STUDY OF AFLATOXIN EXPOSURE IN SEROLOGICALLY POSITIVE HCV EGYPTIAN PATIENTS

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ABSTRACT

*The liver diseases are important health problem in Egypt. The high incidence of hepatitis C virus infection among Egyptian is annoying for the individual as well as the governmental authorities and health care providers.Aflatoxins remain as a threat to the health of livestock as well as humans by their containing intermittent occurrence in both foods and feeds. The present study aimed to study Aflatoxin (AFB1) and its possible role in the pathogenesis of HCV infection among Egyptians .The study conducted on 30 patients with hepatitis C virus infection from Cairo medical center and 20 subjects as a control.All subjects were subjected to the following: clinical examination, abdominal ultrasonography, liver biopsy for patients and laboratory tests which include liver function tests, serum HCV-Ab by ELISA, HCV-RNA-PCR & Serum AFB1. **Results:** The number of contaminated samples with AFB1 in chronic hepatitis C patients were (23) ,with a mean level of 16.5 ng/ml and a percentage of contamination of 76.7% which were significantly higher than control group, Pvalue(0.04). This present study demonstrated that no statistical significant difference between male and female as regarding contamination with AFB1 and no statistical significant difference between contaminated and non contaminated cases with AFB1 as regarding the mean age. The serum samples of HCV patients which were contaminated with AFB1 showed higher levels of ALT, AST, ALP than non contaminated similar cases. The present work could demonstrate a positive correlation between HCV-RNA-PCR (copy / ml) and ALT and AST. Also between aflatoxin B1 and HCV-RNA-PCR (copy /ml). In addition to a highly significant positive correlation between modified fibrotic score and AST and ALT levels. **Conclusion :** the present work could demonstrate a correlation between AFB1 and HCV which indicate additional factor to our public problems of HCV increased prevalence in Egypt.*

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INTRODUCTION

The liver diseases are important health problem in Egypt. The high incidence of hepatitis C virus infection among Egyptians which is about 8.7%[25], 9.22%[35] and 19.2%[39] is annoying for the individual as well as the governmental authorities and health care providers.

The complications of HCV infections which vary from chronic liver illness to hepatocellular carcinoma are frequently represented daily at any general hospital all over Egypt.

The mode of transmission of hepatitis C virus infection in Egypt and the reasons of its high incidence are not completely understood. The Aflatoxins are a group of potent hepatotoxic and hepatocarcinogenic secondary fungal metabolites which can be widespread in human foods [11]. Aflatoxin B1 (AFB1) is metabolized predominantly in hepatocytes by microsomal mixed-function oxygenase enzyme system to various reduced and oxidized derivatives including

an unstable reactive AFB1-8,9-epoxide, which can bind covalently to nucleophilic sites of biologically macromolecules including nucleic acids and proteins[20]. The formation of AFB1- guanine adducts has been shown to be critical for the carcinogenesis induced by AFB1 in animals[29]. Measurement of AF exposure was relatively crude in all studies, in addition the available data provide no information on the biologically effective dose of AF at the individual level. i.e. the amount of the activated agent that has actually reacted with critical cellular targets. such as DNA or RNA, but only on individual exposure, i.e., the concentration of a particular chemical to which the individual is subjected based on estimates in the food. This former parameter may be influenced by intake, distribution, metabolic (in) activation and excretion of AF, also the interaction with other risk factors for primary heaptocellular carcinoma (PHC), such as HBV, alcohol and tobacco and HCV infection[34].

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Aim of the work:

The aim of this work is to study AFB1 and its possible role in the pathogenesis of HCV infection among Egyptians. The presentation of a relation between AFB1 and HCV infection may have an input in the proper diagnosis, treatment and prognosis of hepatitis C virus disease.

SUBJECTS AND METHODS

Subjects:

This study was conducted on 30 patients with hepatitis C virus infection from Cairo Medical center. They were 22 males and 8 females and the age of the study group was ranging from 34 to 65 years. This study also included twenty subjects as reference group of matched age and sex. All subjects were subjected to the following :

1- Clinical examination:(Chest-Heart-Abdomen)

2- Abdominal ultrasonography.

3- Liver biopsy for patients.

4- Laboratory tests:

- Liver function tests:

* ALT(Alanine aminotransferase).*AST (A spartate aminotransferase).

