http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 10 (9): 1422-1429, 2007
ISSN 1028-8880

Detection of Aflatoxin among Hepatocellular Carcinoma Patients in Egypt

[1]M. Mokhles, [2]M.A. Abd El Wahhab, [3]M. Tawfik, [1]W. Ezzat, [4]K. Gamil and [2]M. Ibrahim
[1]Department of Internal Medicine National Research Center
[2]Department of Food Toxicology and Contaminants, National Research Center
[3]Department of Tropical Medicine Beni Souif University
[4]National Liver Institute, Menoufia University, Egypt

Abstract: The present research were tempted to investigate whether Aflatoxin is an additive factor in development of HCC through detecting its metabolite Aflatoxin M1 in serum and urine of HCC and cirrhotics in Egypt. Present study comprised (46) Hepatocellular Carcinoma (HCC) patients with mean age (56.28+8.08), 30 males and 16 females, (12) cirrhotic patients with mean age (47.83±18.20), 7 males and 5 females and (12) sex and age matched healthy controls. All were exposed to, liver function tests, abdominal ultrasonography and detection of Aflatoxin metabolite M1 in serum and urine by means of the reverse phase HPLC device. Aflatoxin M1 was detected in sera of HCC group, cirrhotics and controls (57.8%) (5.61±17.21 ng mL^{-1}), (91.7%) (19.23±20.42 ng mL^{-1}) and (50%) (0.66±0.84 ng mL^{-1}), respectively and in urine (41.3%) (3.82±8.03 ng mL^{-1}) (91.7%) (43.22±45.02 ng mL^{-1}) and (50%) (0.98±1.4 ng mL^{-1}), respectively representing significant increase in the serum of the cirrhotic group ($p<0.02$) and a high significant increase in urine of the cirrhotic group ($p<0.0001$). Among HCC group patients, there is high significant value of M1 concentration in urine of upper Egypt residents compared to those of lower Egypt ($p<0.002$). The mean value of Aflatoxin M1 concentration among females of the HCC group was significantly higher than that among males ($p = 0.006$). There is higher statistical significance of aflatoxin prevalence and concentration in serum and urine of cirrhotics than HCC patients and controls and in concentration in urine of HCC patients from upper than lower Egypt.

Key words: Aflatoxin, hepatocellular carcinoma, Egypt

INTRODUCTION

Hepatocellular Carcinoma (HCC) is the fifth most common malignancy in the world (El-Serag, 2002) complicating liver cirrhosis in most cases. Its incidence is increasing worldwide ranging between 3 and 9% annually (Velazquez *et al*., 2003). In Egypt, HCC was reported to account for about 4.7% of Chronic Liver Disease (CLD) patients where its epidemiology of HCC is characterized by marked demographic and geographic variations. (El-Zayady, 2001). Aflatoxin is a common contaminant of foods, particularly in the staple diets of many developing countries. This toxin is produced by fungal action during production, harvest, storage and food processing and it is considered by the US Food and Drug Administration (FDA) to be an unavoidable contaminant of foods. The FDA's goal has been to minimize contamination; this goal was realized by implementing regulations that required special attention to the management of the problem (Rodgers *et al*., 2002). Aflatoxins were first isolated some 40 years ago after outbreaks of disease and death in turkeys and of cancer in rainbow trout fed on rations formulated from peanut and cottonseed meals (Rucker *et al*., 2002). The toxins are produced as secondary metabolites by *Aspergillus flavus* and *Aspergillus parasiticus* fungi when the temperatures are between 24 and 35°C and they will form within many commodities whenever the moisture content exceeds 7% (10% with ventilation). The fungi responsible are ubiquitous and can affect many of the developing-country dietary staples of rice, corn, cassava, nuts, peanuts, chilies and spices (Williams *et al*., 2004). Where several studies showed that Aflatoxin M1 is found to contaminate dairy products (Srivastava *et al*., 2001; Galvano *et al*., 2001; Oruc and Sonal, 2001) Fungal invasion and contamination often begin before harvest and can be promoted by production and harvest conditions. Genotypes (Mehan *et al*., 1986), drought (Sanders *et al*., 1993), soil types (Mehan *et al*., 1991) and insect activity (Lynch and Wilson, 1991) are important in determining the likelihood of preharvest contamination (Cole *et al*., 1995). Timely harvest and

