Carcinogenic effects of aflatoxin B1 among wheat handlers

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Background: Epidemiological studies have demonstrated that serum aflatoxin B1 (AFB1) is a hepatocarcinogenic mycotoxin and contributor to the high rate of hepatocellular carcinoma (HCC). The prevalence of liver cancer in Egypt is particularly worrisome. In a registry-based analysis of occupational risk for HCC, significant excesses were observed especially for grain mill workers.
Objective: The aim of this study was to assess the hepatic carcinogenicity of AFB1 in wheat handlers.
Methods: Serum AFB1/albumin (AFB1/Alb), alpha-fetoprotein (AFP), alpha-l-fucosidase (AFU), and arginase were estimated in exposed wheat handlers including millers and bakers. The control group was composed of non-occupationally exposed workers.
Results: AFB1/Alb and AFU were significantly higher among workers employed as bakers compared to mill workers and controls. Mill workers had higher levels of AFB1/Alb than the controls. AFB1/Alb, AFP, and AFU were all significantly higher and arginase was significantly lower among HCC cases compared to the other groups. There was a significant correlation between AFU and AFB1/Alb in bakers and between AFP and AFB1/Alb in HCC cases. Arginase was inversely correlated with AFB1/Alb in HCC cases. AFB1/Alb was significantly correlated with the duration of exposure in bakers.
Conclusion: Wheat handlers exposed to *Aspergillus flavus* have a high risk of elevated serum AFB1/Alb levels and AFU.

Keywords: Wheat handlers, Hepatocellular carcinoma, Aflatoxin B1, Alpha-fetoprotein, Alpha-l-fucosidase, Arginase

Introduction

Hepatocellular carcinoma (HCC) is one of the most common cancers worldwide and the third most common cause of cancer-related death.[1] Data from multiple studies demonstrate that the incidence of HCC has been increasing, with approximately 500 000 new cases and more than 600 000 deaths annually.[2] The risk of liver cancer in Egypt is particularly worrisome, especially in comparison with other countries. The Middle East Cancer Consortium found that livers cancer accounted for 12.7% of male cancers and 3.4% of female cancers in Egypt.[3] Risk factors for HCC include chronic hepatitis B or C infection, cirrhosis, non-alcoholic fatty liver, alcohol induced liver disease, and exposure to aflatoxin and/or other carcinogens.[4] Aflatoxin B1 (AFB1) is a mycotoxin produced by *Aspergillus flavus* and *Aspergillus parasiticus,* which are widespread in nature. The mycotoxin is found in foodstuffs, such as corn, rice, oil seeds, dried fruits, and peanuts that have been improperly stored in hot, humid, and unsanitary conditions.[5]

The isolation of aflatoxin biomarkers in human biological samples such as serum AFB1-DNA adduct, AFB1-lysine adduct, and other metabolites of AFB1 in urine and feces, such as AFM1 and AFB1-mercapturic acid, can be used to measure aflatoxin exposure. Exposure assessment is essential for understanding the extent of aflatoxin exposure in a population.[6] AFB1/albumin (AFB1/Alb) adduct is formed following metabolism of aflatoxin in the liver, and previous studies have found it to be a valid indicator of the formation of hepatic aflatoxin DNA adducts in animals and humans.[7] In prior research, the authors have found that chronic occupational exposure to *Aspergillus flavus* resulted in a significant elevation of serum levels of AFB1/Alb in workers exposed to wheat flour dust and of urinary AFM1 (the metabolite of AFB1) in textile workers exposed to cotton dust.[8,9]

Reports from epidemiological studies have demonstrated that AFB1 is a hepatocarcinogenic mycotoxin and the primary contributor to the high rate of HCC.[10] The International Agency for Research on

