Elevated Aflatoxin Exposure and Increased Risk of Hepatocellular Carcinoma

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To elucidate the importance of aflatoxin in the etiology of hepatocellular carcinoma (HCC), a community-based cohort study combined with molecular dosimetry of aflatoxin exposure was performed in the Penghu Islets where the HCC mortality rate is highest in Taiwan. A total of 6,487 residents aged 30 to 65 years were recruited in the two-stage screening survey and underwent regular follow-up examination. Among 33 newly-diagnosed HCC cases, 31 (94%) were chronic hepatitis B surface antigen (HBsAg) carriers and 3 (9%) were positive for antibodies against hepatitis C virus (HCV). Among 20 HCC patients and 86 matched healthy controls whose serum samples were tested for aflatoxin B_1 (AFB_1)-albumin adducts by competitive enzyme-linked immunosorbant assay (ELISA), 13 (65%) HCC patients and 32 (37%) matched controls were seropositive, showing a statistically significant multivariate-adjusted odds ratio of 5.5 with a 95% confidence interval of 1.2 to 24.5. The results imply the elevated risk of HCC among Penghu residents may be attributable to their heavy exposure to aflatoxins and high HBsAg carrier rate. (HEPATOLOGY 1996;24:38-42.)

Both viral and chemical carcinogens are involved in the multistage process of human hepatocarcinogenesis. Hepatocellular carcinoma (HCC), one of the major malignant neoplasms in the world,[1-3] is the leading cause of cancer-related death among men and the third among women in Taiwan, where hepatitis B virus (HBV) infection is hyperendemic.[4-6] The HBV surface antigen (HBsAg) carrier status has been well documented as one of the most important risk factors for HCC in Taiwan as in other countries.[7,8] In addition to chronic HBV infection, several other factors, including hepatitis C virus (HCV) infection, habitual alcohol intake, cigarette smoking, family history of HCC, low vegetable consumption, elevated serum testosterone level, and low serum retinol level, have been reported as HCC risk factors in Taiwan.[9-14]

Aflatoxin is a well-documented hepatocarcinogen in animals. A significant association between aflatoxin exposures and human HCC has been reported in several ecological[15-21] and molecular epidemiological studies.[22-24] However, the importance of aflatoxin in the development of HCC in Taiwan remains to be elucidated. A quantitative indirect immunofluorescence method has been developed[25] and used to detect aflatoxin B_1 (AFB_1)-DNA adducts in surgically removed or needle-biopsy liver tissues of HCC patients in Taiwan. A detectable level of AFB_1-DNA adducts was observed in frozen sections of tumor tissues from 8 of 27 (30%) HCC patients in northern Taiwan[26] and in smeared tumor tissues from 35 of 50 (70%) HCC patients in southern Taiwan.[27] Furthermore, there was no difference in positivity of AFB_1-DNA adducts between tumor and nontumor tissues of HCC patients.[26] AFB_1-DNA adducts in tumor tissues of HCC patients may be used as a biomarker for the exposure to aflatoxin.

The age-adjusted mortality rate of patients with liver cancer in the Penghu Islets has been reported to be the highest in Taiwan.[4-6] Recent surveys have shown that more than one third of peanuts in Penghu were heavily contaminated by aflatoxins, with an average aflatoxin content of 167 μg/kg for contaminated peanuts.[28] This study was performed to assess the aflatoxin exposure for residents in Penghu by molecular dosimetry of the serum level of AFB_1-albumin adducts of HCC patients and matched healthy controls.

PATIENTS AND METHODS

Study Area. The Penghu Islets are located to the west of main Taiwan Island. There are six administrative areas including Makung, Huhsi, Paisha, Hsiyu, Chimei, and Wanan. Makung, Huhsi, and Paisha were chosen as the study area. There are about 100,000 residents who live in an area of 127 km^2. Most men in the Penghu Islets are fishermen, salesmen, and farmers, and most women are housewives. Peanuts are major agricultural products in the Penghu Islets.

