

## AFLATOXIN AS AN ENVIRONMENTAL RISK FACTOR ATTRIBUTABLE TO LIVER CANCER IN NILE DELTA

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### Abstract

#### Keywords:

AflatoxinB1;  
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(ClinicalTrials.gov Identifier: NCT02461966).

**Background and Aim:** The burden of hepatocellular carcinoma (HCC) has been increasing in Egypt. This fact raises the question, does the high prevalence of HCC in Egypt is related only to HCV? or is it augmented by AFB1 exposure in our patients? The aim of this study was to identify the role of aflatoxin as an environmental risk factor attributable to liver cancer in Nile delta in Egypt.

**Patients and Methods:** This cross sectional study was carried out in tropical medicine department in Tanta university hospital on 160 patients with HCC, 80 cirrhotic patients. Sixty individuals were invited to share in the study as a control group. All patients and control were evaluated for age, sex, residence, occupation, viral markers, liver functions and serum level of aflatoxinB1.

**Results:** Aflatoxin level in serum was significantly higher in HCC patients when compared to cirrhotics and to controls. The mean age of HCC patients was  $58.575 \pm 9.583$  years. HCC was much higher in males than females with male to female ratio 4.7:1. Concerning smoking, 45% of HCC patients were smokers. DM was diagnosed in 42.5% of HCC cases. Anti HCV-Ab was present in 95% of HCC cases.

**Conclusions:** Environmental aflatoxin seems to be a major risk factor for HCC in Nile delta. The high prevalence of HCC in Nile delta in Egypt is related to HCV and it is augmented by AFB1 exposure.

### Introduction

Liver cancer in adult men is the fifth most frequently diagnosed cancer worldwide, and is the second leading cause of cancer-related death in the world. In adult women, it is the seventh most commonly diagnosed cancer and the sixth leading cause of cancer death<sup>1</sup>.

In Egypt, there is a remarkable increase in HCC incidence among chronic liver diseases (CLD) patients. The incidence doubled from 4.0% in 1994 to 7.2% in 2005<sup>2</sup>. In a recent study, There is a much higher incidence of HCC among cirrhotic patients in Egypt reaching about 21%. This increase can be attributed to the improvement of screening programs and diagnostic tools of HCC, and increased survival rate among patients with cirrhosis to allow time for some of them to develop HCC<sup>2, 3</sup>.

Hepatocellular carcinoma is multifactorial in etiology and complex in pathogenesis, the blend of risk factors differs in different parts of the world, and this may explain in part the diverse biologic characteristics of HCC in different populations<sup>4</sup>.

In Egypt which has the highest prevalence of HCV worldwide, HCC occurs almost in post hepatitis C cirrhosis 2. However, many non-viral factors like obesity and diabetes, which operate via NASH, alcohol, tobacco and aflatoxin B have been implicated in the development of HCC 5.

AflatoxinB1 (AFB1), derived from *Aspergillus flavus* and *Aspergillus parasiticus*. Once ingested, AFB1 is metabolized to an active intermediate which can bind to DNA and cause damage, including producing a characteristic mutation in the p53 tumor-suppressor gene 4.

This mutation has been observed in 30–60% of HCC tumors in aflatoxin endemic areas 6. AFB1 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, resulting in a guanine cytosine (GC) to thiamine adenine (TA) transversion. AFB1 selectively targets the third base position of codon 249 of the human p53 gene, a known mutational hotspot in human hepatocellular carcinoma (HCC) 7. A significant association between aflatoxin exposure and HCC has been reported in hyperendemic areas 8. Chinese patients with chronic HBV and aflatoxin exposure have about 60 fold increased risk of developing HCC 8. Also, Aflatoxin exposure may be associated with advanced liver disease in chronic hepatitis C (HCV) patients 9.

These facts raise the question, does the high prevalence of HCC in Egypt is related only to HCV? or is it augmented by AFB1 exposure in our patients?

So, we design this cross sectional study to compare the serum level of AFB1 in HCC patients, their non hepatic house relatives, and non HCC cirrhotic patients.

## Materials and methods

### Study design and sampling:

After the approval of research ethics committee at Tanta Faculty of Medicine, this study was conducted in tropical medicine department, Tanta university hospital on 300 individuals; 160 patients diagnosed as HCC were included as group I. Their household relatives sharing same economic and environmental conditions were invited to share in the study. All of them gave an informed consent to share in the study; 80 cirrhotic patients who were included as group II, and 60 healthy non hepatic individuals were included as control (group III).