Corresponding Author: Mohamed Mokhles, 45 Zahraa El Helmia Towers, Gesr El Suez,
Postal Code 11321 Cairo, Egypt Tel: +2012-3407520 Fax: +202-4016995

rapid and adequate drying before storage are also important (Mehan *et al*., 1986). Even commodities dried to a satisfactory degree before storage can develop local pockets favorable to aflatoxin growth as a result of moisture generated by insect respiration and local condensation (Mehan *et al*., 1991). The liver is the primary site of biotransformation of ingested AFB_1 where the predominant human cytochrome P450 CYP450 isoforms involved in human metabolism of AFB_1 are CYP 3A4 and CYP 1A2 that catalyze the biotransformation of AFB_1 to the highly reactive *exo*-8, 9-epoxide of AFB_1, CYP 1A2 is also capable of catalyzing the epoxidation of AFB_1 to yield a high proportion of *endo* epoxide and hydroxylation of AFB_1 to form aflatoxin M1 (AFM1) which is a poor substrate for epoxidation (Guengerich *et al*., 1998), less potent than AFB_1 (Wild and Turner, 2002) and is generally considered a detoxification metabolite, while CYP 3A4 can also form AFQ1 a less toxic detoxification metabolite and CYP 3A5 metabolizes AFB_1 mainly to the *exo* epoxide and some AFQ1 (Wang *et al*., 1998). Polymorphism studies with CYP 3A5 reveal that this isoform is not expressed by most people and in particular about 40% of African-Americans do not express this enzyme (Wild *et al*., 1993). Studies with Gambian children reveal that Aflatoxin can cross the placenta and be transported into the new born (Kitada *et al*., 1989). Thus CYP 3A7 a major cytochrome P450 in human fetal liver, has the capacity to activate AFB_1 to the 8, 9-epoxide (Wild *et al*., 1993). Epoxidation of AFB_1 to the exo-8,9-epoxide is a critical step in the genotoxic pathway of this carcinogen. The epoxide is highly unstable and binds with high affinity to guanine bases in DNA to form afltoxin-N^7guanine (Guengerich, 2001). The afltoxin-N^7-guanine has been shown to be capable of forming guanine (purine) to thymine (pyrimidine) transversion mutations in DNA (Bailey *et al*., 1996). As an alternative mechanism to the formation of 8,9epoxide from activation of AFB_1, formation of Reactive Oxygen Species (ROS) has been demonstrated in several models (Yang *et al*., 2000; Lee *et al*., 2005). ROS has been shown to be mutagenic and may thus contribute to the process of cancer formation. HBV has also been shown to generate ROS (Liu *et al*., 1994). Synergistic interaction of both AFB_1 and HBV via ROS formation may be a major mechanism by which they induce HCC. Furthermore, induction of phase 2 detoxification enzymes such as the GST families has been described in AFB_1 and HBV hepatocarcinogenesis (Sun *et al*., 2001). Studies *in vitro* and animal models as well as epidemiological studies have revealed a high incidence of this transversion mutation occurring at codon 249 of the p53 tumor suppressor gene a region corresponding to the DNA binding domain of the corresponding protein (Sudakin, 2003). Thus, it is well established that Exposure to aflatoxin is an additional risk factor for the development of HCC, through damage of DNA in liver cells and mutation in p53 tumor suppressor gene. So, we were tempted to investigate whether Aflatoxin is an additive factor in development of HCC in Egypt through detecting its metabolite Aflatoxin M1 in serum and urine of HCC and cirrhotics to add to the study carried in Assiut University in Egypt by E.F. Abdo (1998) that looked for the Aflatoxin metabolites in viral hepatitis and cirrhotic patients but not in HCC patients and consequently to answer the question should the conditions of storage, harvesting and importing commodities in Egypt be reconsidered.

MATERIALS AND METHODS

After having the Ethical Committee of the National Research Center approval we started recruiting our patients from the Hepatology Outpatient Clinic of the Internal Medicine Department at the National Research Center and the outpatient clinic of Professor Dr. Yaseen Abd El-Ghaffar Charity Center for Liver Diseases and research, throughout the period from December 2004 till August 2005 where after signing the informed consent all the patients were subjected to the following :

Full history taking including past history of schistosomiasis, thorough clinical examination, laboratory investigations; liver function tests (Aspartate Transaminase (AST), Alanine Transaminase (ALT), serum bilirubin (total and direct), serum total proteins, serum albumin, prothrombin concentration, abdominal ultrasonography. Patients showing focal hepatic lesions were subjected to triphasic spiral abdominal Computerized Tomography (CT) and alphafetoprotein (if not previously done) Then, patients were enrolled into the study according to the following inclusion/exclusion criteria:

A-inclusion:

- Age >18 years <80.
- Both sexes.
- Cirrhotic liver disease.
- Malignant focal hepatic lesion

B-exclusion:

- Systemic diseases: Renal, Cardiac, Respiratory …etc.
- Malignancies other than hepatic.
- Hepatic encephalopathy at time of the study.

Finally we were left with (46) patients having Hepatocellular Carcinoma (HCC) with mean age (56.28±8.08) 30 males and 16 females, (12) patients having liver cirrhosis with the mean age of (47.83±18.20) 7 males and 5 females and (12) sex and age matched healthy controls. All patients and controls were subjected to Collection of serum and urine samples to detect aflatoxin M1 according to the following methodology:

PROCEDURES FOR ANALYSIS OF SERUM AND URINE SAMPLES

Extraction of AFM_1 from serum samples was modified from the method of (El-Nezami *et al*., 1995) and extraction of AFM_1 in urine samples was done according to the methods described by Mykkanen *et al.* (2005).