Subject Recruitment. Seemingly healthy residents recruited in a community-based two-stage screening program were chosen as the population of this study. They were selected from an eligible roster of residents obtained from household registration offices in which sociodemographic characteristics including birth, death, marriage, divorce, migration, education, and employment of each resident are registered and annually double-checked by mandate. Both men and women aged between 30 and 65 years were recruited for the screening program. All study subjects participated in this free screening program on a voluntary basis with an informed consent.

Questionnaire Interview and Biospecimen Collection. Participants were personally interviewed based on a structured questionnaire at recruitment. Information obtained included sociodemographic characteristics, cigarette smoking, alcohol drinking, betel nut chewing, consumption frequency of various food items, and personal and family history of major diseases. Questionnaire interviews were performed by public health nurses who were well trained to standardize their interview techniques. Blood samples were collected from each study subject. Aliquots of serum, buffy coat, plasma, and red blood cells separated from blood samples were all stored at −70°C, and urine samples were stored at −30°C. They were transported in dry ice to the central laboratory in the National Taiwan University College of Public Health where all biospecimens were kept in deep freezers until examination.

Abbreviations: HCC, hepatocellular carcinoma; HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; HCV, hepatitis C virus; AFB_1, aflatoxin B_1; ALT, alanine transaminase; AST, aspartate transaminase; AFP, α-fetoprotein; anti-HCV, antibodies to HCV; ELISA, enzyme-linked immunosorbent assay.

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Two-Stage HCC Screening. In the two-stage screening program, study subjects were first screened by serological markers, including alanine transaminase (ALT), aspartate transaminase (AST), α-fetoprotein (AFP), antibodies to HCV (anti-HCV), and HBsAg. Both ALT and AST levels were determined by serum chemistry autoanalyzer (Hitachi Model 736; Hitachi Co., Tokyo, Japan) using commercial reagents (Biomerieux, Mercy l'Etoile, France), whereas AFP, anti-HCV, and HBsAg were tested by enzyme immunoassay using commercial kits (Abbott Laboratories, North Chicago, IL). Any subject who had an elevated level of ALT ($\geq$45 IU/mL), AST ($\geq$40 IU/mL), or AFP ($\geq$20 ng/mL), a positive status of anti-HCV or HBsAg or a family history of HCC or liver cirrhosis among parents and siblings was referred for the second-stage screening by upper abdominal ultrasonography. The abdominal ultrasonography was performed by board-certified gastroenterologists who were well experienced in ultrasonographic examinations using a Toshiba SAL-38B ultrasonographic apparatus with 3.75 MHz real-time linear and sector probes (Toshiba, Japan). According to a standard procedure, both probes were used in the upper abdominal ultrasonography, which was also video recorded. The video records were used as the reference for the follow-up of focal lesions, such as HCC, hemangioma, fat-free area or pseudotumor formation in fatty liver, and liver cyst. They were also used for the peer reviews of parenchymal changes including fatty liver, cirrhosis, and chronic liver diseases.

Confirmatory Diagnosis. Any subjects who had an ultrasonographic image compatible with HCC were referred to one of several general teaching medical centers in Taiwan for further confirmations depending on the choice of the suspected cases. HCC was confirmed by computed tomography, digital substracted angiogram, and aspiration cytology. Cytological examinations were independently performed by the teaching medical centers and Taipei Institute of Pathology. There were two criteria to diagnose HCC: (1) positive findings on cytological or pathological examinations or (2) typical images compatible with HCC with an AFP level $\geq$400 ng/mL. Confirmed HCC cases were further treated by surgical resection or transcatheter arterial chemo-embolization. Pathological examinations were also performed for surgically treated HCC patients.

Follow-up Study. Patients who were found to be affected with liver cirrhosis by ultrasonography were intensively followed up every 3 months, whereas others were regularly examined annually. The intensive follow-up of cirrhotic patients was performed by the examinations of serum AFP level and upper abdominal ultrasonography. The annual regular follow-up for noncirrhotic subjects was performed using the afore-mentioned two-stage screening protocol. Any suspected HCC cases thus identified were referred for confirmatory diagnosis as described before.

