Hepatotoxic effects of aflatoxin in workers exposed to wheat flour dust

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Abstract

Aflatoxins are a group of mycotoxins produced by *Aspergillus flavus* and *Aspergillus parasiticus* . They are potent inducers of hepatotoxicity. Those toxic compounds are ubiquitously found in food commodities such as nuts, cereals, spices and milks and dairy products. Aflatoxin B1 (AFB1) has been classified by the International Agency for Research on Cancer (IARC) as Group 1 carcinogen. Once AFB1 is ingested by humans, it is metabolized by liver enzymes into many metabolites. The study aimed to estimate the hepatotoxicity of AFB1 in workers occupationally exposed to wheat flour dust. Statistical analysis of the results revealed that Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), γ-glutamyl transpeptidase (GGT), Alkaline phosphates (ALP) levels in the exposed workers were significantly higher compared to their controls. In the exposed workers, there were significant correlations between AFB1/Alb and the duration of exposure, and between the levels of AST and ALT and the level of AFB1/Alb. The present study that the serum AFB1/Alb was significantly correlated with the duration of exposure in workers exposed to wheat flour dust, and such elevation in AFB1/Alb levels have hepatotoxic effects in the exposed workers.

Keywords; Aflatoxin; Aspartate aminotransferase; Alanine aminotransferase; γ-glutamyl transpeptidase; Alkaline phosphates.

Introduction

Mycotoxins produced by fungi are commonly found in a wide range of food commodities. Aflatoxins (AFs) are produced by *Aspergillus* species of fungi; mainly *Aspergillus flavus*, *Aspergillus paraciticus* and *Aspergillus nominus* **(Reddy et al., 2011)**. Several studies were carried out to evaluate the level of AFs in food products in different governorates in Egypt. From Qaluobia and Kafr El-Sheikh Governorates, 100 samples of imported and local wheat grains were collected and examined for the natural occurrence of AFs during 2000–2001. Results indicated that both local and imported samples were positive for AFB1 (17.5% and 20% respectively), and the concentration ranged from 3 to 25 μg/kg. The level of AFs was dependant on the area of collection as well as the season of the year **(Amra et al., 2007)**.

AFB1 is first metabolized (Phase 1) by the Cytochrome P450 enzyme (CYP450) system found in the microsomes, producing a variety of intermediary metabolites such as AFB1 epoxide and other hydroxylated metabolites like aflatoxin M1 (AFM1), aflatoxin P1 (AFP1), aflatoxin Q1 (AFQ1) and aflatoxicol. AFB1 epoxide is highly reactive and

relatively unstable with inbuilt capacity to bind to cellular macromolecules like DNA, RNA, lipids and proteins, initiating the vicious cycle of lipid peroxidation and culminating in cellular injury (**Stresser et al., 1994)**. AFs are responsible for a wide range of pathological abnormalities in humans and animals. Aflatoxin-albumin adduct (AFB1/Alb); a biomarker for aflatoxin exposure, was found to be higher in the serum of individuals at risk for hepatocellular carcinoma, thus, indicating its importance in human health and diseases (**Sun et al., 2002).** The present study was carried out to evaluate the hepatotoxic effects of aflatoxins in workers exposed to wheat flour dust.

Subjects and methods

Subjects

Eighty five flour milling workers and 64 apparent healthy males not occupationally exposed to wheat flour dust were included in the present study, after exclusion of the workers with positive viral hepatitis (HBV or HCV). The two groups were matched for age, residential area and socioeconomic status of the workers.

Blood Samples

Random Venous blood samples (5 ml) were collected from all study subjects by sterile disposable syringes. Blood samples were left to clot for 30 minute at 37°C and then centrifuged at 3,000 rpm for 10 minutes to isolate the sera.

AFP B1 was firstly extracted using EASI-EXTRACT® Aflatoxin immune affinity column (Scotland). AFB_1 concentrations of the samples were analysed by microtitre plate Enzyme linked immunosorbent assay (ELISA) method using RIDASCREEN® (Germany) **(R-Biopharm GmbH, 2004).** Serum albumin was measured in all serum samples using the Human kits for kinetic determination of Albumin and the AUTOLAB selective access batch auto-analyzer (from Boehringer Mannheim Lab Diagnostics).

Serum aminotransferases (ALT and AST) were estimated by the method of Provisional Recommendations on IFCC methods for the measurement of catalytic concentrations of enzymes **(Bergmeyer et al., 1977).** Serum Gamma glutamyl transferase (GGT) was measured using the method of **Szasz** in **(1969)** and serum alkaline phosphate (ALP) was measured by method of **Belfield and Goldberg (1971).**

Results

There was no significant difference in the age between the workers and their controls (44.5±7.7 and 42±7.5 years respectively). There was also no significant difference in the smoking habits and smoking index between the workers and the controls (6.3±1.1 and 4.2±1.6 package/ year respectively). The duration of exposure of the workers were in the range 7-50 years; with average 25.8±8.8 years. Table (1) shows that the serum levels of AFB1/Alb, and the liver enzymes (ALP, ALT, AST, and GGT) were significantly higher in the workers compared to the control group.

