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A Survey on Relationship between Diet and Urinary Excretion of Aflatoxin M_1: A Screening Pilot Study on Iranian Population

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Abstract

Background: Aflatoxins are found in the most types of foods such as corn, peanuts, pistachio, rice, milk, and etc. These toxins may cause several adverse health effects, notably cancer. The main aim of this study was to evaluate excretion level of aflatoxin M_1 (AFM_1), as a biomarker of aflatoxin B_1 exposure, in urine samples of Iranian population. Also, relationship between diet and urinary excretion of AFM_1 has been discussed.

Methods: From June to August 2014, this study was carried out among 70 staffs from Faculty of Health, Shahid Sadoughi University of Medical Sciences, Yazd, Iran. From each participant, 72-h dietary recall was asked and recorded. Then, urine samples were collected separately in the sterile plastic falcon and transferred immediately to the laboratory and stored at -20 °C. AFM_1 was assessed in each sample using ELISA procedure. The data were analyzed by Kruskal–Wallis one-way analysis of variance test using SPSS software (16.0).

Results: AFM_1 was detected in 15 of 70 (21%) urine samples. The mean levels of contamination in faculty members and non-faculty members were 0.6 and 1.7 pg/ml, respectively. There was a significant difference between the excretion of AFM_1 and consumption of nuts as well as Iranian traditional confection ($p<0.05$). No relationship was found between the demographic factors and excretion of AFM_1 ($p>0.05$).

Conclusions: Considering adverse health effect, aflatoxin exposure in this Iranian population should be reduced. So, comprehensive training of the people is one of the most practical and useful methods for reducing aflatoxin health risks.

Introduction

Aflatoxin (AF) is a group of mycotoxin compounds, which is produced in foods by three main species of *Aspergillus* genus including *A. flavus*, *A. parasiticus* and *A. nomius* (Atanda et al., 2007; Ehrlich et al., 2007; Khodadadi et al., 2014). AFs are mainly found in rice (Feizy et al., 2010), sesame (Asadi et al., 2011), nuts (Pour et al., 2010), and milk as well as dairy products (Rahimi et al., 2010).

Typically, there are four types of AFs including AFB_1, AFB_2, AFG_1 and AFG_2 that are recognized as high toxic compounds. Liver is the main organ affected by these carcinogenic toxins (Groopman and Kensler, 2005). The existence of AFs in foods may cause chronic health effects including immune-system suppression (Shuaib et al., 2010), impaired childhood development (Sherif et al., 2009) and notably cancer. Also, acute toxicity can lead to

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death (Iha et al., 2011; Smith et al., 2012; Yazdanpanah et al., 2013).

Exposure assessment is one of the most important parts of the risk assessment process (Sherif et al., 2009; Wu et al., 2009). Dietary intake of AF contaminated food is the main source of human exposure to AFs (Egal et al., 2005; Lewis et al., 2005). Human exposure to AFB_1 has been determined by excretion of its metabolites in urine, milk and blood. Approximately, 1.2-2.2% of the AF received through food is excreted as AFM_1 in urine of human (Asadi et al., 2011; Cheraghali et al., 2007; Leong et al., 2012; Mahdavi et al., 2010; Pour et al., 2010; Rahimi et al., 2010; Romero et al., 2010; Sadeghi et al., 2009; Zaghini et al., 2005).

The presence of AFM_1, a hydroxylated metabolite of AFB_1 and one of the most important AF metabolites, in breast milk provides main source of its exposure for lactating newborn infants and breast feeding (Afshar et al., 2013; Battacone et al., 2005; Iha et al., 2011; Leong et al., 2012; Mahdavi et al., 2010; Mckean et al., 2006; Partanen et al., 2009; Polychronaki et al., 2008; Sadeghi et al., 2009; Yazdanpanah et al., 2013; Zaghini et al., 2005).

Among some previous similar published studies, Sabran et al. (2012) found significant relationship between consumption of milk and dairy products and excretion of AFM_1. Romero et al. (2010) evaluated excretion of AFM_1 in 69 people in Brazil, 45 of them (65%) had contamination. Polychronaki et al. (2008) detected AFM_1 in 38% of Egyptian and also 86% of Guinean urine samples.

As it was thought the staffs of Faculty of Health had enough knowledge of AF and its risks; it was expected that they may have less exposure to it in comparison with others. Therefore, in this study, we decided to investigate relationship between diet and urinary excretion of AFM_1 in staffs of Faculty of Health, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

Material and methods

Ethical approval

This research was approved by Shahid Sadoughi University of Medical Sciences, Yazd, Iran (Protocol No. 3493).

Informed consent was obtained from each participant before beginning the study.

Sampling

This study was a cross sectional-descriptive study that was done from June to August 2014. Seventy people were selected among the eligible employees including academic and non-academic staffs.

Urine samples were collected separately in the plastic falcons and transferred immediately to the laboratory and stored at -20 °C.