Control Selection. To examine the association between serum AFB_1-albumin adducts level and HCC risk, controls were selected from cohort subjects who were not affected with HCC through the follow-up period. Controls were matched with cases on age ($\pm$5 years), sex, residential villages, and date of blood collection (within 3 months).

AFB_1-Albumin Adducts in Serum. An enzyme-linked immunosorbent assay (ELISA) was used to determine the level of AFB_1-albumin adducts in serum samples. To prepare an antiserum for the detection of AFB_1-albumin adducts, human serum albumin (HSA), and bovine gamma globulin (BGG) were modified with AFB_1 epoxide synthesized by the method of Baertschi et al.[29] The modification levels ranged from 0.6 to 2.3 mol AFB_1/mol protein. New Zealand white rabbits were immunized by intramuscular injection at four sites with 1 mg of the modified BGG (2.3 mol/mol) in complete Freund's adjuvant, followed by monthly boosting with 1 mg protein in incomplete adjuvant. Antiserum (#7) was characterized by competitive ELISA. For the standard curve in the ELISA, [^{3}H] AFB_1-HSA was digested with proteinase K as described later for the human samples and adducts isolated by Seppak C18 (Waters, Milford, MA) extraction using cartridges that were prewashed with 10 mL chloroform, 10 mL methanol and 10 mL water. After application of the sample in phosphate-buffered saline and washing with 10 mL water and 5 mL 5% methanol, the adducts were isolated with 5 mL 80% methanol. Adducts levels were determined from the specific activity.

Albumin was prepared from plasma essentially as described previously[30] and concentration determined with bicinchoninic acid (BCA Reagent, Pierce, Rockford, IL). For the digestion, 2 mg albumin and 0.5 mg proteinase K were incubated for 15 hours at 37°C. Adducts were isolated by the procedure described by Sheabar et al.,[31] dissolved in 0.5 mL phosphate-buffered saline containing 1% fetal calf serum and 1 mmol/L phenylmethylsulfonylfluoride to inhibit residual protease activity, and assayed by competitive ELISA. This assay had 50% inhibition of antiserum binding at 10 to 20 fmol AFB_1 adduct per well. The limit of sensitivity (20% inhibition), when assaying the equivalent of 200 μg albumin/well, was 0.01 fmol/μg. Samples were assayed by duplicate analysis in duplicate wells; samples with less than 20% inhibition were considered nondetectable. Two control samples were analyzed with each batch of sera, a pooled sample of plasma from nonsmoking American subjects and a positive control of serum from a rat treated with 1.5 mg AFB_1.

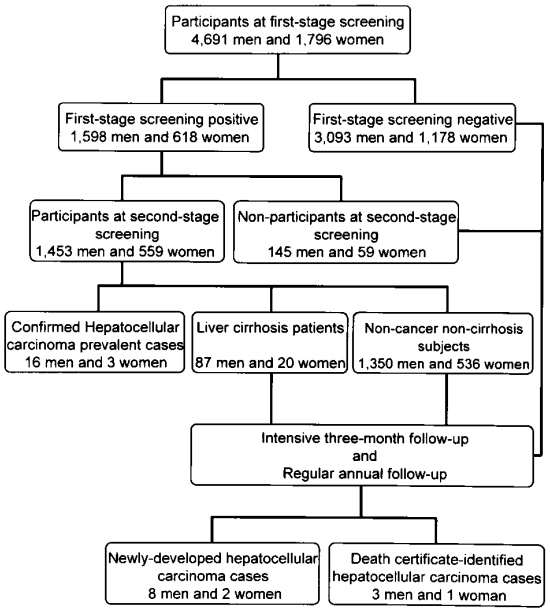

FIG. 1. Flow chart of two-stage HCC screening survey and follow-up examination.