HPLC analysis: Reverse phase HPLC (model LC-10ADvp solvent delivery system; model SIL-10Advp auto injector, Shimadzu, Japan; ODS-5 C18 Brownlee reverse phase column (220×4.6 mm, particle size 5 μm) with a C18 guard column (Perkin Elmer, Norwalk, Conn.) was used to determine the level of AFM_1 in serum and urine extracts. An isocratic system with water: methanol: acetonitrile 66:17:17, flow rate 1 mL min^{-1}, oven temperature 40°C and injection volume of 30 μL were used. Detection was by excitation at 360 nm and emission at 440 nm (Fluorescence-10A XL, Shimadzu detector). The concentrations of AFM_1 in serum and urine were estimated from a standard curve 0.04-10 ng mL^{-1}, prepared from AFM_1 in chloroform (9.93 μg mL^{-1}) reference material RM 423 (LGC Promochem AB, Borås, Sweden). An AFM_1 standard was injected every 10 injections as a quality control, AFM_1 was stored at -20°C in a sylilated vial wrapped in aluminium foil. Since AFM_1 is a possible carcinogen, care was exercised to avoid personal exposure and proper decontamination procedures with 10% sodium hypochlorite were used. HPLC grade methanol, acetonitrile and dichloromethane were purchased from Sigma Chemical Co. (St Louis MO, USA).

RESULTS

The present study which is case control study comprised of 46 patients suffering from Hepatocellular Carcinoma (HCC) twelve patients suffering from liver cirrhosis and 12 normal controls. Demographic data comprising distribution of age, sex, smoking and residence between HCC group and cirrhotics is represented in Table 1. where the mean age of HCC patients (56.28±8.08) was significantly higher than that of cirrhotics (47.83±18.20) (t-value = 2.08 p = 0.04). Distribution of sex among the HCC patients showed that

Table 1: Distribution of age, sex and residence and smoking between HCC group and cirrhhotics

Data		HCC patients (n:46)		Cirrhotics (n:12)	
Age (mean±SD)		56.28 ±8.08		47.83 ±18.20	
	<30	0	0.00%	2	16.70%
	30-50	13	27.40%	2	16.70%
	>50	33	72.20%	8	69.10%
Sex					
	Malen (%)	30	65.20%	7	58.30%
	female n (%)	16	34.70%	5	41.70%
Smoking					
	Yes (%)	11	23.90%	1	8.30%
	Ex (%)	14	30.40%	0	0.00%
	n (%)	21	45.70%	11	91.70%
Residence					
	lower Egypt n (%)	37	80.40%	3	25.00%
	Cairo n (%)	5	10.90%	6	50.00%
	upper Egypt n (%)	4	8.70%	3	25.00%

Table 2: Results of ultrasonographic data of HCC group

		HCC patients	
Data		No. 46	(%) within
FL	RT lobe	4	8.7
	LT lobe	5	10.9
	Both lobes	37	80.4
Cirrhosis			
	Yes	42	91.3
	No	4	8.7
PVT			
	Yes	14	30.4
	No	32	69.6

FL: Focal Lesions, PVT: Portal Vein Thrombosis, RT: Right Lobe, LT: Left Lobe

percentage of males (65.2%) is higher than that of females (34.7%). Then we tabulated the radiological (Ultrasound and spiral CT) data concerning the focal hepatic lesions in Table 2 where we found that, the focal lesions in right lobe is significantly higher than both bilateral multiple focal lesions and focal lesions in left lobe, (Z = 7.2, Z = 7.9, respectively) ($p<0.0001$). The percentage of patients having HCC on top of liver cirrhosis (91.3%) is significantly higher than that of those without liver cirrhosis (8.7%) (Z = 9.4, $p<0.0001$) yet absence of portal vein thrombosis is significantly higher than its presence (Z = 2.88, $p<0.001$). Table 3 shows the classification of patients according to Child Pough scoring system. Among HCC group, the percentage of grade C patients (47.8%) was the highest followed by grade B (34.8%) then grade A (17.4%). However, the only significance was detected between percentages of patients in grades BandA (Z = 1.75, $p<0.05$) and patients in grades C and A (Z = 2.76, $p<0.01$). Among the group of cirrhosis, there were no significant differences as regards Child classification.

