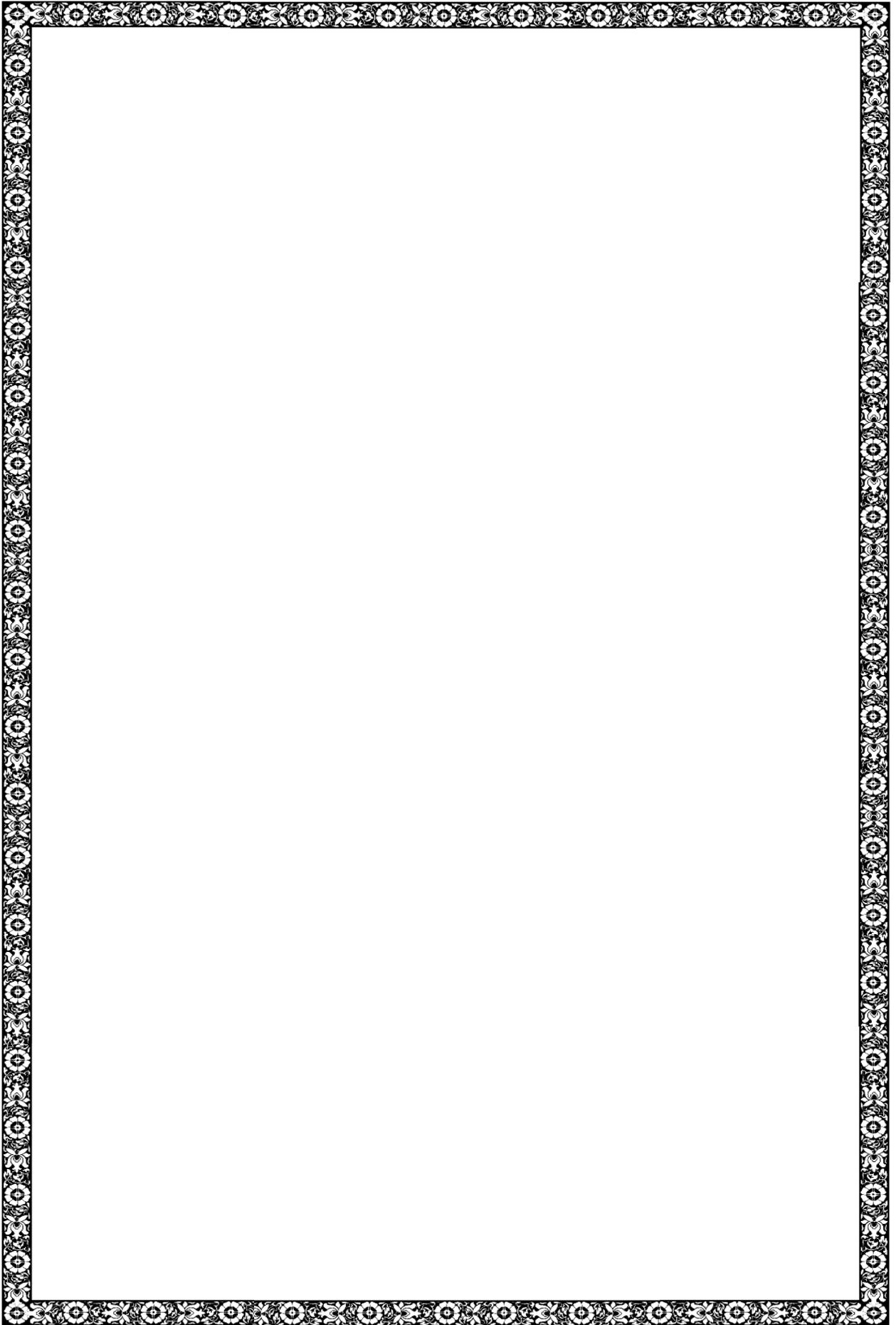




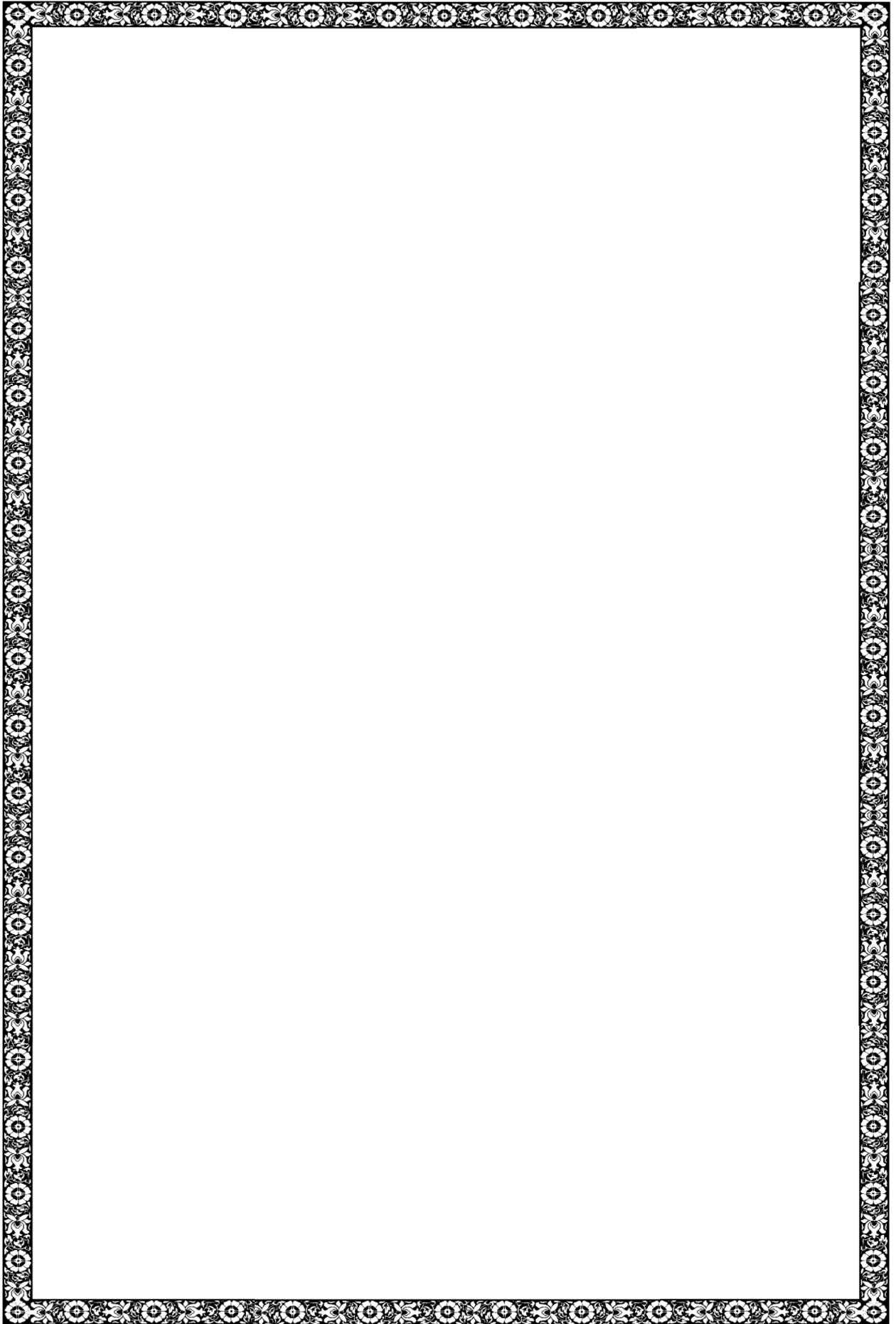
الإدارة العامة للمشروعات البيئية

# أبحاث كلية الطب





## الإدارة العامة للمشروعات البيئية



*Serum Aflatoxin level as a predictor of  
Hepatocarcinogenesis in HCV-infected Egyptians*

**Mohamed A. El-Farrash\*, Mohamed Abdel-Wahab\*\* and Mohammad Sabri Rizk\***

**Department of Medical Microbiology and Immunology\* and Gastroenterology Surgery  
Center\*\*, Faculty of Medicine Mansoura University, Mansoura City, Egypt.**

**Corresponding Author:**

*Professor Mohammed A. EL-Farrash,*

**Department of Medical Microbiology and Immunology,**

**Faculty of Medicine. Mansoura University, Mansoura, Egypt.**

**E-mail: [mfarrash@hotmail.com](mailto:mfarrash@hotmail.com).**

**Tel: 0101563888.**

**Running Title:**

Afltoxin levels and HCC in Egypt.