Data Analysis and Statistical Methods. In the data analysis for the association between HCC risk and serum AFB_1-albumin adducts level, the serum level was categorized into two groups, detectable and nondetectable, using the detection limit of 0.01 fmol/μg as the cut-off value. Multiple logistic regression analysis was used to calculate odds ratios and their 95% confidence intervals for HCC risk factors including serum AFB_1-albumin adducts level after adjustment for age and sex. The HBsAg carrier status, anti-HCV seropositivity, and family history of liver cancer and cirrhosis among parents and siblings were further included in the multiple logistic regression to derive multivariate-adjusted odds ratio with its 95% confidence interval for serum level of AFB_1-albumin adducts.

RESULTS

As shown in Fig. 1, 4,691 male and 1,796 female residents in the Penghu Islets were recruited for the first-stage screening survey from March 1, 1991 through June 30, 1992. There were 1,019 (22%) men and 294 (16%) women who were HBsAg-positive, 190 (4%) men and 231 (13%) women were anti-HCV-positive, 337 (7%) men and 128 (7%) women had an ALT level $\geq$45 IU/mL, 306 (7%) men and 149 (8%) women had an AST level $\geq$40 IU/mL, 56 (1%) men and 16 (1%) women had an AFP level $\geq$20 ng/mL, 313 (7%) men and 107 (6%) women had a family history of HCC and liver cirrhosis. In total there were 1,598 (34%) men and 618 (34%) women with positive results for at least one of the six first-stage criteria. They were referred for the second-stage screening by abdominal ultrasonography, and 1,453 men and 559 women participated the ultrasonographic examinations, a response rate of 91% and 90%, respectively. Those who had ultrasonographic findings compatible with HCC were referred to teaching medical centers in main Taiwan Island for further confirmation examinations, and 16 male and 3 female subclinical HCC patients were thus diagnosed. In the intensive 3-month

TABLE 1. Characteristics of 33 Newly Diagnosed Subclinical HCC Cases in the Penghu Islets

Case No.	Sex	Age	HBsAg	Anti-HCV	AFP (ng/mL)	AST (IU/L)	ALT (IU/L)	Family History	Tumor Type	Largest Tumor Size (cm)	Portal Vein Tumor Thrombi
1810436	M	48	+	−	11.2	45	62	Yes	M	2.0	No
1810542	M	59	+	−	194.3	26	29	No	M	1.7	No
1810887	M	49	−	−	15.6	15	4	No	M	>10.0	Yes
1811537	M	58	+	−	>1,000	78	26	No	MS	10.0	Yes
1812628	M	62	+	−	307.0	23	15	No	M	>10.0	Yes
1813874	M	62	+	−	215.4	108	58	No	M	2.0	No
1814712	M	35	+	−	14.5	32	30	No	S	5.0	No
1821777	F	64	+	−	115.8	30	18	No	M	5.0	Yes
1823810	F	65	+	−	19.0	34	15	No	S	6.0	No
1824510	F	56	+	−	4.6	42	33	No	M	2.4	No
4810265	M	36	+	−	1.3	25	47	No	M	6.0	No
4814026	M	59	+	−	24.2	36	33	Yes	M	3.0	No
4814101	M	59	+	−	1.8	24	21	No	S	4.8	No
4814239	M	55	+	−	1.1	26	28	No	MS	2.0	No
4814294	M	57	+	+	>1,000	22	17	No	S	3.1	No
4814944	M	53	+	−	2.1	50	36	No	MS	4.0	No
4815106	M	54	+	−	14.0	25	17	No	M	2.0	No
4815168	M	54	+	−	10.4	93	32	No	MS	2.3	No
4818066	M	50	+	−	>1,000	24	28	No	S	3.0	No
4910405	M	38	+	−	>1,000	24	25	Yes	MS	6.0	Yes
4913556	M	53	+	−	6.2	22	35	No	M	2.5	No
4914011	M	63	+	−	>1,000	28	32	Yes	M	6.0	No
5810014	M	46	+	−	8.3	16	19	No	M	5.5	No
5810737	M	43	+	−	>1,000	20	15	Yes	MS	8.0	No
5810816	M	60	+	−	3.4	45	32	No	S	4.8	No
5811691	M	50	+	+	4.9	37	39	No	S	2.0	No
5812491	M	52	+	−	6.0	60	64	No	S	5.0	No
5812658	M	52	−	−	28.5	24	44	No	D	6.0	Yes
5812733	M	60	+	−	6.7	16	7	No	MS	4.1	No
5813587	M	53	+	−	4.4	42	44	No	M	3.0	No
5820166	F	56	+	−	0.8	47	35	No	S	3.3	No
5821037	F	56	+	+	5.4	36	23	No	S	2.0	No
5823255	F	57	+	−	184.3	60	47	Yes	NA	NA	NA