The results of *Urinary* and serological detection of aflatoxin M1 within the 3 groups were represented in

Table 3: Classification of patients according to child pough scoring system among the two studied groups of HCC and cirrhotics

	HCC patients		Cirrhotics	
Child pough score	No. 46	(%)	No.12	(%)
A	8	17.4	4	33.3
B	16	34.8	3	25.0
C	22	47.8	5	41.7

Table 4: Values of AFM_1 in serum and urine among the three studied group

	HCC patients	Cirrhotics	Controls
AFM_1 (ng mL^{-1})	No. 46 (%)	No.12 (%)	No. 12 (%)
Serum	5.61±17.21	19.23±20.42	0.66±0.84
Urine	3.82±8.03	43.22±45.02	0.98±1.4

Table 5: Prevalence of AFM_1 in serum among the three studied groups

Presence of AFM_1 in serum	HCC patients No. 46 (%)	Cirrhotics patients No. 12 (%)	Controls No.12(%)
Yes (%)	27 (58.7)	11 (91.7)	6 (50)
No (%)	19 (41.3)	1 (8.3)	6 (50)
Total (%)	46 (100)	12 (100)	12 (100)

Table 6: Prevalence of AFM_1 in urine among the three studied groups

Presence of AFM_1 in urine	HCC patients No. 46 (%)	Cirrhotics patients No. 12 (%)	Controls No.12(%)
Yes (%)	19 (41.3)	11 (91.7)	6 (50)
No (%)	27 (58.7)	1 (8.3)	6 (50)
Total (%)	46 (100)	12 (100)	12 (100)

Table 7: Correlations between mean values of AFM_1 in serum and age, sex, smoking, residence and child score among HCC group

Variables		AFM_1 in serum	p-value
Age			
	<30	11.2±30.7	0.177
	30-50	3.1±6.8	
	>50		
Sex			
	Males	5.7±18.2	0.889
	Females	4.6±5.3	
Smoking			
	No	3.5±5.0	0.666
	Ex	2.6 ±8.0	0.203
	Smokers	13.2 ±32.9	0.254
Residence			
	Lower Egypt	5.4±17.7	0.726
	Upper Egypt	9.0 ±7.8	
Child			
	A	2.4±5.0	0.442
	B	10.9±27.9	0.897
	C	2.7±4.8	0.388

Table 4 that revealed a significant increase in the mean value of aflatoxin M1 in the serum of cirrhotic patients (19.23±20.42) in comparison with HCC group (5.6±17.21) and controls (0.66±0.84) ($p<0.02$). Also, the mean value of aflatoxin M1 in the urine among the cirrhotic group (43.22±45.02) was significantly higher than that among the HCC group (3.82±8.03) and controls (0.98±1.4) ($p<0.0001$) Table 5 and 6 showed that the prevalence of Aflatoxin M1 in serum and urine among the cirrhotic group was significantly higher than that of the HCC group and than that of the control group. Then we focused on the HCC

Table 8: Correlations between mean values of AFM_1 in urine and age, sex, smoking, residence and child score among HCC group

Variables		AFM_1 in urine	p-value
Age			
	<30		0.922
	30-50	3.7±6.5	
	>50	3.4±8.1	
Sex			
	Males	2.7±5.7	0.006**
	Females	12.9±16.9	
Smoking			
	No	5.9±9.7	0.046
	Ex	0.4±1.1	0.622
	Smokers	4.1±8.7	0.128
Residence			
	Lower Egypt	2.7±6.8	0.002**
	Upper Egypt	15.3±11.8	
Child			
	A	6.2±8.6	0.506
	B	2.8±6.2	0.726
	C	3.7±9.1	0.521

** Highly significant values

Table 9: Cut-off level of aflatoxin in urine of HCC and cirrhotic patients

Cut of level of AFM_1 in urine		Sensitivity (%)	Specificity (%)	% of agreement
HCC	34	97.8	50	87.7
Cirrhosis	2	83.3	83.3	83.3

group searching for a correlation between Aflatoxin M1 mean levels (urine and serum) and age, sex smoking, laboratory investigations, ultrasonographic data and Child's scores Table 7 and 8. where we found that the mean value of aflatoxin in urine among upper Egypt residents was significantly higher than its value among lower Egypt and Cairo residents ($t = 4.36$, $p = 0.002$), the mean value of aflatoxin in urine among females was significantly higher than its value among males ($t = 2.89$, $p = 0.006$).

Table 9 showed that at a cut off level of 2 ng mL^{-1} of Aflatoxin in urine there is a 83.3% sensitivity and 83.3% specificity for presence of cirrhosis while at a cut off level of 34 ng mL^{-1} there is a 97.8% sensitivity and 50% specificity for presence of HCC.