**Key words:**

HCC, aflatoxin, HCV, risk factors, Egypt

## ABSTRACT

Egypt has a very high prevalence of Hepatitis virus type C (HCV) infection and an increasing incidence of hepatocellular carcinoma (HCC) in a younger age group. As alcoholism is rare in Egypt, the main risk factor for carcinogenesis in HCV infected patients is supposed to be mutation induced by aflatoxin or its metabolites in hepatocytes. The environmental exposure to aflatoxins in foods or feeds may be reflected on the level of circulating aflatoxin (AFB1) in blood. The levels of albumin-abducted AFB1 were measured using a quantitative ELISA test in the sera of 80 Egyptian patients diagnosed as HCC, 40 HCV infected non malignant subjects and 40 healthy control individuals. The mean value of albumin-abducted AFB1 in the sera of HCC patients was significantly higher than the control groups ( $P < 0.05$ ). Farmers coming from rural areas had significant rise in the AFB1 compared to other patients coming from urban areas or having other jobs ( $P < 0.01$ ). The level of AFB1 was noticed to be significantly higher in patients having multiple lesions and also in patients presenting with tumor sizes more than 5 cm ( $P < 0.05$ ). HCV antibody and/or RNA were detected in all examined HCC patients. Exposure to environmental aflatoxin seems to be a major risk factor for HCC in HCV-infected Egyptians. HCV chronic hepatitis could render the liver less capable of intoxication and removal of AFB1 from the body. Then the accumulated AFB1 may induce mutation in p53 paving the way for HCV to induce HCC.

## INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most common malignancy in the world complicating liver cirrhosis in most cases <sup>(1)</sup>. Its incidence is increasing worldwide ranging between 3% and 9% annually <sup>(2)</sup>. In Egypt, HCC was reported to account for about 4.7% of chronic liver disease (CLD) patients <sup>(3)</sup>. The epidemiology of HCC is characterized by marked demographic and geographic variations. The main risk factors of HCC are the hepatitis B (HBV) and the hepatitis C (HCV) viruses, which together account for three quarters of all cases worldwide <sup>(4)</sup>.

HCV is a positive-stranded RNA virus which belongs to the *Flaviviridae* family. HCV infection, in addition to being a major cause of chronic liver disease, is a major cause of liver cancer <sup>(5)</sup>. Egypt has one of the highest prevalence rates of HCV infection in the world <sup>(6)</sup>; however, the risk and attribution related to HCV in Egyptian patients with HCC remains unknown. HCC is the ultimate complication of chronic HCV infection. It occurs at an annual incidence of 1–4% in patients with HCV-related cirrhosis <sup>(7)</sup>. Cirrhosis appears to be the main risk factor for hepatocellular carcinoma in HCV-infected individuals. Other known risk factors of HCC, including dietary aflatoxin B1 (AFB1) intake, cigarette smoking or heavy alcohol consumption, can have synergistic effects <sup>(8)</sup>.

The aflatoxins including AFB1 are secondary metabolites of some strains of the molds *Aspergillus flavus* and *Aspergillus parasiticus* species. Those fungi may contaminate human and animal foods, such as peanuts and corn, during plant growth and after harvest. The highest exposure to AFB1 has been

observed in parts of Africa, China, and Southeast Asia, which are also characterized by a high incidence of HCC <sup>(9)</sup>. In Egypt, 2 studies had found high content of aflatoxins both in seeds and in serum and urine of some patients <sup>(10, 11)</sup>. Those studies raised the probability of high AFB1 intake in HCV-infected individuals as the main risk factor for occurrence of HCC which is now seen in relatively young Egyptian patients. It has been reported that AFB1 exerts liver-specific carcinogenicity by inducing a guanine to thymine substitution at codon 249 on the p53 gene <sup>(12)</sup>. AFB1 is metabolized by the mixed-function oxidase system to a number of hydroxylated metabolites and to AFB1 8, 9-epoxide, which binds to DNA, and forms the promutagenic N7dG adducts <sup>(13)</sup>. AFB1 can also cause oxidative and nitrosative stress, and may indirectly induce p53 mutations by lipid peroxidation <sup>(14)</sup>.

This study was conducted to clarify the role of AFB1 as a risk factor for developing HCC in HCV-infected Egyptian patients.

