

## **IS AFLATOXIN B<sub>1</sub> A COMMON RISK FACTOR FOR HEPATOCELLULAR CARCINOMA?**

*BY*

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### **ABSTRACT**

*The burden of hepatocellular carcinoma (HCC) has been increasing in Egypt with a doubling in the incidence rate in the past 10 years. This has been attributed to several factors. The aim of this study was to evaluate aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) in serum of HCC patients as a risk factor compared to their relatives. AFB<sub>1</sub> was determined by a quantitative thin layer chromatography. Results of this study showed that HCC was highly correlated to viral hepatitis C (HCV), viral hepatitis B (HBV) and anti-bilharzial antibodies. However, all patients and their relatives, unexpectedly, showed negative AFB<sub>1</sub> in their blood. It can be concluded that AFB<sub>1</sub> is not necessarily present in all cases of HCC as a predisposing factor and that other factors as viral hepatitis and bilharziasis are more commonly correlated to HCC.*

**Key words:** *Aflatoxin B<sub>1</sub> - Hepatocellular carcinoma - HCV- HBV.*

### **INTRODUCTION**

The use of biomarkers in molecular epidemiology studies for identifying the progression of health effects of environmental agents provides important information. Investigations of aflatoxins probably represent one of the most extensive data sets in this field (Groopman and Kensler, 2005).

Aflatoxins are naturally occurring my-

cotoxins found in different foods and feed stuffs (Abdelhamid et al., 1996) in endemic status (Abdelhamid and Saleh, 1996) and they have been demonstrated to be carcinogenic in many experimental models (Kumar et al., 2008). As a result of nearly 30 years of study, experimental data and epidemiological studies in human populations, aflatoxin B<sub>1</sub> (AFB<sub>1</sub>), the most prevalent form (Cullen and Newberne, 1993) was classified as carcinogenic to humans by the International

Agency for Research on Cancer (Williams et al., 2004).

AFB<sub>1</sub> is metabolized to exo-8, 9-epoxide by cytochrome P450, and the metabolite reacts with the guanine residue to form the aflatoxin-N7-guanine adducts (AFB<sub>1</sub>-N7-guanine), resulting in a guanine cytosine (GC) to thiamine adenine (TA) transversion (Gallagher et al., 1994). Clinical studies have shown that AFB<sub>1</sub> selectively targets at the third base position of codon 249 of the human p53 gene, a known mutational hotspot in human hepatocellular carcinoma (HCC) (Ozturk, 1991). A significant association between aflatoxin exposure and HCC has been reported in hyperendemic areas (Qian et al., 1994). A synergistic interaction between AFB<sub>1</sub> exposure and viral hepatitis B (HBV) infection on HCC risk has been reported in several epidemiologic studies (Sun et al., 1999).

Aflatoxin exposure may be associated with advanced liver disease in chronic hepatitis C (HCV) patients. Levels of AFB<sub>1</sub>-albumin/albumin were significantly related to ultrasonographic hepatic parenchyma scores in anti-HCV-positive subjects (Chen et al., 2007).

Liver cancer is one of the most frequent solid cancers that kill more than 650,000 people around the world each year (Mazzanti et al., 2008). So, understanding the

pattern of the possible risk factors implicated in the development of HCC in Egypt was the aim of this research.

## ***SUBJECTS AND METHODS***

### **Study design and sampling:**

This study was applied on 46 hepatocellular carcinoma (HCC) patients from Mansoura Gastroenterology Center from January 2007 till January 2008. Diagnosis of HCC based on histopathological examination and/or detection of hepatic lesion(s) by imaging (ultrasound, CT scan, MRI) plus  $\alpha$ -feto-protein above 200 ng/ml. Data were collected from their files (age, gender, occupation, residence, serological tests for HCV, HBV and bilharziasis and liver function tests). Cigarette smokers were excluded. All individuals in every patient house- who share the same quality of life - were invited to share in the study, as a control group (50 individuals). Blood samples were collected, after their consent, for detection of AFB<sub>1</sub> using quantitative thin layer chromatography (TLC).