Table (1): Comparisons of AFB1/Alb and the liver enzymes between the exposed workers and the controls

	Workers (85)		Controls (64)		Independent t-test	
	Mean	SD	Mean	SD	t-test	P-value
AFB1/ Alb (ng/g)	0.06	0.03	0.04	0.02	5.403	P< 0.0001
ALP (IU/L)	96.1	7.34	71.8	21.58	2.929	P< 0.01
ALT (U/L)	37.5	10.66	5.3	1.83	31.386	P< 0.0001
AST (U/L)	26.5	8.70	3.2	1.05	23.159	P< 0.0001
GGT (U/L)	22.3	1.78	6.0	3.67	4.113	P< 0.0001

In the exposed workers, the AFB1/Alb was significantly correlated with the duration of exposure (r= 0.3, P< 0.05). The AST and ALT were significantly correlated with the serum levels of AFB1/Alb (Table 2). But, there was no significant correlation between ALP and GGT levels and the AFB1/Alb levels. Table (2) also showed that AST was significantly correlated with the duration of exposure, but, there was no significant relationship between the other liver enzymes and the duration of exposure.

Table (2): Relationships between the liver enzymes and the serum levels of AFB1/Alb and the duration of exposure

	AFB1/Alb (ng/g)		Duration of exposure (years)	
	r=	P-value	r=	P-value
AFB1/Alb (ng/g)	-		0.5	P< 0.01
ALP (IU/L)	0.04	NS	0.2	NS
ALT (U/L)	0.3	P< 0.05	0.3	NS
AST (U/L)	0.5	P< 0.01	0.4	P< 0.05
GGT (U/L)	0.1	NS	0.01	NS

Discussion

In occupational settings the preferential route of exposure to AFs is through inhalation **(Viegas et al., 2012).** AFs are possibly carried to the workers' breathing zone by dust, and therefore, dust exposure promotes exposure to AFs. **Brera and his colleagues (2002)** found AFs in airborne dust samples from different occupational settings. Workers with occupational exposure airborne grain dust, are at risk of ingesting, transmucosally absorbing, and inhaling AFB1 released during product preparation or processing **(Wang et al., 2008; Traverso et al., 2010; Yang et al., 2013).**

The results in the present study revealed that the serum levels of AFB1/Alb was significantly higher in the workers compared to the control group. In previous study, occupational chronic exposure to the high concentrations of *Aspergillus flavus* in the workplaces caused significant elevation in the levels of serum AFB1/Alb, and these elevations were significantly increased with the increase in the environmental exposures to *Aspergillus flavus* **(Saad-Hussein, 2010-2013)**. Several studies proved that poorly ventilated workplaces with high concentrations of airborne aflatoxin producing strains

of *Aspergillus flavus* resulted in elevation in the blood levels of AFB1 in the exposed workers (**Oluwafemi et al., 2012).**

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST), are commonly measured clinically as a marker for the liver health **(Hou et al., 2013).** Where alterations in serum levels of ALT and AST are liver specific and have been considered as a tool for studying varying cell viability and changes in cell membrane permeability **(El-Sayed and Khalil, 2009).** In addition, GGT is a prime marker of bile duct epithelial proliferation that is typical of aflatoxicosis **(Kramer, 1989). Adedara et al. (2010)** found that an increase in hepatic ALP activity was well supported by the parallel increase in GGT activity following exposure to AFB1.

The present study revealed that there was significant elevation in the liver enzymes (ALT, AST, ALP and GGT) of examined workers compared to their controls. In agreement with the present results, the activities of ALT and AST were significantly higher in aflatoxin-treated animals compared to the control animals **(Yassein and Zghair, 2012)**. In contrast to our study, **Fu et al. (2013)** found that, there were no significant effects of AFB1 on the liver enzymes ALT, AST, ALP and GGT, and they attributed that to the short duration of exposure (42 days). They attributed these results to the short duration of exposure that may not have been great enough to elicit substantial changes in these indices.

In the present study, there was significant correlations between AFB1/Alb serum levels and the liver enzymes (ALT and AST) of the exposed workers, and between their duration of exposure to wheat flour dust and the AFB1/Alb and AST levels. The significant elevation in these liver enzymes could be attributed to the long duration of occupational exposure to wheat flour dust that lead to significant elevation in serum AFB1/Alb levels. This was also confirmed by the previous study, that detected significant elevation in the liver enzymes (ALT, AST, ALP and GGT) in wheat milling workers with high levels of serum AFB1/alb and high oxidative stress biomarker (MDA) compared to the controls **(Saad-Hussein et al., 2014)**.

The present study concluded that the serum AFB1/Alb was significantly correlated with the duration of exposure in workers exposed to wheat flour dust, and such elevation in AFB1/Alb levels have hepatotoxic effects in the exposed workers.