From all participants thorough medical history was taken from them and they received full clinical examination, ten ml peripheral venous blood samples were drawn from each participant, five ml were used for routine laboratory investigations in the form of complete blood picture (CBC), erythrocyte sedimentation rate (ESR), renal functions tests, liver functions tests and blood glucose level, and 5ml were centrifuged and serum was kept in 2 eppendorf tubes frozen at -20°C. One serum tube is analyzed using third generation enzyme – linked immunosorbent assay technique (ELISA) to detect HBsAg and HCV antibodies. The other is used to detect Alpha-fetoprotein (AFP) by biocheck AFP enzyme immunoassay for the quantitative determination of the cancer antigen AFP concentration in human serum, and aflatoxin (AFB1) by ELISA.

Real time abdominal U/S was done for all patients and controls included in the study using TOSHIBA ECCOCEE machine with a convex – sector probe (PVF – 375 MT – 3.57 MHz) for evaluation of liver, spleen, Portal vein and Focal lesions. Spiral CT or dynamic MRI was performed for suspected cases to diagnose and grade HCC.

The diagnosis of HCC was based on detection of hepatic focal lesion by one dynamic imaging techniques (dynamic CT, dynamic MRI) typical for hepatocellular carcinoma or alpha-feto protein > 200 if the hepatic focal lesion above 2 cm in cirrhotic liver or two dynamic imaging techniques typical for hepatocellular carcinoma if hepatic focal lesion 1-2 cm in cirrhotic liver. Otherwise, diagnosis of HCC was performed on detection of hepatic focal lesion and alpha-feto protein >400. Cirrhotic patients were diagnosed clinically, laboratory and by imaging.

### Statistical analysis:

Data was statistically analyzed using SPSS (statistical package for social science) program version 13 for windows. Data are shown as mean ± SD for quantitative data. For qualitative data, it was showed as frequency and percent. Chi

square test was done for qualitative variable analysis or Fischer exact test for 2 x 2 tables when expected cell count of more than 25% of cases was less than 5. Kruskal-Wallis test was done to compare the three groups regarding non normally distributed quantitative variables and post hoc test done to detect the relationship between variables within groups. For all used tests, a p value < 0.05 was considered statistically significant.

All data are tested with kolmogorov- Smirnov Z test for normality and most of them were found normally distributed. We use both parametric and non parametric testes accordingly on doing the association. (ClinicalTrials.gov Identifier: NCT02461966).

## Results

This study was performed on 300 individuals grouped into; HCC (n=160), cirrhosis (n=80) and 60 apparent healthy subjects as control group. The comparison between the demographic data of all these studied groups is shown in table (1) and comparison between the groups as regard to age, alfafetoprotein (AFP) and aflatoxin is presented in table (2) and all pairwise comparison of age, AFP and aflatoxin in all studied groups is presented in table (3).

When the 3 groups were compared, only age, alfa-fetoprotein level, HCV positivity and aflatoxin level showed statistically significant difference. The comparison between HCC group and control group revealed age, aflatoxin level, HCV and alfafetoprotein level as the significant variables between both groups.

Then, a comparison was also made between HCC and cirrhotic group only. The comparison between both groups revealed age, and alfa-fetoprotein level as the significant variables between both groups while HCV positivity was not statistically significant between both groups.

## Discussion

The burden of HCC has been increasing in Egypt with a doubling in the incidence rate in the past 10 years. This was attributed to several biological (e.g. hepatitis B and C virus infection) and environmental pollutants e.g. aflatoxins 10.

The primary aim of this study was to identify the role of aflatoxin as an environmental risk factor attributable to liver cancer in Nile delta.

In this study, we included HCC patients and some of their household relatives. So, the participants shared same socioeconomics, same diet and most environmental factors (as they had different occupations). HCC group has more male to female ratio compare to cirrhotic patients and healthy control. Prevalence in males was evidenced in Egyptians 9, 11-13. Most of our HCC patients were farmers this was in accordance with Abdel-Wahab et al. 11, but differing from Attalla et al., 14 who found that HCC is more prevalent in urban residents and employees. This difference may be referred to difference in population of the study as most of our cases were from rural areas.

In HCC, male predominance is more obvious in population at high risk with male to female ratio 3.7:1 4. As male to female ratio in our HCC patients was 4:1, this may denote that Nile delta is among the areas of higher risk to HCC.

This ratio was previously reported in Egypt and explained by the higher susceptibility of males to environmental carcinogenic factors and greater exposure to them 15. However, sex hormones and other X-linked genetic factors may also be important. It has been speculated that estrogens and androgens could modulate hepatocarcinogenesis and explain the higher incidence of HCC in men 2. Also, DNA synthetic activities are higher in male than in female cirrhotics, and this might be one of the possible explanations for the gender discrepancy in HCC 16.

