positive Above mean (≥0.53)                | 22   | 19      | 17.28 (6.45, 46.32)** | 19.01 (6.67, 54.17)** |

aAdjusted for smoking, alcohol, BMI, urinary AFB1 metabolites and urinary 8-oxodG.

**P value < 0.0001.

Table IV. The combined effect of urinary biomarker levels and risk of HCC

| Urinary 15-F2t-IsoP (nmol/mmol creatinine) | Urinary 8-oxodG (nmol/mmol creatinine) | Case | Control | OR (95% CI) | OR (95% CI) a |
|-------------------------------------------|---------------------------------------|------|---------|-------------|--------------|
| Below mean (<0.53)                        | Above mean (≥24.70)                   | 8    | 51      | 1.0         | 1.0          |
| Below mean (<0.53)                        | Below mean (<24.70)                  | 22   | 135     | 0.85 (0.32, 2.24)  | 1.17 (0.41, 3.35) |
| Above mean (≥0.53)                        | Above mean (≥24.70)                   | 15   | 35      | 2.29 (0.88, 5.98)  | 2.99 (1.00, 8.98) |
| Above mean (≥0.53)                        | Below mean (<24.70)                  | 29   | 69      | 2.21 (0.87, 5.62)  | 2.69 (0.97, 7.67) |

P trend = 0.004

| Urinary 15-F2t-IsoP (nmol/mmol creatinine) | Urinary AFB1 (fmol/mmol creatinine) | Case | Control | OR (95% CI) | OR (95% CI) a |
|-------------------------------------------|------------------------------------|------|---------|-------------|--------------|
| Below mean (<0.53)                        | Below mean (<5.12)                | 22   | 150     | 1.0         | 1.0          |
| Below mean (<0.53)                        | Above mean (<5.12)                | 8    | 36      | 1.69 (0.68, 4.19)  | 1.58 (0.58, 4.31) |
| Above mean (≥0.53)                        | Below mean (<5.12)                | 21   | 48      | 3.05 (1.51, 6.16)** | 2.76 (1.24, 6.13)** |
| Above mean (≥0.53)                        | Above mean (<5.12)                | 23   | 56      | 2.63 (1.26, 5.17)** | 2.86 (1.26, 6.49)** |

P trend = 0.001

aAdjusted for HBsAg status, smoking, alcohol and BMI.

**P value < 0.05.

***P value < 0.005.
8-oxodG as well as AFB1 exposure. Moreover, the ORs for detection of urinary 15-F2t-IsoP above the mean showed a dose-dependent increase with level of urinary AFB1 (Table I). These results provide evidence that AFB1 exposure increase oxidative stress in humans. ROS generated in inflamed tissues can cause injury to target cells and also damage DNA and may be involved in the progression of viral hepatitis as well as hepatocarcinogenesis (6).

Chronic HBV infection is a major risk factor associated with the development of HCC. Although the underlying mechanisms that lead to malignant transformation of infected cells remain unclear, several direct and indirect mechanisms have been described for HBV-induced hepatocarcinogenesis [reviewed in ref. 20]. HBV has a direct oncogenic effect through integration of HBV DNA into the cellular DNA resulting in genetic alterations. In a transgenic mouse model, the accumulation of toxic levels of HBsAg is followed by liver injury, inflammation and HCC formation (21). The continuous association between ROS due to chronic inflammation may be part of indirect mechanisms of HBV-induced HCC. We did not observe an association between chronic viral infection and urinary 15-F2t-IsoP level; however, the combination of urinary 15-F2t-IsoP above the mean and chronic HBV infection resulted in an OR of 19.01 (95% CI = 6.67–54.17) compared with those with low urinary 15-F2t-IsoP and without HBV infection, supporting the multiple mechanisms of HBV-related HCC.

It is well known that the formation of trans-8,9-dihydro-8-(7-guanyl)-9-hydroxy–AFB1 adducts is a critical step in AFB1 genotoxicity (22). Recently, we reanalyzed the effect of AFB1 exposure on HCC risk with additional cases and controls and a longer period of follow-up compared with that in our previous study (2). The effect of AFB1 on HCC risk remains statistically significant with an OR of 1.76 (95% CI = 1.22–2.55) for those with urinary AFB1 level above the mean compared those with AFB1 level below the mean (data not shown). Our demonstration that AFB1 is associated with oxidative DNA damage (7) and lipid peroxidation (current study) suggests that increased oxidative damage may be partly responsible for AFB1-induced hepatocarcinogenesis. This is also supported by the analysis of the combined effects of urinary AFB1 and 15-F2t-IsoP (Table IV). We observed that the 15-F2t-IsoP effect was more prominent among subjects with high 8-oxodG and low AFB1 levels. These observations are consistent with the notion that the three factors, namely, AFB1, 8-oxodG and F2-Isop, are reflective of a common mechanistic pathway showing a threshold dose in relation to risk. However, our findings should be interpreted with caution since the small sample size in the present study might result in an unstable estimation of the combined effect.

