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MONITORING OF AFLATOXIN EXPOSURE IN URINE OF EGYPTIAN SCHOOL CHILDREN OF DIFFERENT SOCIOECONOMIC STANDARDS

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ABSTRACT

Aflatoxins are Fungal metabolites commonly found as Toxic contaminants of food commodities. They are produced by several species of Aspergillus flavus moulds when growing under conditions of high temperature and high relative humidity and contain a group of highly toxic secondary mould metabolites which have been, identified as potent hepatocarcinogens. The present study was aimed to estimate urine level of Aflatoxin B1 in school children of different socioeconomic standards. For this purpore, 100 of apparently healthy school children were enrolled in this study, 54 females (28 from low socioeconomic standard level and 26 from high socioeconomic standard level) and 46 males (21 from low socioeconomic standard and 25 from high socioeconomic standard level), the age of the study group ranging from 6-16 years divided into 2 groups, less than 9 years (27) and more than 9 years (73), urine samples were submitted To extraction then clean up using column chromatography followed by drivatization and HPLC determination of Aflatoxin B_1

Results: Total number of contaminated samples was 36 with a percent of contamination of 36%. In the female group it was 31.4% while in the male group it was 41.3% which was higher. From socioeconomic view, percent of contamination was 23.5% and 49% in high and low socioeconomic standard groups respectively. In the group less than 9 years, percent of contamination was 29.6% while it was 38.4% in the group more than 9 years. So percent of contamination increases in males than females, in low socioeconomic standard than high and in the age group more than 9 years. This study can conclude that school age children in Egypt in under potential risk of exposure to AFB1which threaten their liver health status by away or the other, the extent of the problem in not well defined but the hazards are many.

INTRODUCTION

Aflatoxin are mycotoxins produced by several species of Aspergillus flavus moulds. They have been found in various plants products including peanuts, copra, Soya, or in cereals such as maize, rice and wheat. Aflatoxin B1 (AFB1) is the most commonly occurring variety and one of the most carcinogenic *(Bean and Yourtee, 1989).*

Several epidemiological studies have found positive associations between AFB1 dietary exposure and an increased risk of human liver cancer *(Zhu et al, 1987).*

El-Khadem et al, (1975) have reported that more than 50% of the Aspergillus flavus strains isolated from peanuts collected from 3 provinces of Egypt were good aflatoxin producers. The temperature, humidity conditions in Egypt and conditions under which food stuffs were produced, stored, and processed led to the conclusion that in Egypt the potentiality for the contamination of food stuffs by aflatoxin exists.

At present, the biological samples of interest for human monitoring of AFB1 are urine, blood, milk and tissue samples.

Among the hydroxylated urinary metabolites, AFB1 was found to be an excellent marker with a linear relationship between its excretion and the absorbed dose ***(Groopman et al 1992).***

The possibilities for the measurement of AF exposure in biological samples include the measurement of parent AF and/or their metabolites in these samples or the measurement of AF adducts with DNA or protein which are of major interest because they are direct products of damage to a critical cellular macromolecular target ***(Ozturk and Collaborators, 1991).***

The availability of data to elucidate the relative contribution of AF exposure and HBV infection and their mechanisms of interaction in the liver carcinogenesis will influence decisions regarding the most appropriate public health measures for prevention of hepatocellular carcinoma in any given country ***(Makaranada et al, 1998).***

No available data about the relationship of Aflatoxin to other types of hepatitis, HCV that considered as a national health problem in Egypt might carry certain relation to mycotoxin exposure. The exposure starts from childhood period.

The repeated exposure to different mycotoxins was demonstrated to prepare the hepatic parenchymal media to hepatitis ***(Makaranada et al 1998).***

AIM OF THE WORK

To investigate AFB1 level in urine of school children of different socioeconomic standards as a result of contamination of their food to find basic data for such type of research.

SUBJECTS AND METHODS

Subjects: This study conducted on 100 of apparently healthy school children from Kalubya and Bohera governorates, 54 females, (28 from low socioeconomic standard level, and 26 from high socioeconomic standard level) and 46 males (21from low socioeconomic standard level and 25from high socioeconomic standard level). The age of the study group ranging from 6-16 years divided into less than 9 years (27) and more than 9 years (73).

Sampling: 100ml of morning urine sample was collected (not related to any preparatory stage or timing) from each child in a sterile disposable container. The samples were put in icebox and transferred to the laboratory for the extraction and determination of AFB1which was done in central lab. of residue analysis of pesticides &heavy metals in food. (Agriculture research center, ministry of agriculture)

Method of analysis: In this method there are some methods were combined together ***(AOAC1995, Farag et, al., 1989 and CEN/TC 275 N 281 Method 1998)*** and some modifications were used to proportion with the laboratory facilities. The method of analysis included extraction then clean up using column chromatography followed by drivatization and HPLC determination.