DISCUSSION

Exposures to aflatoxin clearly have a major toxicological effect and may cause a range of consequences: 1) large doses lead to acute illness and death, usually through liver cirrhosis; 2) chronic sublethal doses have nutritional and immunologic consequences; and 3) all doses have a cumulative effect on the risk of cancer. (Cullen and Newberne, 1993). In addition, it was reported that Gambian children and adolescents chronically infected with HBV have higher concentration of AFB_1 adducts than uninfected individuals (Chen *et al*., 2001; Turner *et al*., 2000). The currently favored method of measuring human exposure consists of the analysis of body fluids for the presence of aflatoxin derivatives (Groopman, 1993) Each biochemical process

results in derivatives that have a characteristic half-life within the body and thus the exposure over a period of days, weeks, or months can be assessed. Recent exposure to aflatoxin is reflected in the urine as directly excreted AFM_1 and other detoxification products, but only a small fraction of the dose is excreted in this way. Measurements of aflatoxin and its byproducts in urine have been found to be highly variable from day to day, which reflects the wide variability in the contamination of food samples (Makarananda *et al*., 1998; Wild *et al*., 1998. We found a highest significant increase in urinary excretion of aflatoxin M1 among cirrhotic patients in comparison with HCC group and controls ($p<0.0001$) and significant increase also of serological level of aflatoxin among cirrhotic patients, ($p<0.02$). Those results match with those of a previous study done in Assuit university, where one hundred and three samples of serum as well as 24 h collected urine samples were collected from 49 HbsAg and HCV Ab seropositive cases (first group) 35 liver cirrhosis patients (second group) and 19 healthy persons (third group). Samples were analyzed for detection of aflatoxin B1 in serum and aflatoxins B1 and M1 in urine. The mean values of aflatoxin B1 and aflatoxin M1 in urine were significantly higher in liver cirrhosis patients (0.2±4.99 and 2.5±0.4 ng mL^{-1}, respectively) than in seropositive cases (0.2±4.08 ng mL^{-1} and 0.5±0.5 ng mL^{-1}, respectively) and control healthy group (0.06±0.04 ng mL^{-1} and 0.3±0.01 ng mL^{-1}, respectively). While there were no statistically significant difference between mean values of aflatoxins B1 and M1 in urine in seropositive cases and control healthy group (Abdo, 1998) Aflatoxin B1 has been linked to hepatocellular carcinoma from previous studies reported in the medical literature as follows: An increased incident (10% excess) of hepatocellular carcinoma was reported in south-eastern portion the U.S. in areas of high daily intake of aflatoxin B1. The daily intake of B1 in the south-eastern subjects was 13-197 ug kg body weight as compared to those in northern and western areas with a daily intake of 0.2-0.3 ug kg body weight and in China a strong correlation between the intake of peanut, peanut oil and corn and increased mortality rates for liver cancer were reported in five groups of inhabitants from four villages where the mortality rates were 125, 97.40, 41.65, 24.01 and 1.05, respectively. The median intake of aflatoxin B1 for each group was 6.05, 6.36, 2.69, 1.83 and 0 ug day. The median daily urine concentrations of M1 metabolite were 16.46, 8, 29, 4.78 and 1.21 ng person (Parkin *et al*., 1998). Where in our study the concentration of M1 in serum and urine among the HCC group was higher than that among the control group although it did not reach statistical significance, this could be explained by the fact that our study was a case control study while the Chinese study correlated HCC to the amount of Aflatoxin intake and the American one compared the rate of HCC in two different geographical areas. When comparing the 3 groups as regards the prevalence of Aflatoxin we reached the same results where the highest significance was in the cirrhotic group and although the prevalence of Aflatoxin presence among the HCC group was higher than that of the control group, yet this did not reach statistical significance which could be explained by the conclusion reached by Qian *et al*. (1994) that stated that not all individuals who consume AFB_1-contaminated foodstuff have the same risk of HCC where studies have reported a significant association between AFB_1 markers in biosamples and HCC, the same studies have failed to demonstrate a correlation between AFB_1 in foodstuffs and AFB_1 biomarkers suggesting that differences in an individual's abilities to metabolize AFB_1 may be related to HCC susceptibility Correlating Aflatoxin levels in serum and urine of the HCC group to different factors as age,sex, smoking residence we found that levels of Aflatoxin in serum and blood of inhabitants of upper Egypt was significantly higher than that of inhabitants of lower Egypt which could be attributed to the fact that at temperatures between 24 and 35°C and when the moisture content exceeds 7% (10% with ventilation) aflatoxins will grow within many commodities (Williams *et al*., 2004). Factors fundamental to a nation's ability to protect its population from aflatoxin include the following. First, a nation must have the political will to address the issue of aflatoxin exposure. Most nations are signatories to Codex Alementarius (WHO/FAO documents that deal with food quality in traded commodities) and subscribe to the need to limit exposure of their populations to aflatoxins (Jonathan, 2004). The traditional approach to preventing exposure to aflatoxin has been to ensure that foods consumed have the lowest practical aflatoxin concentrations. In developed countries, this has been achieved for humans largely by regulations that have required low concentrations of the toxin in traded foods (Galvano *et al*., 1993). Where several countries have put a minimum permissible level for food contamination by Aflatoxin, as in Kuwait where it is 0.2 μg mL^{-1} for M1 in dairy products. (Srivastava *et al*., 2001) and in Brazil it is 0.5 μ g mL^{-1} limit (Sassahara *et al*., 2005) and Turkish tolerance limit of 50 ng L^{-1} (Oruc and Sonal, 2001). Chemoprotection against aflatoxins has been demonstrated with the use of a number of compounds that either increase an animal's detoxification processes (Hayes *et al*., 1998; Abd El Aziz *et al*., 2005; Abd El Wahhab *et al*., 2005; Abd El Wahhab and Aly, 2005; Abd El Wahhab *et al*., 2006) or prevent the