Although 8-oxodG is a biomarker indicating oxidative DNA damage, a recent study (23) suggests that urinary 8-oxodG level is an index measuring both oxidative DNA damage and oxidative DNA repair capacity. In a previous study, we showed a slightly decreased risk of HCC in those with a higher level of urinary 8-oxodG (7). In the present study, we found ORs (95% CIs) of 2.99 (1.00–8.98) and 2.69 (0.97–7.67) for those with 8-oxodG above and below the mean among those with urinary 15-F2t-IsoP above the mean. In order to fully understand the relationship between urinary 8-oxodG and 15-F2t-IsoP on human diseases, DNA repair capacity should be taken into consideration.

To the best of our knowledge, no published studies have measured urinary 15-F2t-IsoP to investigate the effect of AFB1 exposure on lipid peroxidation in humans. The use of urinary 15-F2t-IsoP as a biomarker of oxidative damage resulting from AFB1 exposure has several advantages: (i) urinary 15-F2t-IsoP is a specific product of lipid peroxidation; (ii) the antibody-based method of quantitation does not require extraction or purification of the biological samples prior to analysis making it suitable for application in large clinical or experimental studies; (iii) there is no evidence of artifactual formation of isoprostanes during handling and storage of urine, unlike plasma (10); (iv) urinary 15-F2t-IsoP excretion is not confounded by the lipid content of the diet (24) and (v) since 15-F2t-IsoP is metabolized rapidly in the body and a large quantity of this compound is excreted from the circulation within a few minutes, it is adequate to measure the compound in urine (25).

Although the use of urinary 15-F2t-IsoP as an index of lipid peroxidation remains attractive, our results must be interpreted with caution. First, the putative causal role of AFB1 exposure in increasing urinary 15-F2t-IsoP could not be verified. The association between urinary 15-F2t-IsoP and AFB1 metabolites was examined using a cross-sectional study design that only measured levels of both analytes at baseline, making temporal separation of cause and effect difficult. A longitudinal rather than a cross-sectional study should be conducted to ascertain the possible association between AFB1 exposure and lipid peroxidation. Nevertheless, the strong association indicates the presence of oxidative lipid damage in persons with high AFB1 metabolites. Further investigations, incorporating prospective and dietary intervention studies, are required to confirm AFB1-related HCC via oxidative lipid damage. Second, levels of urinary 15-F2t-IsoP represent a steady-state concentration that is dependent on production (degree of lipid peroxidation) versus metabolism and excretion (26). Third, measuring the levels of urinary 15-F2t-IsoP by single spot samples might not be representative of individual levels of oxidative lipid damage. There was, however, no diurnal variation in the urinary 15-F2t-IsoP (27), suggesting that the collection of a single urinary sample would be adequate. Currently, there is no information available pertaining to the stability of isoprostanes in urine and the urinary samples examined in our study were collected nearly 15 years ago. Nevertheless, since we treated urine samples of cases and controls identically in terms of process, storage and assay, any effect on the biomarker levels might be disease blind. Fourth, there is concern that because of the small sample size in our case–control study, the statistically significant finding may be due to the chance. However, given the strong linear correlation with urinary AFB1 metabolites, as well 8-oxodG, and statistically significant high mean level of 15-F2t-IsoP among cases, the possibility of a false-positive finding may be small. Also, due to the small sample size, the results of the combined effect of urinary 15-F2t-IsoP with either HBV infection (Table III) or other urinary biomarkers (Table IV) must be interpreted with additional caution. In addition, the controls with available urine may not be representative of the general reference population due to the higher frequencies of younger as well as HbsAg-negative subjects. However, the cases and controls were comparable with regard to sociodemographic characteristics such as age or gender (7) that may affect the HCC risk or levels of urinary 15-F2t-IsoP. In this case, selection bias is unlikely to occur. Finally, levels of 15-F2t-IsoP are not a quantitative marker of damage to lipid by all reactive species (13). Future epidemiological studies should measure multiple oxidative stress markers in order to understanding the role of oxidative stress in HCC.

In summary, we found, among controls, a statistically positive association of urinary 15-F2t-IsoP, a biomarker of oxidative stress with urinary AFB1 metabolites, a biomarker of AFB1 exposure and with urinary 8-oxodG, a biomarker of oxidative DNA damage. These results strongly suggest that AFB1 exposure may result in an increased risk of oxidative damage. In terms of HCC risk, a significant positive relationship between urinary 15-F2t-IsoP was observed. Our results provide information on the application of biomarkers in human populations at high risk for cancer and that AFB1-induced lipid peroxidation may, in addition to the formation of AFB1-DNA adducts, have an important role in AFB1 carcinogenicity.

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