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Cancer has classified AFB1 as a Group1 carcinogen for HCC.[11] Many studies have demonstrated the association between the ingestion of aflatoxin-contaminated foods and the risk of HCC, yet few studies have measured the risk of HCC among people occupationally exposed to aflatoxin. In a registry-based analysis of occupational risks for primary liver cancer in Sweden, significant excesses were observed in both male and female workers in grain mills. This finding was associated with potential exposures to the hepatotoxins, aflatoxins, parasites, pesticides, and fumigants.[12] In a previous study, we found that the serum P53 was significantly higher in wheat mill workers with high serum levels of AFB1 compared to non-occupationally exposed controls.[13]

The primary tumor biomarker for HCC is alpha-fetoprotein (AFP), a single polypeptide chain glycoprotein, and early diagnosis of HCC improves the survival of patients. Alpha-l-fucosidase (AFU) is a lysosomal enzyme found in mammalian cells and is a proposed tumor marker for HCC. Previous studies have found increased AFU serum levels in patients with cirrhosis and HCC.[14,15] Arginase is a hydrolase typically found in the liver, where it catalyzes the final reaction in the synthesis of urea, the so-called liver-type arginase.[16] Concurrently, the rise of extra-hepatic arginase can increase the level of polyamines, compounds crucial for cell proliferation. Thus, both arginase isoenzymes seem to participate in liver cancerogenesis.[17]

The objective of this study was to assess the carcinogenic effect of AFB1 on the liver of wheat handlers occupationally exposed to high concentrations of *Aspergillus flavus*.

Methods

Study participants

The study was a cross-sectional comparative study. Ethical approval was granted by the Ethical Committee of the National Research Center in Egypt.

The study population was comprised of 190 wheat handlers: 100 flourmill workers and 90 bakers from seven bakeries located in Helwan District, Cairo, Egypt. The flourmill workers and the bakers were occupationally exposed to high concentrations of *Aspergillus flavus* (on average 95.1 and 487.2 cfu/m^3 respectively).[8] The control population included 64 apparently healthy subjects from the National Research Center (normal controls) and 32 HCC-positive controls from the National Cancer Institute in Cairo, Egypt. The positive controls were included to compare the levels of selected tumor markers. The normal control subjects were not occupationally exposed to wheat or other organic dusts. They were exposed to *Aspergillus flavus* in the range of 10.0–12.8 cfu/m^3 (average 11.5 ± 1.41 cfu/m^3).[8] Hepatitis B virus- or hepatitis C virus-positive subjects were excluded from the study.

Questionnaire

A questionnaire including questions about demographics, smoking history, and a detailed occupational history was administrated to all participants by the authors. Written informed consent was obtained from participants before the questionnaires were administered. Smoking Index was calculated as the number of cigarette packs per year smoked according to Aslam *et al.*[18]

Blood samples

Five ml of blood were collected from all participants in sterile dry tubes. They clotted for 30 minutes at 37°C and centrifuged at 3000 rev/min for 10 minutes. The separated sera were stored at −20°C until tested.

Estimation of AFB1

AFB1 was extracted using EASI-EXTRACT® Aflatoxin immunoaffinity column (Scotland) and quantitatively determined using RIDASCREEN® Aflatoxin B1 30/15 ELISA (Germany).

Estimation of serum Alb

Serum albumin was determined using the colorimetric method described by Doumas and Biggs.[19] AFB1 results were expressed as ng/g albumin.

Estimation of tumor biomarkers

AFP testing was based on the principle of a solid phase enzyme-linked immunosorbent assay using the AFP ELISA test kit (BioCheck, Inc., Foster City, CA, USA).[20]

AFU testing was based on the enzymatic cleavage of the synthetic substrate p-nitro phenyl a-L-fucopyranoside to p-nitrophenol and L-fucose.[21] Estimation of AFU by colorimetric assay was performed using kits from Biodiagnostics in Egypt.