production of the epoxide that leads to chromosomal damage (Kensler *et al*., 1993; Abd El Wahhab *et al*., 1998). One technical solution is drug therapy, because several compounds, such as oltipraz and chlorophyll, are able to decrease the biologically effective dose. Enterosorption: Another approach has followed the discovery that certain clay minerals can selectively adsorb aflatoxin tightly enough to prevent their absorption from the gastrointestinal tract (Mayura *et al*., 1998; Abd El Wahhab *et al*., 1999). Whereas many toxins are adsorbed to surface-active compounds, such as activated charcoal, the bonding is not often effective in preventing uptake from the digestive system. Thus, we concluded from our study, that Aflatoxin M1 is prevalent among cirrhotic patients in Egypt and showed highest concentration in the urine and serum among the same group of patients compared to HCC patients and controls. Also, the prevalence and concentration of Aflatoxin M1 in the serum and urine of the HCC group was higher than that among the control group, yet, this was not of statistical significance. Also the concentration of Aflatoxin M1 in serum and urine of HCC patients from upper Egypt was significantly higher than that of lower Egypt.

So, we recommend performing studies on the Egyptian commodities both local and imported for evaluating their contamination status and consequently setting strict rules for storage conditions. Also, it is of extreme importance to put a permissible level for Aflatoxin existence in dietary products in Egypt. Finally, we strongly recommend extending this study on a larger scale of HCC patients representing all regions of Egypt, on both clinical and molecular levels to determine the role of Aflatoxin in the pathogenesis of the disease.

REFERENCES

Abdel-Aziz, M.T., H. Salamas, M. Abdel-Aziz, H.H. Fouad, L.A. Rashed, S. Abd-Alla, M.A. Abdel-Wahhab and T. Ahmed, 2005. Interferon-α gene therapy prevents aflatoxin and carbon tetrachloride promoted hepatic carcinogenesis in rats. Intl. J. Mol. Med., 15: 21-26.

Abdel-Wahhab, M.A., S.A. Nada, I.M. Farag, N.F. Abbas and H.A. Amra, 1998. Potential protective effect of hscas and bentonite against dietary aflatoxicosis in rat: With special reference to chromosomal aberrations. Nat. Toxins, 6: 211-218.

Abdel-Wahhab, M.A., S.A. Nada and H.A. Amra, 1999. Effect of aluminosilicates and bentonite on aflatoxin-induced developmental toxicity in the rats. J. Applied Toxicol., 19: 199-204.

Abdel-Wahhab, M.A. and S.E. Aly, 2005. Antioxidant Property of *Nagilia sativa* (Black cumin) and *Syzygium aromaticum* (Clove) in rats during Aflatoxicosis. J. Applied Toxicol., 25: 218-223.

Abdel-Wahhab, M.A., A. Saeed and A. Hufner, 2005. NMR and radical scavenging activities of patuletin from *Urtica urens* L. against aflatoxin B_1. Pharmaceutical Biol., 43: 515-525.

Abdel-Wahhab, M.A., H.H. Ahmed and M.M. Hagazi, 2006. Prevention of aflatoxin B_1-initiated hepatotoxicity in rat by marine algae extracts. J. Applied Toxicol., 26: 229-238.

Abdo, E.F., 1998. Aflatoxins in the Serum and Urine of HbsAg Sid and HCV Seropositive Cases. Assiut University.

Bailey, E.A., R.S. Iyer, M.P. Stone, T.M. Harris and J.M. Essigmann, 1996. Mutational properties of the primary aflatoxin B1-DNA adduct. Proc. Natl. Acad. Sci. USA., 93: 1535-1539.

Chen, S.Y., C.J. Chen, S.R. Chou, L.L. Hsieh, L.Y. Wang, W.Y. Tsai, H. Ahsan and R.M. Santella, 2001. Association of aflatoxin B(1)-albumin adduct levels with hepatitis B surface antigen status among adolescents in Taiwan. Cancer Epidemiol Biomarkers Prev., 10: 1223-1226.

Cole, R.J., J.W. Dorner and C.C. Holbrook, 1995. Advances in Mycotoxin Elimination and Resistance. In: Advances in Peanut Science. Stillwater, Pattee, H.E. and H.T. Stalker (Eds.). Ok: American Peanut Research and Education Society, Inc., pp: 456-474.

Cullen, J.M. and P.M. Newberne, 1993. Acute Hepatotoxicity of Aflatoxins. In: The Toxicology of Aflatoxins: Human Health, Veterinary and Agricultural Significance. Eaton, D.L. and J.D. Groopman (Eds.). London: Academic Press., pp: 1-26.