## **MATERIALS AND METHODS**

### **Patient Selection:**

The study included 2 groups of patients and a control group. All patients in the study and control groups agreed to be included in the study and signed consents after knowing what is going to be done. The Ethical Committee of Mansoura University Hospitals approved the work protocol. The groups were:

**A. HCC patients:** Eighty patients (66 male and 14 female) with HCC were recruited from those attending the outpatient clinic of the Gastroenterology Surgery Center at Mansoura University Hospital, Mansoura city in Northern Egypt. Patients previously diagnosed as HCC were selected. All of them had been diagnosed clinically and on basis of abdominal ultrasonic examination, computerized tomography (CT) scanning and elevated feto-protein (AFP). Most of patients were attending the outpatient clinic for follow up or to be evaluated for liver transplantation. The age of the patients ranged from 45 to 68 years.

**B. HCV antibody-positive individuals:** Forty persons who were diagnosed as HCV-antibody positive during blood donation were included. All of them had normal liver function tests and ultrasonic examination excluded the presence of HCC. The general conditions of them were generally good and they were selected with their age between 45 and 60 to match with the HCC patient group. The male to female ratio was also matched to the HCC patient group (32 males and 8 females).

**C. Control group:** Forty persons who were HCV-antibody negative and matched for age with both groups were selected from persons attending the laboratory for routine investigations. The criteria of inclusion were to be HCV-antibody negative, HBsAg negative, normal liver function tests and abdominal ultrasonic examination revealing normal liver. The male /female ratio was also matched to both groups (34 males and 6 females).

From all groups, blood samples were collected, sera were separated by centrifugation, divided 1ml /tube in sterile eppendorf tubes and kept frozen at -40°C till used.

## **Viral Hepatitis Markers:**

A. HCV antibody detection: The HCV antibody status was determined using the Murex anti-HCV (Version 4) (from Murex Biotech S.A.) according to the manufacturer's recommendations. Only samples with clear-cut results (positive or negative) were included in the HCV or the control group.

B. HBs antigen detection: The HBs antigen was tested using the commercially available HBs Enzyme Immunoassay kit (from DiaSorin S.R.L. Saluggia, Italy). The method is a qualitative HBsAg assay based on the ELISA technique.

C. HBV core Total Antibody (IgG and IgM): The HBc antibody was tested using the Anti-HBc Reagent Pack (*Vitros Immunodiagnostic Products*) for the *in vitro* qualitative detection of total antibody (IgG and IgM) to hepatitis B core antigen (total anti-HBc) in human serum.

D. HCV RNA detection: HCV RNA was detected by RT-PCR using the Biosewoom HCV PCR kit (from Biosewoom Inc. Seoul Korea). Total RNA was prepared from serum samples according to the manufacturers' instructions. RNA extraction from all samples in both groups was done under the same conditions. In each time 20 samples (10 samples from cases with HCC and 5 samples from HCV or control group) were extracted to equalize conditions for both groups.

## **AFB1 measurement by ELISA:**

The AFB1 level in serum samples was determined using the Ridascreen Aflatoxin B1 (from R-Biopharm AG, Demstadt, Germany). The test is a

competitive enzyme immune assay for the quantitative analysis of Aflatoxin B1. The kit included positive controls AFB1 containing 4000, 2000, 1000, 500, 250 or 0 ng/kgm in methanol/water (10/90). For serum samples preparation, samples were diluted 1:4 with the dilution buffer (8.75 ml sample buffer of Ridascreen and 1.25 ml methanol). The final sample contained 10% methanol and the dilution factor was 1:5.

**Albumin measurement:**

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) as described by the manufacturers. The mean albumin value in mg/dl in each group was calculated and used to get the mean AFB1/mg of albumin.

**Statistical Analysis:**

Statistical package for social sciences (SPSS, version 10) was used for data management.

## RESULTS

### **All HCC patients were HCV antibody positive:**

All patients with HCC in the studied group were HCV-antibody positive. Although it was expected that a high percentage of HCC patients would be infected with HCV, the 100% HCV positivity was a surprise. It should be mentioned that selection of patients in the HCC group was done regardless to their HCV infection status. The criteria of inclusion did not include a positive HCV test. The RT-PCR technique to amplify and detect the presence of HCV revealed that the HCC group had significant higher incidence of HCV viraemia when compared to the HCV control group. The RT-PCR was positive in 56 samples (70%) in the HCC group while it was positive in 25% of the non malignant group. Ten samples of the control group were also tested by RT-PCR and all of them gave negative results as expected. Table 1 is showing the results of HCV markers in all groups.

### **HBV infection was less common in HCC Egyptian patients:**

Among the HCC group 6 patients were positive for HBV surface antigen. The HCV non-malignant group and the healthy control group were negative for the HBV surface antigen. When all samples were tested for HBV core antibody (total IgM and IgG) the 6 HBV surface antigen positive samples and other 12 samples were positive. HBV core IgG indicates remote infection by the HBV. Table 1 summarizes the HCV and HBV serological markers in the examined groups.