The average age of HCC was around 66 years 17 or in the seventh or eighth decades of life [18]. In our HCC patients, mean age was lower (58.57 years) which can again suggest Nile delta as high risk area as reported by Kew 4 who stated that the incidence of HCC increases progressively with advancing age, however, in areas with high risk the mean age is definitely younger. Also similar results were reported by Mohamed et al. 15 who reported that the

peak age was 56 years, Tangkijvanich et al. 16 with mean age 52.6 years, and El-Zayadi et al. 2 with most predominant age group (40-59) years.

Despite the relative young age of HCC patients it was still older than cirrhotic patients (50 years) and healthy controls. This was expected as HCC develops on top of cirrhosis in the majority of our cases and by turn, patients with HCC are older than those of cirrhosis.

In this study, 80% of HCC cases were from rural areas, these results agree with that of El-Zayadi et al. 2 who stated that most of HCC cases (75.2%) resided in rural areas.

In this study, 57.5% of our HCC cases were farmers, this result was higher than that of Mohamed et al. 15 who reported only 23.3% of HCC cases were farmers. However, Mohamed et al. 15 also stated that any patient, whatever his job is can be affected if exposed to carcinogenic factors. Carcinogenic factors including environmental pollutants, and exposure to aflatoxins that are found in contaminated foods such as corn, peanuts, various other nuts, soy sauce and fermented soy beans 20.

Concerning smoking, our study showed that, 45% of HCC cases had history of smoking, half of them were heavy smokers, a nearly similar result was reported by Mohamed et al. 15 who reported that 49.6% of HCC cases had history of smoking. Although Kew 4 reported that heavy smokers have an 50% higher risk to HCC than non smokers, Kensler et al. 20 documented that smoking cause DNA damage and cell proliferation, and El-Zayadi 21 reported that smoking yields chemicals with oncogenic potential that increase the risk of HCC.

Although alcoholism is a well identified risk factor for HCC and in one study alcoholic liver disease accounted for 32% of all HCCs 22, and a recent study showed synergism between alcohol drinking and HBV or HCV infection 23, however this role was not clear in our study as none of HCC cases in our study had history of alcohol intake and nearly similar result of 3.9% was reported in Egyptian series by Mohamed et al. 15. This may be due to different socioeconomic and religious status that forbidden alcohol intake.

Concerning diabetes mellitus (DM), our study showed that 40% of HCC cases had history of DM. This was in agreement with Davila et al., 24 who stated that diabetes is associated with a 2-3 fold increase in the risk of HCC, regardless of the presence of other major HCC risk factors.

Extensive collaborative research was carried out to explore the independent and combined effects of HBV and HCV and other factors in the etiology of HCC. Although HBV is considered worldwide as a major risk factor for liver cirrhosis and HCC, the prevalence of HBV infection in Egypt has been declining over the last two decades, while HCV has increased. Egypt has possibly the highest HCV prevalence worldwide, estimated among the general population to be around 14%.

Concerning seroprevalence of HBsAg positivity among HCC cases (2.5%) of cases were positive, this was much lower than results reported by Mohamed et al. 15 who reported that 17.5% of HCC cases were HBsAg positive. This decrease of HBsAg positivity may be partially attributed to successful control measures of blood transfusion and vaccination and partially to the development of mutant or occult HBV infection, which requires costly assays for diagnosis 2.

In our study, regarding the seroprevalence of HCV antibody positivity among HCC cases (95%) of cases were positive, this was higher than that reported in Egyptian study by Mohamed et al. 15 who reported 64.4% of HCC cases were positive and near to results by El-Zayadi et al. 2 who reported 87.9% of HCC cases were positive, our results was in agreement with Michielsen et al. 23 who stated that in areas with an intermediate rate of liver tumours such as southern Europe, Egypt and Japan, HCV is the predominant cause of HCC. Here, HCC is mostly discovered in patients with long standing cirrhosis due to HCV.