El-Nezami, H.S., G. Nicoletti, G.E. Neal, D.C. Donohue and J.T. Ahokas, 1995. Aflatoxin M1 in human breast milk samples from Victoria, Australia and Thailand. Food Chem. Toxicol., 33: 173-179.

El-Serag, H.B., 2002. Hepatocellular carcinoma: An epidemiologic view. J. Clin. Gastroenterol., 35(Suppl 2): S72-78.

El-Zayadi, A., H. Abaza, S. Shawky, M.K. Mohamed, O.E. Selim and H.M. Badran, 2001. Prevalence and epidemiological features of hepatocellular carcinoma in Egypt-a single center experience. Hepatol. Res., 19: 70-179.

Galvano, F., A. Piva, A. Ritieni and G. Galvano, 2001. Dietary strategies to counteract the effects of mycotoxins: A review. J. Food. Prot., 64: 120-131.

Groopman, J.D., 1993. Molecular Dosimetry Methods for Assessing Human Aflatoxin Exposures. In: The Toxicology of Aflatoxins: Human Health, Veterinary and Agricultural Significance. Eaton, D.L. and J.D. Groopman (Eds.). London: Academic Press, pp: 259-279.

Guengerich, F.P., W.W. Johnson, T. Shimada, Y.F. Ueng, H. Yamazaki and S. Langouet, 1998. Activation and detoxication of aflatoxin B1. Mutat. Res., 402: 121-128.

Guengerich, F.P., 2001. Forging the links between metabolism and carcinogenesis. Mutat. Res., 488: 195-209.

Hayes, J.D., D.J. Pulford and E.M. Ellis, 1998. Regulation of rat glutathione S-transferase A5 by cancer chemopreventive agents: Mechanisms of inducible resistance to aflatoxin B1. Chem. Biol. Interact., 111-112: 51-67.

Jonathan, H.W., D.P. Timothy, E.J. Pauline, K.S. Jonathan, M.J. Curtis and A. Deepak, 2004. Human aflatoxicosis in developing countries: A review of toxicology, exposure, potential health consequences and interventions American J. Clin. Nutr., 80: 1106-1122.

Kensler, T.W., E.F. Davis and M.G. Bolton, 1993. Strategies for Chemoprotection Against Aflatoxin-Induced Liver cancer. In: The Toxicology of Aflatoxins: Human Health, Veterinary and Agricultural Significance. Eaton, D. and J.D. Groopman (Eds.), London: Academic Press, pp: 281-306.

Kitada, M., M. Taneda, H. Ohi, M. Komori, K. Itahashi, M. Nagao and T. Kamataki, 1989. Mutagenic activation of aflatoxin B1 by P-450 HFLa in human fetal livers. Mutat. Res., 227: 53-58.

Lee, J.K., E.H. Choi, K.G. Lee and H.S. Chun, 2005. Alleviation of aflatoxin B1 induced oxidative stress in HepG2 cells by volatile extract from Allii Fistulosi Bulbus. Life Sci., 77: 2896-2910.

Liu, R.H., J.R. Jacob, J.H. Hotchkiss, P.J. Cote, J.L. Gerin and B.C. Tennant, 1994. Woodchuck hepatitis virus surface antigen induces nitric oxide synthesis in hepatocytes: Possible role in hepatocarcinogenesis. Carcinogenesis, 15: 2875-2877.

Lynch, R.E. and D.M. Wilson, 1991. Enhanced infection of peanut, *Arachis hypogaea* L. seeds with *Aspergillus flavus* group fungi due to external scarification of peanut pods by the lesser cornstalk borer, *Elasmopalpus lignosellus* (Zeller). Peanut Sci., 18: 110-116.

Makarananda, K., U. Pengpan, M. Srisakulthong, K. Yoovathaworn and K. Sriwatanakul, 1998. Monitoring of aflatoxin exposure by biomarkers. J. Toxicol. Sci., 23: 155-159.

Mayura, K., M.A. Abdel-Wahhab and K. McKenzie, 1998. Prevention of maternal and developmental toxicity in rats via dietary inclusion of aflatoxin sorbents: Hidden risks. Toxicol. Sci., 41: 175-182.

Mehan, V.K., D. McDonald, N. Ramakriahna and J.H. Williams, 1986. Effects of genotype and date of harvest on infection of peanut seed by *Aspergillus flavus* and subsequent contamination with aflatoxin. Peanut Sci., 13: 46-50.

Mehan, V.K., C.D. Mayee, S. Jayanthi and D. McDonald, 1991. Preharvest seed infection by *Aspergillus flavus* group fungi and subsequent aflatoxin contamination in groundnuts in relation to soil types. Plant Soil., 136: 239-248.

Mykkanen, H., H. Zhu, E. Salminen, R.O. Juvonen, W. Ling, J. Ma, N. Polychronaki, H. Emilainen, O. Mykkanen, S. Salminen and H. El-Nezami, 2005. Fecal and urinary excretion of aflatoxin B1 metabolites (AFQ1, AFM1 and AFB-N7-guanine) in young Chinese males. Intl. J. Cancer, 115: 879-884.