### **Method :**

Five ml blood samples were drawn from each participant, all samples were kept frozen until analysis. The quantitative determination of AFB<sub>1</sub> by TLC was carried out according to the method of Eppley (1968), modified by Abdelhamid (1981). The laboratory studies were undertaken in the mycotoxin laboratory of Prof.

Dr. Abdelhamid, A. M., Faculty of Agriculture, Mansoura University. All chemicals and solutions used were purchased from United Co. for Chemical and Medical Preparations, Cairo, Egypt. Mycotoxin standard used was from Makor Chemicals Co., Jerusalem. The detection level is 0.5 ppb.

Blood was extracted with chloroform in a separate funnel, filtrated on anhydrous sodium sulfate and then the filtrate was evaporated under vacuum at 45°C till dryness using electric vacuum evaporator from Sartorius, Switzerland. The residues were dissolved in 100µl chloroform for spotting on a TLC plate against external standard. The plate was developed in toluene/acetic acid/formic acid (6/3/1, v/v/v) for 45 min, air dried, sprayed with sulfuric methanol and observed under UV lamp at 366 nm for qualitative determination. For the quantitative estimation, the spots of the samples as well as of the standard were scratched separately, dissolved in similar volumes of chloroform on filter papers onto test tubes and then the optical density (OD) was measured spectrophotometrically for all extracts using the following equation:

The concentration of AFB<sub>1</sub> (ppb) in a sample = OD of the standard X ppb of the standard / OD of a sample.

#### **Statistical analysis:**

Data were compared by using Chi

square (X<sup>2</sup>) for qualitative data (frequency and proportion). These data were run on an IBM compatible personal computer by using Statistical Package for Social Scientists (SPSS) for windows 11 (SPSS Inc., Chicago, IL, USA).

### **RESULTS**

Forty six HCC patients aged 45.1 ± 11.69 y shared in this study. Males represented 78.3% while females were 21.7 %. As shown in table (1), the number of males was significantly higher than females. Urban residents and employees were significantly higher than rural residents and farmers. The number of patients with right lobe HCC was insignificantly higher than left lobe HCC patients. The number of patients with positive HCV was insignificantly higher than patients with negative HCV while that of patients positive for HBV was significantly higher than those negative for HBV. The number of patients positive for bilharziasis antibodies was significantly lower than patients negative for it.

Table (2) shows that the number of patients positive for HCV, HBV and bilharziasis was higher in males than females. Patients positive for HCV and HBV were more in urban than rural residents and in employees more than farmers while it did not differ in bilharziasis. Patients with

right lobe HCC were more in patients positive for HBV (18/46) than patients positive for HCV (12/46) and least in patients positive for bilharziasis (4/46). Meanwhile, patients with left lobe HCC were more in patients positive for HCV and HBV (18/46 each) than bilharziasis (4/46). About 65.2 % (30/46) were positive for both HCV and HBV while only 13 % (6/46) were positive for both bilharziasis and HCV and 13 % (6/46) were positive for both bilharziasis and HBV. All HCC patients and their relatives were negative for AFB<sub>1</sub>.

### **DISCUSSION**

Hepatocellular carcinoma (HCC) was reported to account for about 4.7% of chronic liver disease patients (El-Zayadi et al., 2001). The burden of HCC has been increasing in Egypt with a doubling in the incidence rate in the past 10 years. This has been attributed to several biological (e.g. hepatitis B and C virus infection) and environmental pollutants e.g. aflatoxins, pesticide residues and heavy metals (Anwar et al., 2008). A meta-analysis study showed that, the mean proportion of HCC with the 249ser mutation was positively correlated with aflatoxin exposure (Stern et al., 2001).

The present research aimed at evaluating AFB<sub>1</sub> as risk factor for HCC compared to other risk factors in Egypt.