* ALP (Alkaline phosphatase).*GGT (Gamma Glutamyl transferase).

* Serum total bilirubin.

- Serum HCV antibody by (ELISA).

- HCV RNA PCR

- Serum AFB1.

Sampling:5ml of venous blood was collected from each subject included in the study in a sterile tube without any additives. After coagulation at room temperature, serum was separated from the clot by centrifugation. Samples were stored at -20°C for determination of liver functions, serum HCV antibody by ELISA, HCV RNA PCR and serum aflatoxin B1.

Methods:

Liver biopsy: Ultrasound (US) guided liver biopsy was done to all patients. The bleeding profile (platelets count, bleeding time and prothrombin time and concentration) was checked, and corrected, if necessary. A consent was taken from each patient and the biopsy was performed after an over night fast. Using a 18 G Hepafix needle

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biopsy, a core was taken from the hepatic focal mass (es). The biopsies were fixed in formalin, paraffin embedded, and histological sections prepared and stained, each biopsy was graded using the guidelines set out by Ishak et al., 1995[26].

AST and ALT: the tests were done by a colorimetric determination of the enzyme activity according to the Reitman and Frankel method[36].

ALP: the test was done by a colorimetric determination of the enzyme activity according to Kind and King method [30].

GGT: the test was done by a kinetic determination of the enzyme activity using carboxy substrate according to Szasz et al method[45].

Total bilirubin : the test was done according to Jendrassik and Grof et al method[27].

HCV Ab: The test was done by ELISA of Murex VK 47 third generation[24]

HCV - RNA by Reverse transcriptase polymerase chain reaction (RT - PCR) [21]:

(Monitor, Roche Molecular systems, Nuteley, NJ).

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Enzyme immunoassay for the quantitative analysis of aflatoxin B1[9]:

The basis of the test is the antigen -antibody reaction. The wells in the microtiter strips were coated with specific antibodies to aflatoxin B1. By adding aflatoxin B1 standards or the sample solution and enzyme labelled aflatoxin B1 (enzyme conjugate), free and enzyme labelled toxin competed for the antibody binding sites. Any unbound enzyme conjugate was then removed in a washing step. Enzyme substrate (urea peroxide) and chromogen (tetramethylbenzidine) were added to the wells and incubated. Bound enzyme conjugate converted the colorless chromogen into a blue product. The addition of the stop reagent led to a color change from blue to yellow. The measurement was made photometrically at 450 nm. The absorption was inversely proportional to the aflatoxin B1 concentation in the sample. N.B: The samples were diluted 1+4 with the following buffer (8.75 ml sample buffer +1.25ml methanol 100%). A calibration curve was constructed, the AFB1 concentration in ng/ml was read from the curve .

RESULTS

Table 1: Percentage of contamination with Aflatoxin B1 in patients and control groups.

Group		Aflatoxin B1 positivity		Total	Chi square	P value
		None	Contaminated			
Control	Count	11	9	20	3.9	0.04
	%	55.0%	45.0%	100.0%		
Chronic hepatitis C	Count	7	23	30		
	%	23.3%	76.7%	100.0%		
Total	Count	18	32	50		
	%	36.0%	64.0%	100.0%		

This table showed that there was a statistically significant difference between patients and control cases as regarding the contamination with aflatoxin B1 (p value 0.04).

Table 2: Aflatoxin B1 (ng/ml) levels in control and patients .

Group	N	Mean	Std. Error	Unpaired t	P value
Control	20	1.24	0.347	2.4	0.01
Chronic hepatitis C	30	16.565	4.817		

This table showed that there was a statistically significant difference between control and patients with HCV positive cases as regarding the mean Aflatoxin B1 (p value 0.01).

Table 3: Sex distribution as regarding contamination with Aflatoxin B1 among cases with chronic hepatitis C.

Sex		Aflatoxin B1 positivity		Total	Chi-square	P value
		None	Contaminated			
Female	No	3	5	8	0.3	>0.05
	%	37.5%	62.5%	27%		
Male	No	4	18	22		
	%	18.2%	81.8%	73%		
Total	No	7	23	30		
	%	23.3%	76.7%	100%		

This table showed that there was insignificant difference between males and females as regarding contamination with Aflatoxin B1 (p value >0.05).

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