**Table1:** Viral hepatitis markers in the studied groups

	HCV Markers		HBV Markers	
	HCV Ab	HCV RNA	HBsAg	HBcAb
HCC group	100%	70%	7.5%	22.5%
HCV control	100%	25%	0%	0%
Healthy control	0%	0%	0%	0%

**High AFB1 levels in all groups:**

The absolute values of AFB1 extracted from the calculation curve showed significant higher AFB1 concentrations in samples obtained from the HCC group when compared with the HCV non malignant or the healthy control group. The mean AFB1 value in the serum of healthy control group was 7.3 ppb (part per billion). Among the 40 persons included in the control group 26 persons had serum AFB1 values less than the mean value, 13 had values above the mean but by less than 2-folds. Only one person had a high level of AFB1 reaching 3.5-fold the mean value.

In the HCV positive non-malignant group the mean value of AFB1 was 12.8 ppb which is higher than the mean value of the healthy control. Among persons included in this group 23 persons had AFB1 values less than the mean of the healthy control, 8 persons had values raised by less than 2-folds, 6 persons had values raised by 2 – 3 folds and 3 persons had values raised by more than 3-fold than that of the mean value for the healthy control.

The HCC patients group had a significant higher mean value of AFB1 in their samples when compared to the control group ( $P = < 0.05$ ). The mean value for 79 persons was 17.9 ppb. One sample had AFB1 above the upper limit of the curve (more than 400 ppb). If this sample is considered just 400 ppb and included the mean for the HCC group may rise to 30.4 ppb. We preferred to exclude that sample for the more logic comparison. The mean value of AFB1 (17.9 ppb) for the HCC group was about 2.5-fold higher than the mean for the healthy control. It was also higher than the mean of the HCV positive non-malignant group by about 1.4-fold. Interestingly, only 4 patients in the HCC group had AFB1 values equal or less than the mean for the healthy control. The other 76 patients had values of AFB1 higher than the mean of the healthy control. Among them 26 patients had levels about 3-fold that of the mean for healthy controls and 22 patients had elevated AFB1 level by more than 3-fold higher than the mean value in the healthy control group. Table 2 is showing the AFB1 levels in different groups.

Because the ELISA test is measuring the albumin-abducted AFB1, we decided to adjust the obtained AFB1 values according to the albumin levels as described previously <sup>(11)</sup>. The mean value of albumin in sera of the healthy control group was 48 mg/ml and the adjusted AFB1 was calculated to be 0.15 ng AFB1/mg of Albumin. When the adjusted value of the HCV non malignant group was calculated it was found to be 0.32 ng of AFB1/ mg of albumin as the mean value of albumin level in this group was 40 mg/ml. Because the albumin level was low among the HCC group (26 mg/ml) the adjusted mean AFB1 was much higher in this group reaching 0.69 ng of AFB1/mg of albumin.

Table 2 shows the adjusted AFB1 values in the studied groups which had statistically significant differences ( $P = < 0.005$ ).

**Table 2:** Serum levels of AFB1 in the studied groups

	Serun Aflatoxin B1 (ppb*)	Serum Aflatoxin B1 (per mg of Albumin)
HCC group	17.9	0.69
HCV control	12.8	0.32
Healthy control	7.3	0.15
*ppb: part per billion.		

**AFB1 was higher in male farmers from rural areas:**

We analyzed the difference between AFB1 levels in different patients with HCC. Table 3 is summarizing some of the differences among different patients according to the level of AFB1 (per mg of albumin). Farmers coming from rural areas had significant rise in the AFB1 compared to other patients coming from urban areas or having other jobs. The patients coming from Kafr El-Sheikh governorate (the most north-mid area of Nile Delta) had significant high levels of AFB1 which may be related to some environmental or nutritional factors in this locality.