Arginase assay was based on colorimetric determination of urea by condensation with diacetylmonoxime in an acid medium in the presence of ferric chloride (oxidant) and carbazide (accelerator), using kits from Biodiagnostics in Egypt.[22]

The sensitivity of tumor detection using AFP was increased from 68.2% to 88.6% when AFP was evaluated in conjunction with AFU.[15] Therefore, AFP and AFU tumor biomarkers were used to screen for HCC among the workers exposed to high concentrations of *Aspergillus flavus.*

Statistical analysis

All analyses were performed using SPSS v.18. Results were expressed as means ± standard deviations. Analysis of variances (ANOVAs) and a *post-hoc* test of least-significant differences (LSDs) were used to test for statistically significantly differences between the exposed groups (flourmill workers and bakers)

Table 1 Comparison of the AFB1/Alb and the tumor markers in the four examined groups

	Normal controls (n=64)		Milling workers (n=100)		Bakers (n=90)		HCC cases (n=32)		ANOVA	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	*F*-ratio	*P*-value
AFB1/Alb (ng/g) LSD	0.03 (M, B, HCC)	0.02	0.06 (C, B, HCC)	0.03	0.11 (C, M, HCC)	0.05	0.65 (C, M, B)	0.47	128.2*	<0.0001
AFU (U/l) LSD	3.4 (B, HCC)	0.31	3.6 (B, HCC)	0.51	11.3 (C, M, HCC)	0.75	35.9 (C, M, B)	4.80	64.7	<0.0001
AFP (ng/ml) LSD	1.3 (HCC)	0.30	3.9 (HCC)	0.35	2.2 (HCC)	0.55	119.3 (C, M, B)	23.09	46.7	<0.0001
Arginase (U/l) LSD	333.6 (HCC)	66.06	340.5 (HCC)	98.48	326.9 (HCC)	61.33	261.9 (C, M, B)	106.95	6.5	<0.0001

Note: C: normal controls; M: mills; B: bakers; HCC: hepatocellular carcinoma cases.
*Kruskal–Wallis and Mann–Whitney *U* tests were used.

and the control groups. Kruskal–Wallis and Mann–Whitney *U* tests were used to compare non-parametric data. Correlation coefficient was used to study the relationships between the different studied parameters. A *P*-value $\leq$0.05 was considered statistically significant.

Results

There were no significant differences between the workers, normal controls, and HCC controls regarding their age (47.3$\pm$7.7, 48.7$\pm$7.95, and 49.4$\pm$4.5 years, respectively) or Smoking Index (6.6$\pm$11.4, 5.22 $\pm$8.3, and 6.3 $\pm$6.8 cigarette package/year, respectively). The flourmill workers and the bakers were occupationally exposed to high concentrations of *Aspergillus flavus* for more than 10 years (19.3$\pm$6.5 and 22.0$\pm$8.7 years, respectively).

Table 1 shows that HCC group had the highest levels of AFB1/Alb, AFU, and AFP. Bakers had significantly higher levels of AFB1/Alb and AFU than the flourmill workers (P<0.0001). Arginase was significantly lower in the HCC compared to the other groups (P<0.0001).

There was a significant correlation between AFU and AFB1/Alb in bakers, and between AFP and AFB1/Alb in HCC cases. Arginase was inversely correlated with AFB1/Alb in HCC controls (Table 2). There was no significant correlation between the duration of exposure and tumor markers in flourmill workers or bakers, but there was a significant correlation between the duration of exposure and AFB1/Alb level in bakers (r=0.04, P<0.05).

Discussion

AFB1/Alb adducts are a long-term marker of AFB1 exposure and provide insight to the accumulated exposure after a two to three month period.[23] The authors have previously found that airborne *Aspergillus flavus* concentrations were higher in bakeries (487.2 cfu/m^3) than in flour mills (storage section: 76.9 cfu/m^3, garbling 76.8 cfu/m^3, grinding 205.1 cfu/m^3, and packaging 21.4 cfu/m^3) in the Helwan district in Cairo, Egypt, and the concentration of *Aspergillus flavus* in the two workplaces was higher compared to that in the control area (11.5$\pm$1.41 cfu/m^3).[8] Chronic occupational exposure to high concentrations of *Aspergillus flavus* was found to cause significant elevation in the serum AFB1/Alb of bakers and flourmill workers compared to the normal controls. This was also true when comparing the bakers to the flourmill workers. The normal control subjects were used to adjust for the confounding effect of dietary aflatoxin. Based on the results, we hypothesize that the significant elevation of AFB1/Alb in the HCC cases compared to the other groups, and in the bakers and the flourmill workers compared to normal control subjects, could denote the important hepatocarcinogenic effect of AFB1 and may be attributed to the decrease in the efficiency of the metabolism of AFB1 in liver cells to be converted to the metabolic form of AFM1 to be excreted.