In the studied group, HCC was more prevalent in males, urban residents and employees. Prevalence in males was evidenced in Egyptians (Abdel-Wahab et al., 2007) and others (Chen et al., 2007 and Stern et al., 2001). However, Abdel-Wahab et al. (2007) found that the prevalence of HCC was more common in rural residents and farmers especially in HCV patients. This may be explained by different inclusion and exclusion criteria.

The present study showed that HCC was more correlated to HBV and HCV than bilharziasis while it was not correlated to AFB<sub>1</sub>. Clinico-epidemiological evidence suggests that hepatitis B virus (HBV) infection is the most important cause of HCC in the world (Robinson, 1994). HBV infection and schistosomiasis are among the most common causes of liver HCC in Egypt (Badawi and Michael, 1999). In Egypt, HBV conferred a higher risk to develop HCC more than HCV (El-Zayadi et al., 2005). HCV mostly plays an indirect role in tumor development by promoting fibrosis and cirrhosis. On the other hand, HCV may play a direct role in hepatic carcinogenesis through involvement of viral gene products in inducing liver cell proliferation (El-Nady et al., 2003). However, it seems that cirrhosis is the common pathway by which several risk factors exert their carcinogenic effect (Fattovich, 1998).

The present study showed that quantitative TLC determination of AFB<sub>1</sub> in sera of patients and their relatives was surprisingly negative. In the studied group, HCC was more common in employees than farmers and in urban residents more than rural. It must be noted that aflatoxins exposure is more common in rural areas and in farmers (Abdelhamid et al., 1999). There are risk factors for HCC in urban areas more commonly found, namely viral hepatitis. The patients included in this study had  $\alpha$  feto-protein level above 200 ng/ml, which represents about 15.6% - 28.9% of HCC Egyptian patients (El-Zayadi et al., 2005).

Although there is strong evidence for an association between dietary aflatoxin exposure and HCC incidence seen in many case-control studies, there have been several investigations that do not support this positive association. Cross-sectional studies revealed a non-association between aflatoxin metabolites in human urine and aflatoxin-albumin adducts in serum and HCC disease rates, while HBsAg status was positively correlated (Campbell et al., 1990 and Srivatana-kul et al., 1991).

This negative aflatoxin in the studied group may be an indicator of a chronic aflatoxicosis, through which the toxin could be deposited in its target organ (in the case of AFB<sub>1</sub> is the liver) and not in the blood

pool as in the acute toxicoses. These negative results does not exclude the role of aflatoxin as a contributing factor for HCC as the cumulative effect of feeding low levels of mycotoxins may contribute to a gradual deterioration of liver functions (Abdelhamid, 2008).

Susceptibility to aflatoxin is greatest in the young and there are very significant differences between species, persons of the same species (according to their differing abilities to detoxify aflatoxin by biochemical processes), and the sexes (according to the concentrations of testosterone) as mentioned in Abdelhamid (2000 and 2005). The toxicity of aflatoxin also varies according to many nutritional factors (Pier et al., 1985). A previous study showed that the serum level of AFB<sub>1</sub> was highly significant in HCC patients compared with control (Abdel-Wahab et al., 2008). That study showed that HCC was higher in Kafer El-Sheikh governorate that is another governorate than Dakahlia governorate, where the present study was performed where nutritional habits differ and concentration of aflatoxins in food stuffs also may differ.

It can be concluded that although AFB<sub>1</sub> is a known hepatic carcinogen, it was not detected in sera of some HCC patients that were included in this study and that other risk factors as HBV, HCV and bilharziasis are more correlated to HCC.