Extraction: The 100-ml of urine was extracted with 50 ml Chloroform and another two portions of 50 ml Chloroform. The combined extracts were pass through anhydrous sodium sulfate and evaporated by rotatory evaporator under vacuum at 40°C.

Column preparation: A ball of glass wool was placed into the bottom of chromatographic Column (plastic syringe 5ml), 0.5 gm of anhydrous sodium sulfate, 2ml of dichloromethan, then 0.5 gm of silica gel were added and the sides of column were washed with 1ml of dichloromethan. The dichloromethan was drained to the top of silica gel layer, then 0.5 gm of sodium sulfate was added and 1ml of dichloromethan was added to the top of sodium sulfate layer.

Clean up using Column chromatography: The extract was dissolved in 2 ml dichloromethan and added to the prepared

column, another 2ml of dichloromethan was added to get all the residues from the flask and the residues was transferred to the column. The solvent gets out from the column with medium flow rate (2ml/min).

The sides of the flask were washed with two portions of 5ml dichloromethan and added to the column. The last 10ml of dichloromethan was get out of the column with maximum flow rate (5ml/min.), then 10ml of benzene/ acetic acid (9+1), 10 ml hexane were added and discarded all with maximum flow rate (5ml/min.).

The aflatoxin was eluted with 10ml methanol-Chloroform (3 + 97) which received in 10ml glass tube with medium flow rate (2ml/min.), then tube evaporator with nitrogen was used for evaporation of the eluent.

Drivatization: 200ul of hexane and 50 ul of trifloro acetic acid (TFA) were added to the previous tube. The tube was capped and vortexied vigorously for 30 s exactly the layer was left to stand 5 min. 1.95ml acetonitrile-water (1:9) was added and the tube was capped and vortxied for 30 s exactly. The layer was left to separate for 10 min. The lower aqueous layer was used for HPLC determination.

The mobile phase consisted of water – methanol–acetonitrile (10:17:17 v/v/v), which injected into HPLC column (O.D.S. C 18% cartilage type), at elution rate 0.8-1 ml/ min and pressure 3000 psi. The aflatoxin B1 was detected at 360 nm (Fluorescence detector). The chromatographic peaks of the tested samples were compared with peaks resulting from injecting a positive standards into the HPLC columns and then qnantitated (positive standards were supplied by Sigma lab. USA) when the signal from the detector is potted as a function of time, distance or volume, the detected aflaloxins were quantitated.

Results calculation. The analyte concentration in sample Cs (ng/ml) is calculated as following:

Cs = Hs / H st X C st / Sv X D/I

Cs =concentration of the toxin in sample ng/ml.

Hs =peak height of the standard.

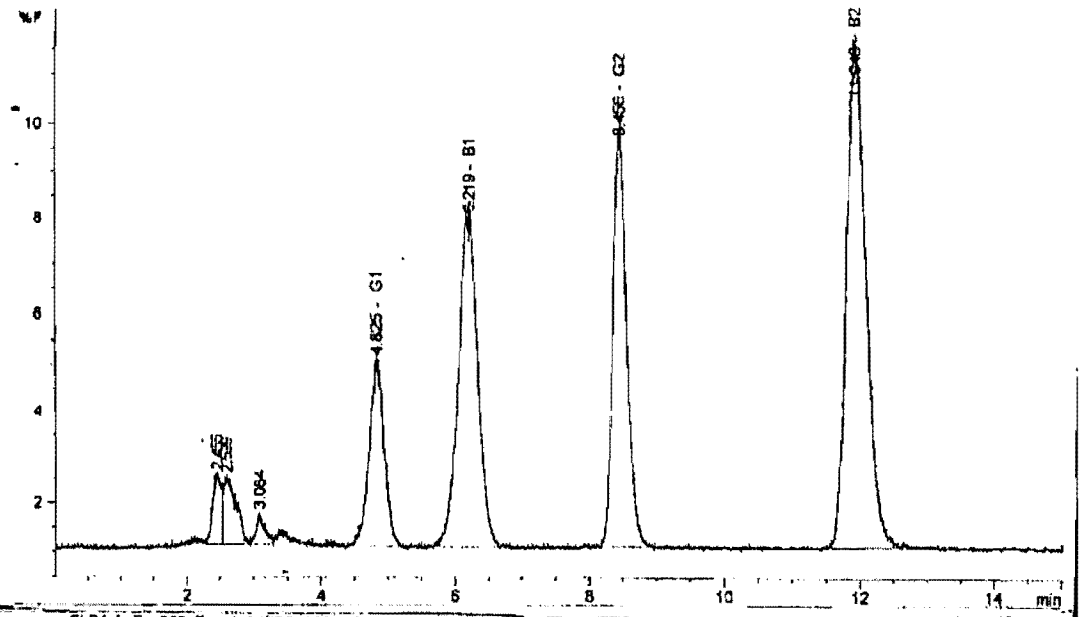
Cst =standard concentration (ng)

Sv = sample volume (ml).