Abdel-Wahab et al., 25 in a study performed on 80 cases with hepatocellular carcinoma diagnosed in the Gastroenterology center, Mansoura University, Egypt and 20 healthy control found that Aflatoxin B1 may play an important role in the occurrence of HCC in the north Nile delta area and especially in males, farmers, and rural residents, HCV infection, cirrhotic liver and multifocal hepatoma patients. Aflatoxin B1 in high concentration is associated with high incidence of chronic HCV, and affects hepatic parenchyma and multifocal lesions. Mohamed et al., 15 detected a significant higher percent of aflatoxins in the serum of Egyptian patients with HCC compared to their controls; with a twofold increased risk.

the other hand, there have been several investigators that do not support this positive association. Mokhles et al., 26 found that there is higher statistical significance of aflatoxin prevalence and concentration in serum and urine of cirrhotics than HCC patients and controls. In our study, despite the same diet and environment serum aflatoxin level was significantly higher in HCC patients more than cirrhotics and in cirrhotic more than control. This may be attributed to the cumulative effect of AFB exposure as age advanced; there is an evidence for an association between dietary aflatoxin exposure and HCC incidence seen in some case-control studies.

Turner et al., 27 have measured the level of aflatoxin-albumin (AF-alb) adducts as a validated biomarker to assess exposure. In this pilot survey, a limited number of sera samples, available from a hepatocellular carcinoma (HCC) case-control study in Egypt, were analysed. AF-alb was detected in 24/24 samples from HCC-negative individuals (geometric mean 9.0 pg/ mg; range 3.5-25.8pg/ mg), while 7/22 samples from HCC-positive cases had detectable AF-alb (geometric mean 2.6 pg /mg; range: non-detectable-32.8 pg/ mg). These AF-alb data do not represent a case-control comparison due to inherent difficulties in comparing markers of dietary intake between controls and patients with disease. They recommended that although these data are limited, the potential health consequences of aflatoxin exposure in this region merit further investigation.

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) 28,29.

Based on our study, the high prevalence of HCC in Nile delta in Egypt is related to HCV and it is augmented by

AFB1 exposure. There is more than synergy between HCV and Aflatoxin as chronic liver disease could render the liver less capable of removal of Aflatoxin from the body. Also, Aflatoxin may induce mutation in p53 preparing the way for HCV to induce HCC.

Our study has some limitations. This was a single center study of a tertiary care setting, raising the question of generalizability. Also, larger studies on larger group of patients are needed to confirm the results.

#### Footnotes

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Conflict of Interest: None declared.

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**Table (1): Demographic data of all studied groups**

	Groups						X <sup>2</sup> test	P-Value
	HCC group (N= 160)		Cirrhosis (N=80)		Control (N=60)			
	No	%	No	%	No	%		
Male-Female	132 28	82.5 17.5	56 24	70 30	36 24	60.0 40.0	3.23	> 0.05
Rural-Urban	128 32	80 20	52 28	35 65	48 12	80 20	1.81	> 0.05
Employee Farmer	68 92	42.5 57.5	40 40	50 50	44 16	73.3 26.7	4.15	> 0.05
Non smoker Smoker	88 72	55 45	56 24	70 30	44 16	73.3 26.7	2.19	> 0.05
No DM DM	92 68	57.5 42.5	68 12	85 15	48 12	80 20	5.75	> 0.05
No PVT PVT	148 12	92.5 7.5	76 4	95 5	60 0	100 0	1.22	> 0.05
No HCV HCV	8 15	5 95	8 72	10 90	52 8	86.7 13.3	44	< 0.01
No HBV HBV	156 4	97.5 2.5	72 8	90 10	60 0	100 0	2.73	> 0.05

**Table (2): Age, AFP and aflatoxin in all studied groups**

Studied variables (Mean ± SD)	HCC	Liver cirrhosis	Healthy controls	Kruskal Wallis test	P-value
Age	58.58±9.58	50.00±8.72	27.80±12.06	36.67	< 0.01
AFP	18.0±0.83	1.05±0.22		11.83	< 0.01
Aflatoxin	7.96±2.06	6.10±1.71	4.13±1.67	35.02	< 0.01

**Table (3): All pairwise comparison of Age, AFP and aflatoxin in all studied groups**

Studied variables (Mean $\pm$ SD)	HCC	Liver cirrhosis	Healthy controls	Kruskal Wallis test	P-value	P1	P2	P3
Age	58.58 $\pm$ 9.58	50.00 $\pm$ 8.72	27.80 $\pm$ 12.06	Sig.	Sig.	Sig.	Sig.	Sig.
AFP	18.0 $\pm$ 0.83	1.05 $\pm$ 0.22		11.83	< 0.01	Sig.		
Aflatoxin	7.96 $\pm$ 2.06	6.10 $\pm$ 1.71	4.13 $\pm$ 1.67	35.02	< 0.01	Sig.	Sig.	Sig.

P1=(HCC,cirrhosis), P2=(HCC, healthy control), P3=(cirrhosis, healthy control),sig.=significant