**Table (1):** The percentage and significance of personal data.

<b>Criteria</b>	<b>Variables</b>		<b>Significance</b>
<b>Sex</b>	<b>Male</b>	<b>Female</b>	$X^2 = 7.348$ $p = 0.007 *$
	36 (78.3%)	10 (21.7%)	
<b>Residence</b>	<b>Urban</b>	<b>Rural</b>	$X^2 = 5.261$ $P = 0.022*$
	34 (73.9%)	12 (26.1%)	
<b>Occupation</b>	<b>Employee</b>	<b>Farmer</b>	$X^2 = 5.261$ $P = 0.022*$
	34 (73.9%)	12 (26.1%)	
<b>Site of hepatic carcinoma</b>	<b>Right lobe</b>	<b>Left lobe</b>	$X^2 = 1.087$ $P = 0.297$
	28 (60.9%)	18 (39.1%)	
<b>HCV</b>	<b>Positive</b>	<b>Negative</b>	$X^2 = 2.130$ $P = 0.144$
	30 (65.2%)	16 (34.8%)	
<b>HBV</b>	<b>Positive</b>	<b>Negative</b>	$X^2 = 7.348$ $P = 0.007*$
	36 (78.3%)	10 (21.7%)	
<b>Anti-Bilharzial Ab.</b>	<b>Positive</b>	<b>Negative</b>	$X^2 = 9.783$ $P = 0.002*$
	8 (17.4%)	38 (82.6%)	

 $X^2$  : chi square.\*P : significant when  $< 0.05$ .

**Table (2):** Cross-tabulation of cases.

Items		HCV		HBV		Anti Bilharzial Ab	
		Positive	Negative	Positive	Negative	Positive	Negative
Sex	Male	20	16	26	10	6	30
	Female	10	0	10	0	2	8
Residence	Urban	22	12	28	6	4	30
	Rural	8	4	8	4	4	8
Occupation:	Employee	22	12	28	6	4	30
	Farmer	8	4	8	4	4	8
Site of HCC	Rt	12	16	18	10	4	24
	Lt	18	0	18	0	4	14
HCV	Positive	30	0	30	0	6	24
	Negative	0	16	6	10	2	14
HBV	Positive	30	6	36	0	6	30
	Negative	0	10	0	10	2	8
Anti Bilharzial Ab	Positive	6	2	6	2	8	0
	Negative	24	14	30	8	0	38

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## هل الأفلاتوكسين ب ١ عامل شائع لحدوث ورم الكبد الخبيث ؟

المشتركون فى البحث

- |                              |                                |
|------------------------------|--------------------------------|
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إن الانتشار الواسع لأورام الكبد الخبيثة خلال العشر سنوات الأخيرة قد أعزى لعدة أسباب، منها الالتهاب الكبدى الوبائى ب و س والإصابة بالبلهارسيا وبعض السموم مثل المبيدات الحشرية والسموم الفطرية، ومن ثم كان الهدف من هذا البحث هو بحث مدى علاقة التسمم بالأفلاتوكسين ب ١ بإصابة بأورام الكبد الخبيثة فى مصر.

لقد أجرى هذا البحث على ٤٦ من مرضى أورام الكبد الخبيثة من خلال مركز جراحة الجهاز الهضمى بالمنصورة من يناير ٢٠٠٧ حتى يناير ٢٠٠٨، و ٥٠ من أقاربهم الذين يشاركونهم نفس الأحوال المعيشية والذين لا يعانون من أى أمراض بالكبد، ولقد تم فحص ملفاتهم للحصول على المعلومات الديموجرافية ونتائج التحاليل التى أجريت لهم وكذلك تم سحب عينات من دمائهم ودماء أقاربهم والكشف عن وجود الأفلاتوكسين ب ١ بها.

وقد أثبت هذا البحث أن أورام الكبد الخبيثة أكثر إنتشاراً فى الذكور عن الإناث وفى المدن عن القرى والموظفين عن الفلاحين، وكذلك كان إنتشار هذا المرض الخطير أكثر فى المصابين بالتهاب الكبدى الفيروسى ب عن المصابين بالتهاب الكبدى الفيروسى س أو البلهارسيا، وقد كانت العينات كلها سلبية للأفلاتوكسين ب ١.

ومن هذا نستنتج أن الالتهاب الفيروسى خصوصاً ب والإصابة بالبلهارسيا من العوامل الأكثر شيوعاً فى مصر، عن التسمم بالأفلاتوكسين ب ١ والتى قد تؤدى إلى الإصابة بأورام الكبد الخبيثة.