References

Adedara IA, Owumi SE, Uwaifo AO, Farombi EO (2010): Aflatoxin B1 and ethanol co-exposure induces hepatic oxidative damage in mice Toxicol. Ind. Health.; 26: 717–724.

Amra HA, Masheal SF, Ebba MM, Bakr AA, Nogaim QA, Anwar WA. (2007): Aflatoxins level in some Egyptian crops collected from different governorates: possibilities for detection and prevention (Poster No. 26) 5th International Conference on Environmental Mutagens in Human Populations: "Identification of Environmental Hazards and Promotion of Health", Antalya, Turkey, 20–24.

Belfield A, Goldberg DM. (1971): Revised assay for serum phenyl phosphatase activity using 4-amino-antipyrine. Enzyme.; 12(5):561-73.

Bergmeyer HU, Bowers GN, Hørder JM and Moss DW (1977): Provisional Recommendations on IFCC methods for the measurement of catalytic concentrations of enzymes. Clin Chem.; 23: 887 – 899.

Brera C, Caputi R, Miraglia M, Iavicoli I, Salerno A, Carelli, G. (2002): Exposure assessment to mycotoxins in workplaces: Aflatoxins and ochratoxin A occurrence in airborne dusts and human sera. Microchem. J.; 73:167–173.

El-Sayed YS, Khalil RH. **(2009):** Toxicity, biochemical effects and residue of aflatoxin B(1) in marine water-reared sea bass (Dicentrarchus labrax L.). Food Chem Toxicol.; 47(7):1606-9.

Fu JC, Chen Q, Du J, Shi BM, Shan AS. **(2013):** Effectiveness of maifanite in reducing the detrimental effects of aflatoxin B_1 on hematology, aflatoxin B_1 residues, and antioxidant enzymes activities of weanling piglets. Livestock Science.; 157 (1): 218-224.

Hou YJ, Zhao YY, Xiong B, Cui XS, Kim NH, Xu YX, Sun SC. (2013): Mycotoxin-containing diet causes oxidative stress in the mouse. PLoS One.; 8(3):e60374.

Kramer JW. **Clinical enzymology**. JJ Kaneko (Ed.), Clinical biochemistry of domestic animals (ed 4), Academic Press, San Diego (1989).

Oluwafemi, F, Odebiyi, T, Kolapo, A. (2012): Occupational aflatoxin exposure among feed mill workers in Nigeria. World Mycotoxin Journal.; **5** (4)**: 385-389.**

R-Biopharm GmbH, (2004): Enzyme immunoassay for the quantitative analysis of aflatoxin B1. Art. No.: R1201. R-Biopharm GmbH, Darmstadt, Germany.

Reddy KRN, Farhana NI, Salleh B. (2011): Occurrence of Aspergillus spp. and aflatoxin B1 in Malaysian foods used for human consumption.; Journal of Food Science.; 76:T99–T104

Saad-Hussein, (2010-2013). Final report of fungi causing Cancers as an Environmental Health Problem in Occupations at risk project, funded by National Research Center, Egypt.

Stresser DM, Bailey GS. (1994): Williams D.E. Indol-3-carbinol and beta-naphthoflavone induction of aflatoxin B1 metabolism and cytochromes P-450 associated with bioactivation and detoxication of aflatoxin B1 in the rat. Drug Metab Dispos, 22: 383–391.

Sun CA, Wu DM, Wang LY, Chen CJ, You SL, Santella RM. (2002): Determinants of formation of aflatoxin-albumin adducts: a seven township study in Taiwan.Br J Cancer.; 87: 966–970.

Szasz G. (1969): Determination of gamma-GT. Clin Chem. 15:124-136.

Traverso A, Bassoli V, Cioè A, Anselmo S, Ferro M. (2010): Assessment of aflatoxin exposure of laboratory worker during food contamination analyses. Assessment of the procedures adopted by an A.R.P.A.L. laboratory (Liguria Region Environmental Protection Agency). Med Lav.; 101(5):375-80.

Viegas S, Veiga L, Malta-Vacas J, Sabino R, Figueredo P, Almeida A, Viegas C,Carolino E. (2012): Occupational Exposure to Aflatoxin (AFB) in Poultry Production. Journal of Toxicology and Environmental Health, Part A, 75:1330–1340.

Wang Y, Chai T, Lu G, Quan C, Duan H, Yao M, Zucker BA, Schlenker G. (2008): Simultaneous detection of airborne aflatoxin, ochratoxin and zearalenone in a poultry house by immunoaffinity clean-up and high-performance liquid chromatography. Environ Res.; 107(2):139-44.

Yang J, Dong S, Jiang Q, Si Q, Liu X, Yang J. (2013): Characterization and expression of cytoplasmic copper/zinc superoxide dismutase (CuZn SOD) gene under temperature and hydrogen peroxide (H2O2) in rotifer *Brachionus calyciflorus.* Gene. 15; 518(2):388-96.

Yassein SN, Zghair ZR. (2012): Study of toxicity and pathogenicity of aflatoxin B1 and G1 in mice Al Anbar J Vet Sci.; 5: 23–31.