**Table 3:** Serum levels of AFB1 in different HCC patients

	No	%	Mean AFB1/ mg Albumin	P value
<b>Sex:</b>				
Male	66	82.5	0.783	< 0.05
Female	14	17.5	0.391	
<b>Residence:</b>				
Rural	62	77.5	0.845	<0.01
Urban	18	22.5	0.259	
<b>Governorate:</b>				
Dakahlia	48	60.0	0.448	<0.001
Gharbia	10	12.5	0.310	
Damietta	8	10.0	0.279	
Kafr El-Sheikh	8	10.0	1.368	
Port Saied	6	7.5	0.286	
<b>Occupation:</b>				
Farmer	28	35	1.408	<0.01
Manual worker	10	12.5	0.389	
Medical Staff	4	5	0.275	
Office employee	14	17.5	0.251	
House wife	10	12.5	0.442	
Others	14	17.5	0.336	

**AFB1 was higher in patients with large or multiple lesions:**

The level of AFB1 was noticed to be significantly higher in patients having multiple lesions and also in patients presenting with tumor size more than 5 cm. This may be related to the effect of AFB1 as predisposing factor affecting all the liver homogenously. Table 4 is showing these findings.

**Table 4:** Serum levels of AFB1 in patients with different HCC lesions

HCC lesion	No	%	Mean AFB1/ mg Albumin	P value
<b>Size:</b>				
> 5 cm	66	82.5	0.785	< 0.05
< 5 cm	14	17.5	0.372	
<b>Multiplicity:</b>				
Single	62	77.5	0.411	<0.001
Multiple	16	20.0	1.938	
Diffuse	2	2.5	0.304	

## DISCUSSION

HCC represents more than 5% of all cancers in the world<sup>(1)</sup>. Two major epidemiological facts characterize this cancer; it occurs in a previously diseased liver, and the causes of the underlying liver disease differ according to the geographical distribution. Consequently, the mechanisms of hepatocarcinogenesis and the characteristics of the tumor might vary greatly from one part of the world to another. In Africa and Southern Asia, the role of AFB1 and HBV infection is highly predominant. In these regions, HCC develops often at young age and in the absence of cirrhosis. By contrast, in Japan, Egypt and in Southern Europe, HCV is the main cause of HCC which occurs in older patients, nearly all of them with advanced fibrosis or cirrhosis. Recently HCC is more frequently noticed in younger Egyptian patients and its incidence is rising<sup>(3)</sup>. The cause of this rise in incidence is mainly due to chronic hepatitis caused by the HCV. In this study all cases of HCC were positive for HCV infection. The role played by HCV in hepatocarcinogenesis is not very clear but previously we noticed that HCV core protein is activating telomerase enzyme in cells lacking active p53 or even in hepatocytes which enter the activated stage<sup>(15)</sup>.

The role of HBV in inducing HCC in Egypt may come after that of HCV as in our HCC group HBV surface antigen was only detected in 7.5% of serum samples and the HBV core antibody was detected in 22.55% of all HCC patients. HBV infection comes far behind HCV infection as a triggering virus for HCC in Egypt.

Exposure to environmental aflatoxin has long been implicated as a risk factor for HCC. Studies of individual levels of aflatoxin in regions with varying HCC risk strengthened the evidence for this association <sup>(16)</sup>. In one study, AFB1 intakes in HCC patients was calculated to be 0.42 – 1.88 g/day <sup>(17)</sup>. The cumulative intakes up to the age of HCC diagnosis were calculated to range from 0.13 mg/kg to 0.49 mg/kg (13 – 49 ppb), assuming a weight of 60 kg <sup>(17)</sup>. These levels are very similar to the level of AFB1 found in our HCC group (17.9 ppb) while in the HCV-positive or healthy control the AFB1 level did not exceed 13 ppb (table 2). We noticed that AFB1 levels were high in Egyptian farmers living in rural areas which may be due to faulty storage of grains allowing the growth of *Asprigillus* fungi producing the AFB1. Also it may be related to the type of bread used in rural areas which is not usually made daily but is made in bulks and stored for weeks or longer. Kafr El-Sheikh governorate inhabitants had higher levels of AFB1 compared to patients coming from different governorates. This finding may be explained by the more humid weather in Kafr El-Sheikh or may be related to different ways of storing grains.

AFB1 was higher in patients having multiple lesions or having lesions larger than 5 cm when diagnosed. In patients with high AFB1 levels the probability of more hepatocyte damage and more adverse effect on the liver may be the cause of multiple or larger HCC lesions.

In HCV-positive non-malignant Egyptians, the levels of AFB1 was 12.8 ppb which is very near to the critical limit (13 – 49 ppb) noticed in the study by Ming et al <sup>(17)</sup>. Also the level of AFB1 in healthy controls was 7.9 ppb which is relatively higher than expected. The higher levels of AFB1 in individuals

infected with HCV may raise the concern that hepatitis induced by the virus affects the metabolism of AFB1 in liver leading to more retention of the toxic agent in sera of infected individuals. For Egyptian patients with chronic HCV hepatitis, it seems prudent to recommend foods low in aflatoxin to prevent or reduce the incidence of HCC.