D= dilution (ml).

I =injection volume (ml).

A



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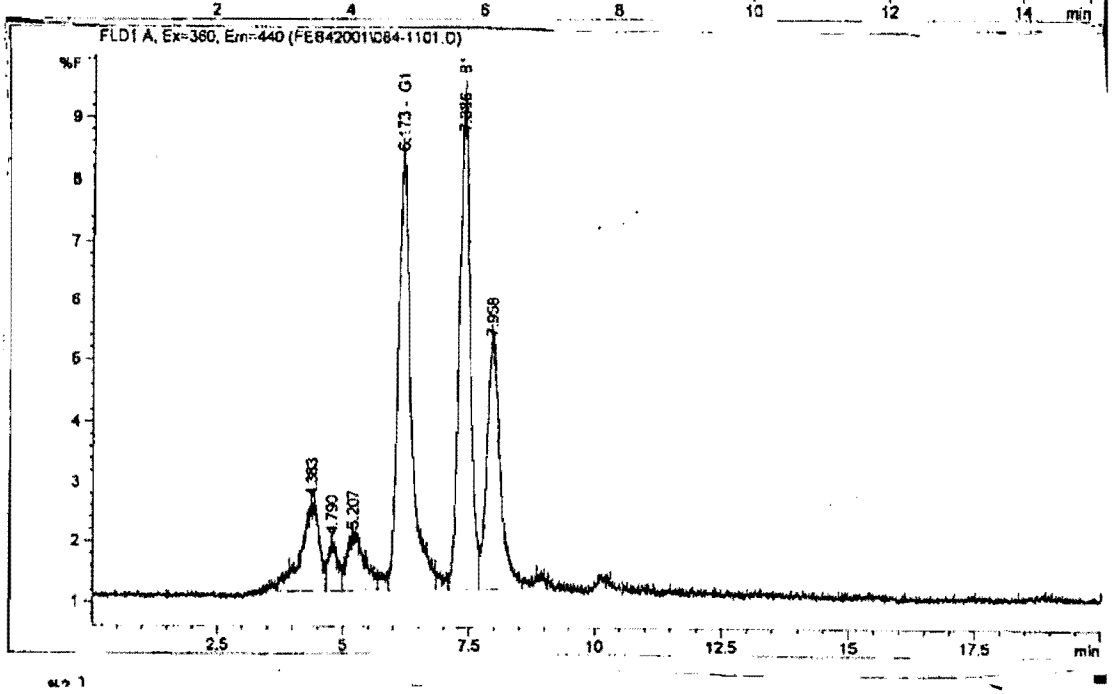

A: Chromatogram of AF standard

B: Chromatogram of urine sample contain High AFβ_1 Level

RESULTS

Table (1): Total number of subjects included in the study with consideration of classification parameters (socioeconomic standard, sex, and age)

Total	male	Female
100	46	54
Total	low	High
100	49	51
Total	<9 years	>9 years
100	27	73

Table (2): Sex consideration in distribution of included subjects in the study and percentage positivity to AFB1

Sex		AFB1		Total
		-ve	+ve	
Males	count	27	19	46
	% within Sex	58.7%	41.3%	100.0%
Females	count	37	17	54
	% within Sex	68.5%	31.5%	100.0%
Total (males&females)	count	64	36	100
	% within Sex	64.0%	36.0%	100.0%

There is no statistically difference between male and female as regards the distribution of positive cases. Fisher exact test p=0.40

Table (3): Socio economic standard consideration in distribution of subjects included in the study and percentage positivity to AFB1

Standard level		AFB1		Total
		-ve	+ve	
High	count	39	12	51
	% within high level	67.5%	23.5%	100.0%
Low	count	25	24	49
	% within low level	51%	49%	100.%
Total	count	64	36	100
	% within all	64.0%	36.0%	100.%

There is a statistically significant difference between standard levels as regards the distribution of positive cases, with higher percentage among the low level. Fisher exact test P= 0.02