Abbreviations: M, multiple; MS, multiple and satellite; S, solitary; D, diffuse; NA, not available; +, positive; −, negative.

follow-up of 87 men and 20 women who were affected with cirrhosis, 10 newly-developed HCC cases were identified before December 31, 1993. Another 4 study subjects were identified from the regular annual follow-up combined with death certification data linkage during a period from July 1, 1992 through December 31, 1993.

Medical records of 33 HCC cases treated in medical centers were reviewed and abstracted according to a standard protocol. Clinical characteristics, including serological markers tested at the recruitment and angiographic findings of these 33 newly-identified HCC cases, are shown in Table 1. All but 2 of them (94%) were HBsAg-positive, 9% were anti-HCV-positive, 39% had an AFP level ≥20 ng/mL, 33% had an AST level ≥40 IU/L, 15% had an ALT level ≥45 IU/L, and 18% had a family history of HCC and cirrhosis among parents and siblings.

Serum levels of AFB_1-albumin adducts in 20 HCC cases and 86 matched controls were determined by competitive ELISA. There were 13 (65%) HCC cases and 32 (37%) matched controls who had a detectable serum level of AFB_1-albumin adducts. Table 2 shows the odds ratios with 95% confidence intervals for HBsAg carrier status, anti-HCV seropositivity, serum level of AFB_1-albumin adducts, family history of liver cancer and cirrhosis among parents and siblings, cigarette smoking habit, and habitual alcohol drinking after adjustment for age and sex. There was a significant association with the development of HCC for HBsAg carrier status, serum level of AFB_1-albumin adducts, family history of liver cancer and cirrhosis, cigarette smoking, and habitual alcohol drinking. The age-sex–adjusted odds ratio (95% confidence interval) of developing HCC was 3.2 (1.1-8.9) for the detectable serum level of AFB_1-albumin adducts.

TABLE 2. Age-Sex–Adjusted Odds Ratios With 95% Confidence Intervals for Hepatitis Infection, Serum Level of AFB_1-Albumin Adducts, Cigarette Smoking, and Habitual Alcohol Drinking in the Penghu Islets

Risk Factor	Healthy Controls (No. [%])	HCC Cases (No. [%])	Odds Ratio (95% confidence interval)
HBsAg carrier status			
Negative	106 (86.2)	2 (6.1)	1.0 (referent)
Positive	16 (13.0)	31 (93.9)	112.1 (23.6-533.8)*
Missing	1 (0.8)	0 (0.0)	
Anti-HCV seropositivity			
Negative	108 (87.8)	29 (87.9)	1.0 (referent)
Positive	12 (9.8)	3 (9.1)	0.9 (0.2-3.6)
Missing	3 (2.4)	1 (3.0)	
AFB_1-albumin adducts			
Negative	54 (43.9)	7 (21.2)	1.0 (referent)
Positive	32 (26.0)	13 (39.4)	3.2 (1.1-8.9)*
Missing	37 (30.1)	13 (39.4)	
Family history of liver cancer and cirrhosis			
No	116 (94.3)	27 (81.8)	1.0 (referent)
Yes	6 (4.9)	6 (18.2)	4.3 (1.3-14.5)*
Missing	1 (0.8)	0 (0.0)	
Cigarette smoking			
Never	63 (51.2)	10 (30.3)	1.0 (referent)
Ever	60 (48.8)	23 (69.7)	3.6 (1.3-10.6)*
Alcohol drinking			
Never	101 (82.1)	23 (69.7)	1.0 (referent)
<20 L/yr	7 (5.7)	2 (6.1)	1.2 (0.2-6.5)
≥20 L/yr	3 (2.4)	4 (12.1)	5.8 (1.2-28.1)*
Missing	12 (9.8)	4 (12.1)	

* $P < .05$.