The high prevalence of HCV in Egypt with the high detected levels of AFB1 may pose a true risk for Egyptian HCV-infected persons to develop HCC. HCV infection may affect the liver in early stages to make it less capable of intoxication and removal of AFB1 from the body. Then the accumulated AFB1 may induce mutation in p53 paving the way for HCV or other risk factors to induce malignancy. Controlling HCV infection and implementation of regulations to minimize the levels of AFB1 in Egyptian foods and also in materials used for feeding chicken or cattle in meat farms may help to lower the incidence of HCC in Egypt.

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نسبة الأفلاتوكسين في المصل كمؤشر لحدوث سرطان الكبد  
في المصريين المصابين بعدوى الفيروس الكبدي "سي"

محمد عبدالرازق الفراش<sup>١</sup>، محمد عبد الوهاب<sup>٢</sup>، محمد صبرى رزق<sup>٣</sup>

قسم الميكروبيولوجيا والمناعة الطبية ومركز جراحة الجهاز الهضمي<sup>٢</sup>.

كلية الطب جامعة المنصورة.

### الملخص العربي

لارتفاع نسبة الإصابة بعدوى الفيروس الكبدي "سي" أصبح من الشائع اكتشاف حالات عديدة من سرطان الكبد بين المصريين في أعمار صغيرة نسبياً. ولما كان تناول الكحوليات لا يشكل خطورة في مصر مقارنة بدول أخرى فإن الاحتمال الأكبر لحدوث سرطان الكبد قد يكون التغير الجيني الناتج عن تعرض المرضى المصريين لجرعات زائدة من الأفلاتوكسين في طعامهم مباشرة أو في أعلاف الدواجن والحيوانات المنتجة للحوم.

وقد تم في هذا البحث قياس منسوب الأفلاتوكسين باستخدام الاختبار اليزا كمي في أمصال ٣ مجموعات من المصريين. المجموعة الأولى شملت ٨٠ مريضاً بسرطان الكبد والمجموعة الثانية شملت ٤٠ شخصاً مصاباً بعدوى الفيروس الكبدي "سي" دون وجود أي أعراض أو علامات مرضية بينما اشتملت المجموعة الثالثة الضابطة على ٤٠ شخصاً صحيحاً.

ووجدت الدراسة ارتفاعاً ملحوظاً للأفلاتوكسين في أمصال مرضى سرطان الكبد عنه في المجموعة الضابطة وكذلك في المصابين بعدوى الفيروس الكبدي "سي". وزادت الأهمية الإحصائية في الزارعين القادمين من الريف المصري وكذلك في المصابين بأورام متعددة أو بورم كبير الحجم.

وتوصلت الدراسة إلى احتمال أن يكون التعرض للأفلاتوكسين في غذاء المصريين من العوامل الأساسية للإصابة بسرطان الكبد خاصة في الأشخاص المصابين بعدوى الفيروس الكبدي "سي" حيث تقل كفاءة الكبد في تخليص الجسم من الأفلاتوكسين فتزداد نسبته في الدم ويؤدى إلى تغير جيني في خلايا الكبد يجعله مهيناً لحدوث السرطان.

## التقرير النهائى

عنوان المشروع : " دراسة تأثير الأفلاتوكسن كعامل ذو خطورة فى إحداث سرطان الكبد "

إسم الباحث الرئيسى : أ.د/ محمد عبد الرازق محمد الفراش

وظيفة الباحث الرئيسى : أستاذ بقسم الميكروبيولوجيا والمناعه الطبية بكلية الطب

١- تم تجميع عينات دم من حالات مصابة بسرطان الكبد كما تم تجميع عينات من حالات مصابة بعدوى الفيروس الكبدى C وحالتهم الصحية مستقرة وجميعهم ليس لديهم دلائل عن إصابة الكبد بأى أورام وكذلك تم تجميع عينات من حالات صحيحة غير مصابة بعدوى الفيروس الكبدى C أو الفيروس الكبدى B

٢- تم قياس نسبة الأفلاتوكسن B المرتبط بالالبومين فى أمصال المجموعات الثلاثة وقد وجد أن نسبة الأفلاتوكسن مرتفعة بصفة عامة فى جميع المجموعات وإن كانت أعلى كثيراً فى مجموعة سرطان الكبد (متوسط ١٧.٩ PPb) والمجموعة المصابة بالفيروس الكبدى C (متوسط ١٢.٨ PPb) عنها فى مجموعة الكنترول (متوسط ٧.٣ PPb) . وعند حساب كمية الأفلاتوكسن بالنسبة للالبومين لوحظ أن النسبة فى الأشخاص الأصحاء ٠.١٥ /مجم وللأشخاص المصابين بعدوى الفيروس C ٠.٣٢ /مجم ولحالات سرطان الكبد ٠.٦٩ /مجم وهو فرق هام إحصائياً .