Oruc, H.H. and S. Sonal, 2001. Determination of aflatoxin M1 levels in cheese and milk consumed in Bursa, Turkey. Vet. Hum. Toxicol., 43: 292-293.

Parkin, D., S. Whelan, J. Ferlay and I. Raymond 1998. Cancer Incidence in Five Continents. Iarc Scientific Publications, Oxford University Press Oxford, 7: 322-325.

Qian, G.S., R.K. Ross, M.C. Yu, J.M. Yuan, Y.T. Gao, B.E. Henderson, G.N. Wogan and J.D. Groopman, 1994. A follow-up study of urinary markers of aflatoxin exposure and liver cancer risk in Shanghai, People's Republic of China. Cancer Epidemiol. Biomark. Prev., 3: 3-10.

Rodgers, A., P. Vaughan, T. Prentice, T.T. Edejer, D. Evans and J. Lowe, 2002. Reducing Risks, Promoting Healthy Life. In: The World Health Report. Campanini, B. and A. Haden (Eds.). Geneva: World Health Organization, 2002.

Rucker, R.R., W.T. Yasutake and H. Wolf, 2002. Trout hepatoma-a preliminary report. Prog Fish Cult., 23: 3-7.

Sassahara, M., D. Pontes Netfto and E.K. Yanaka, 2005. Aflatoxin occurrence in foodstuff supplied to dairy cattle and aflatoxin M1 in raw milk in the North of Parana state. Food Chem. Toxicol., 43: 981-984.

Sanders, T.H., R.J. Cole, P.D. Blankenship and J.W. Dorner, 1993. Aflatoxin contamination of peanuts from plants drought stressed in pod or root zones. Peanut Sci., 20: 5-8.

Srivastava, V.P., A. Bu-Abbas, Alaa-Basuny, W. Al-Johar, S. Al-Mufti and M.K. Siddiqui, 2001. Aflatoxin M1 contamination in commercial samples of milk and dairy products in Kuwait Food Addit Contam 18: 993-997.

Sudakin, D.L., 2003. Dietary aflatoxin exposure and chemoprevention of cancer: Clinical review. J. Toxicol. Clin. Toxicol., 41: 195-204.

Sun, C.A., L.Y. Wang, C.J. Chen, S.N. Lu, S.L. You, L.W. Wang, Q. Wang, D.M. Wu and R.M. Santella, 2001. Genetic polymorphisms of glutathione Stransferases M1 and T1 associated with susceptibility to aflatoxinrelated hepatocarcinogenesis among chronic hepatitis B carriers: A nested case-control study in Taiwan. Carcinogenesis, 22: 1289-1294.

Turner, P.C., M. Mendy, H. Whittle, M. Fortuin, A.J. Hall and C.P. Wild, 2000. Hepatitis B infection and aflatoxin biomarker levels in Gambian children. Trop. Med. Intl. Health, 5: 837-841.

Velazquez, R.E., M. Rodriguez, C.A. Navascues, A. Linares, R. Perez, N.G. Sotorrios, I. Martinez and L. Rodrigo, 2003. Prospective analysis of risk factors for hepatocellular carcinoma in patients with liver cirrhosis. Hepatology, 37: 520-527.

Wang, H., R. Dick, H. Yin, E. Licad-Coles, D.L. Kroetz, G. Szklarz, G. Harlow, J.R. Halpert and M.A. Correia, 1998. Structure-function relationships of human liver cytochromes P450 3A: Aflatoxin B1 metabolism as a probe. Biochemistry, 37: 12536-12545.

Wild, C.P., M. Fortuin, F. Donato, H.C. Whittle, A.J. Hall, C.R. Wolf and R. Montesano, 1993. Aflatoxin, liver enzymes and hepatitis B virus infection in Gambian children. Cancer Epidemiol Biomarkers Prev., 2: 555-561.

Wild, C.P. and P. Pisani, 1998. Carcinogen DNA and protein adducts as biomarkers of human exposure in environmental cancer epidemiology. Cancer Detect Prev., 22: 273-283.

Wild, C.P. and P.C. Turner, 2002. The toxicology of aflatoxins as a basis for public health decisions. Mutagenesis, M17: 471-481.

Williams, J.H., T.D. Phillips, P.E. Jolly, J.K. Stiles, C.M. Jolly and D. Aggarwal, 2004. Human aflatoxicosis in developing countries: A review of toxicology, exposure, potential health consequences and interventions. Am. J. Clin. Nutr., 80: 1106-1122.

Yang, C.F., J. Liu, S. Wasser, H.M. Shen, C.E. Tan and C.N. Ong, 2000. Inhibition of ebselen on aflatoxin B-induced hepatocarcinogenesis in Fischer 344 rats. Carcinogenesis, 21: 2237-2243.