Table 3 shows multivariate-adjusted odds ratios and 95% confidence intervals for HBsAg carrier status, anti-HCV seropositivity, serum AFB_1-albumin adducts level, and family history of liver cancer and cirrhosis. Only HBsAg carrier status and detectable serum level of AFB_1-albumin adducts were significantly associated with an increased risk of HCC. The multivariate-adjusted odds ratio (95% confidence interval) of developing HCC was 5.5 (1.2-24.5) for the detectable serum level of AFB_1-albumin adducts.

DISCUSSION

Chinese throughout the world have a high incidence of HCC that may be attributable to both environmental and genetic risk factors.[10] HBV has been well documented as the most important environmental risk factor for HCC in Taiwan.[4-11] In this study, 94% newly-diagnosed subclinical HCC patients were HBsAg carriers. Chronic HBV infection may increase the probability of accumulating changes in multiple genes, including oncogenes and tumor suppressor genes through specific integration of the HBV genome in hepatocytes or through transactivation by HBV X antigen or truncated products of pre-S2/S genes.[32-37] Chronic HBV infection may also act as a promotor and/or progressor in hepatocarcinogenesis through chronic phasic necroinflammation resulting from the accumulation of HBsAg in hepatocytes.[38]

There are observations suggesting that other factors in addition to chronic HBV infection are important to the development of HCC in Taiwan as in other countries.[8-14] An ecological correlation study also showed a significant association between urinary aflatoxin level and age-adjusted HCC mortality rates at the township level in Taiwan.[21] Residents in the Penghu Islets were reported to have the highest mortality rate caused by liver cancer in Taiwan,[4-6] and peanuts in Penghu Islets were heavily contaminated by aflatoxins.[28] In this study, the HBsAg carrier rate was 22% for men and 16% for women in the Penghu Islets. Because the rates were only slightly higher than that of the general population in Taiwan, the increased HCC risk in Penghu Islets could not readily be explained by its HBsAg carrier rate. Some other environmental factors, especially aflatoxin exposure, may also play an important role in the development of HCC.

AFB_1 is the most potent hepatocarcinogen in a variety of animal species.[39] It is metabolized predominantly in hepatocytes by the microsomal mixed-function oxygenase enzyme system to various reduced and oxidized derivatives including an unstable reactive AFB_1-8,9-epoxide, which can bind covalently to nucleophilic sites of biological macromolecules including nucleic acids and proteins.[40] The formation of AFB_1-guanine adducts has been shown to be critical for the carcinogenesis induced by AFB_1 in animals.[41] AFB_1-8,9-epoxide reacts with albumin in the liver to form AFB_1-albumin adducts that are major protein adducts of AFB_1 found in peripheral blood. The use of aflatoxin-albumin adducts as a biomarker of exposure has several advantages: (1) aflatoxin-albumin adducts reflect DNA damage in hepatocytes as does aflatoxin-N^7-guanine in urine,[42] (2) albumin adducts, at least in experimental animals, are as long-lived as albumin, which has a half-life of 21 days and thus provide a cumulative measure of exposure over a long period of time,[43] and (3) multiple measurements of urinary aflatoxin are required to reflect average exposure, but only a single measurement of albumin adducts is needed to provide a representative average exposure.[44] All previous epidemiological studies on the association between aflatoxin and HCC were based on the urinary aflatoxin level.[22,23] This study, to the best of our knowledge, is the first one reporting a significant association between elevated serum AFB_1-albumin level and HCC risk. The peanuts in Penghu were reported to be heavily contaminated with aflatoxin,[28] and peanuts are frequently consumed by residents in the Penghu Islets according to our questionnaire interview at recruitment. The aflatoxin contamination may vary in different seasons. As the seromarker used in this study may reflect a long-term exposure to aflatoxin, it is considered appropriate to control seasonal variation in aflatoxin exposure by matching date of biospecimen collection.