٣- تم فحص كل عينات الدم لدالات الإصابة بالفيروسات الكبدية النوع C والنوع B وقد وجد أن كل حالات سرطان الكبد فى العينات التى تم فحصها مصابة بعدوى الفيروس الكبدى C بنسبة ١٠٠% بينما كانت معدل الإصابة بالفيروس B كالتالى : ١٥% موجبة HBS Ag و ٤٥% موجبة للأجسام المضادة HBcAb.

٤- تم عمل تحليل PCR لمعرفة مدى نشاط الفيروس الكبدى C فى المجموعات المختلفة ووجد أن الفيروس الكبدى C موجود فى العينات المصابة بسرطان الكبد بنسبة ٣٢.٥% تقريباً بينما كان الفيروس موجود فى الحالات المصابة بعدوى الفيروس الكبدى C بنسبة ٢٥% وهو فرق غير مهم إحصائياً .

٥- أثبتت الدراسة أن أهم العوامل المؤدية إلى زيادة حدوث سرطان الكبد فى العينات التى تم فحصها هى :

أ- الإصابة بالفيروس الكبدى C.

ب- ازدياد نسبة الأفلاتوكسن فى السيرم .

ج- العدوى المصاحبة بالفيروس الكبدى B مع الفيروس الكبدى C .

٦- أثبتت الدراسة ارتفاع نسبة الأفلاتوكسن فى جميع المجموعات التى تم فحصها بدرجات مختلفة مما يلفت النظر لضرورة الانتباه لمتابعة أسباب ذلك وأهمية قياس نسبة هذه المادة السامة فى الأغذية والحبوب المتاحة للاستهلاك البشرى أو المستخدمة فى أعلاف الحيوانات والدواجن التى يستهلكها المصريون .

٧- لوحظ ارتفاع نسبة الأفلاتوكسين فى مجموعة المرضى الذين يسكنون الريف المصرى ويعملون بمهنة الزراعة خاصة فى محافظة كفر الشيخ وذلك قد يكون ناتجاً عن سوء تخزين الحبوب فى الريف المصرى أو بعض الأنماط الغذائية مثل الخبز المخبوز فى المنازل والذى يخترن لفترات طويلة نسبياً .

٨- كما لاحظت الدراسة ارتفاع نسبة الأفلاتوكسين فى الحالات المصابة بأورام متعددة وكذلك فى المرضى الذين لديهم أورام تزيد عن ٥ سم فى قطرها وهذا قد كون بسبب تعرضهم لكميات أكبر من الأفلاتوكسين نتج عنها انتشار الورم وزيادة حجمه .

#### التوصيات :-

١- توعية المواطنين المصريين لتجنب استخدام بعض الحبوب أو الأغذية التى تحتوى على الأفلاتوكسين .

٢- إصدار تشريعات لتحديد أقصى نسبة مسموحة من الأفلاتوكسين فى الأطعمة وحتى فى الأعلاف التى تستخدم فى مزارع الدواجن أو الأسماك أو الأبقار .

٣- إرشاد المرضى المصابين بعدوى الفيروس الكبدى C لضرورة المتابعة حتى يتم تشخيص أى أورام كبدية فى مرحلة مبكرة مما قد يسهل من علاجها .

## ملحوظات :-

- ١- لم نتمكن من توسيع الدراسة لتشمل أعداد أكبر من المرضى أو الحالات الضابطة ضغطاً للنفقات .
- ٢- تخفيض ميزانية المشروع البحثي و صرفها على دفعات متفرقة جعل من الصعب الحصول على كل المواد اللازمة لإجراء التجارب المرتبطة في الوقت المناسب .
- ٣- تأجيل صرف مكافآت العاملين والفنيين المساعدين في المشروع البحثي لنهاية المشروع يؤدي إلى فتور نشاطهم واهتمامهم تدريجياً .