Prior studies have demonstrated that areas with a high AFB1 exposure coincide with a high prevalence of HCC.[24,25] A Taiwanese study found that AFB1 is a reliable predictor of HCC risk, even in cases diagnosed more than 13 years after blood and urine testing for AFB1.[26] Some epidemiological studies detected a statistically significant elevation in the risk of HCC with increasing level of AFB1 exposure.[21,26,27] Textile workers exposed to high concentrations of airborne *Aspergillus flavus* had significant elevations in the urinary AFM1; the metabolite of AFB1, and the

Table 2 Relationship between AFB1/Alb and tumor markers in the study population

	Normal controls (n=64)		Flourmill workers (n=100)		Bakers (n=90)		HCC cases (n=32)	
AFB1/Alb (ng/g)	*r*	*P*-value	*r*	*P*-value	*r*	*P*-value	*r*	*P*-value
AFU (U/l)	0.2	NS	0.2	NS	0.3	P<0.05	0.1	NS
AFP (ng/ml)	0.1	NS	0.2	NS	0.2	NS	0.4	<0.05
Arginase (U/l)	−0.04	NS	−0.1	NS	−0.03	NS	−0.4	0.01

liver tumor biomarkers AFP and AFU compared to a control group, although the tumor markers were generally within normal limits.[28] In the present study, the significant elevation of the serum AFU in the bakers compared to the flourmill workers and the normal controls reflects the high risk of this occupational group of developing HCC. This finding is confirmed by the significant correlation between AFU and AFB1/Alb in the bakers. Murugavel *et al.* found that AFB1-positive non-viral HCC cases showed higher levels of AFP and they postulated that the levels of AFP might be indicative of the severity of the hepatocarcinogenesis, irrespective of the hepatocarcinogen.[29] This may indicate that the severity of hepatocarcinogenesis in HCC cases increases with a rise in the AFB1/Alb levels.

Since HCC is arginine-dependent, and arginine is essential for cell growth, the decrease of arginase may preserve this amino acid within the tumor cells, as arginase isoenzymes seem to participate in liver cancerogenesis. It was found that arginase activity in HCC was lower than in normal livers; however, the amount of hepatic arginase, as well as the expression of arginase mRNA, was lower in HCC compared with normal livers.[17] The serum arginase in the present study was significantly lower in the HCC controls compared to the other three groups, and it was inversely correlated with AFB1/Alb in HCC cases. The low level of arginase and high level of AFB1/Alb in HCC cases may explain the inverse correlation between arginase and AFB1/Alb in HCC cases.

The duration of exposure was not significantly correlated with tumor markers in the wheat handlers. The higher levels of serum AFB1/Alb and AFU in bakers, the significant correlation between AFU and AFB1/Alb, and the significant correlation between their duration of exposure and the AFB1/Alb proved that they were more affected by AFB1 due to their exposure to higher occupational concentrations of *Aspergillus flavus*when compared to the flourmill workers and the normal controls. This means that people exposed to high concentrations of airborne *Aspergillus flavus* run a higher risk of developing elevated tumor biomarkers.

Wheat handlers exposed to high concentrations of *Aspergillus flavus* have a high risk of developing elevated serum AFB1/Alb and elevated AFU, suggesting that they are at high risk of developing HCC. This study recommends estimation of tumor biomarkers during periodic examination of wheat handlers to identify workers susceptible to HCC.

Disclaimer statements

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Conflicts of interest No conflicts of interest.

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