Study subjects participated in this screening program on a voluntary basis, and there may exist some self-selection bias that results in the biased estimation of prevalence and incidence. However, comparability is more important than representativeness in studies designed to assess the asociation between health outcome and risk factor exposure. Because both cases and controls were selected from the screens of this study, they were considered quite comparable in physical and psychological characteristics related to their choice to participate.

Setting the type I error (alpha) level of 0.05, type II error (beta) level of 0.20, exposure rate among controls of 0.40, detectable odds ratio of 5.0, and a case-control ratio of 4.0, the minimum sample size required for cases was 16.[45] Although the sample size was small in this study, the statistical power to detect a significant association between AFB_1-albumin adduct level and HCC risk was as high as >0.80. Furthermore, the odds ratio of developing HCC remained statistically significant after adjustment for other risk factors, indicating the sample size was adequate. In a recent study including only 22 cases and 140 controls,[22] a statistically significant association between urinary aflatoxin level and HCC was observed after multivariate adjustment for HBsAg seropositivity, educational level, cigarette smoking, and alcohol drinking, showing an adjusted odds ratio of 3.8 ($P < .05$). Short-term biomarkers of aflatoxin were used in the previous study, whereas a better long-term marker for biologically effective dose of aflatoxin exposure was used in our study. Despite a small number of HCC cases, a statistically significant multivariate-adjusted odds ratio as high as 5.5 was observed for the seropositivity of AFB_1-albumin adducts in this study.

Study subjects who participated in this study were free from symptoms of liver diseases and did not change their dietary habit and life style at the time of biospecimen collection. Furthermore, a long-term rather than short-term biomarker was used to estimate aflatoxin exposure. It seems reasonable to assume the serum aflatoxin AFB_1-albumin level determined at recruitment may reflect the usual exposure to aflatoxin among study subjects. The association between aflatoxin exposure and HCC risk was analyzed for prevalent and incident case-control sets, respectively. The odds ratios were found comparable but not statistically significant because of the small sample size. It is considered appropriate to combine incident and prevalent case-control sets because of their similarity in the magnitude of association between serum AFB_1-albumin adducts level and HCC risk.

It has been documented that AFB_1 selectively targets at the third base position of codon 249 (G:C to T:A transversion) of the p53 gene.[46] The G-to-T transversion at codon 249 of p53

TABLE 3. Multiple Logistic Regression of Risk Factors for HCC and Matched Healthy Controls in the Penghu Islets

Risk Factors	Group	Odds Ratio (95% confidence interval)
HBsAg	Negative	1.0 (referent)
	Positive	129.4 (25.4-659.2)*
Anti-HCV	Negative	1.0 (referent)
	Positive	1.8 (0.2-13.3)
AFB_1-albumin adducts	Nondetectable	1.0 (referent)
	Detectable	5.5 (1.2-24.5)*
Family history of liver cancer and cirrhosis	No	1.0 (referent)
	Yes	4.2 (0.6-31.3)

* $P < .05$.

has been documented as the mutational hotspot in human hepatocellular carcinoma.[47,48] As p53 is involved in the tumor suppression, it seems reasonable to conclude that AFB_1 plays an important role in the late stage of hepatocarcinogenesis, although an early stage effect may also exist. At least partly attributable to heavy exposure to aflatoxin, the onset age of HCC among residents in Penghu Islets was 10 years younger than that among those who lived in Taiwan Island. A synergistic effect on HCC has recently been observed between HBsAg carrier status and aflatoxin exposure.[22,23] The elevated HCC risk among residents in Penghu Islets seems attributable to their high HBsAg carrier rate and heavy aflatoxin exposure.

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