Determination of the AFM_1

ELISA kit (6827 BN Arnhem, Euro Proxima Company, Arnhem, Netherlands) was used for detection of AFM_1. The sample preparation was performed according to the manufacturer's instructions. All samples were centrifuged for 10 min in 2000 ×g at 4 °C. Each urine sample was diluted 1:1 with sample dilution buffer, mixed well and distributed in 100 µl inside the respective wells of the plate.

According to the manufacturer's instructions, the AFM_1 standards and test urine samples were added in duplicate to each well plate, and then incubated at room temperature (20-25 °C) for 1 h in darkness. After that, the solution was discarded from the microtitre plate and washed 3 times with rinsing buffer. Then, 100 µl of conjugate (Aflatoxin M_1 -HRPO) were added to each well, except zero standard maximal wells. The microtitre plate was shaken for few seconds on a microtitre plate shaker, and then incubated at room temperature for 30 min in darkness. The solution was discarded from the microtitre plate and washed 3 times with rinsing buffer. Amount of 100 µl of substrate solution were added into each well and incubated at room temperature for 30 min.

Finally, 100 µl of stop solution were added to each well. The optical absorbance was read at 450 nm with microplate reader (ELX 800 UV, Bio-Tek Instruments, Inc).

Statistical analysis

Kruskal–Wallis one-way analysis of variance test was used to determine the relationship between type of dairy products, type of rice, place of residence and demographic factors with the concentration of AFM_1. Mann–Whitney test was used to compare the two groups and determine the relationship between food intake and the concentration of AFM_1 in urine. The data were analyzed using SPSS software (16.0).

Results

AFM_1 was detected in 15 of 70 (21%) urine samples of the studied population (Table 1). The mean levels of contamination in academic and non-academic staffs were 0.6 and 1.7 pg/ml, respectively.

Results of this study revealed that no significant relationship was observed between academic and non-academic staffs with excretion level of AFM_1 ($p>0.05$). There was a significant difference ($p<0.05$) between the excretion level of AFM_1 and consumption of nuts as well as Iranian traditional confection (Table 2). No relationship (p>0.05) was found between the socio-demographic factors and excretion level of AFM_1 (Table 1).

Table 1: Occurrence of AFM_1 in urine samples of participants according to socio-demographic factors

Socio-demographic factors	Sample tested No.	Positive samples No.	Min- max (pg/ml)	Mean (pg/ml)
Occupational position				
Academic	9	2	0-5.8	0.6
Non- Academic	61	13	0-42	1.7
Age (year)				
20-30	14	2	0-5.8	0.4
31-40	31	9	0-42	2.1
41-50	25	4	0-32.6	1.4
Professional experience (years)				
<5	26	5	0-8.6	0.7
5-10	8	3	0-42	5.5
>10	36	7	0-32.6	1.3
Educational level				
BSc	35	6	0-32.6	1.3
MSc	27	7	0-42	2.1
PhD	8	2	0-5.8	0.7
Sex				
Male	32	7	0-9.2	0.6
Female	38	8	0-42	2.3
Monthly income				
<387$	23	4	0-9.2	0.6
387-1160 $	38	9	0-42	2.3
>1160 $	9	2	0-5.8	0.6
Marital status				
Married	64	12	0-42	1.6
Single	6	3	0-5.8	1.3

Table 2: Occurrence of AFM_1 in urine samples of participants according to consumed foods in recent 72 h

Consumed foods in recent 72 h	Sample tested No.	Positive samples No.	Min- Max (pg/ml)	Mean (pg/ml)	*P* value
Milk					
Yes	64	14	0-42	1.7	0.5
No	6	1	0-0.1	0.1	
Meat					
Yes	62	12	0-32.6	1.1	0.06
No	8	3	0-42	5.6	
Iranian traditional confection					
Yes	16	6	0-42	5	0.01
No	54	9	0-9.2	0.5	
Nuts					
Yes	24	10	0-42	4	0.01
No	45	4	0-3.6	0.1	
Halva Ardeh (confection made from sesame)					
Yes	5	1	0-1.8	0.3	0.6
No	64	14	0-42	1	
Rice					
Native					
Yes	42	10	0-42	1.9	0.79
No	1	0	0	0	
Imported					
Yes	10	2	0-9.2	1.3	0.72
No	1	0	0	0	
Both					
Yes	16	3	0-8.6	0.9	0.37
No	0	0	0	0	

Discussion

In this study, AFM_1 was found in 21% of participant's urine samples with the range of excreted AFM_1 at 0–42 pg/ml. Lei et al. (2013) detected no significant difference between the excretion level of AFM_1 and sex that is similar with our investigation in which no significant relationship was observed between all socio-demographic factors and AFM_1 excretion level in urine samples of participants.

Several studies have examined the relationship between food intake and urinary excretion of AFM_1. Redzwan et al. (2012) found significant relationship between consumption of milk and dairy products and excretion of AFM_1. In another investigation, Romero et al. (2010) evaluated the presence of AFM_1 in human urine samples from a Brazilian population regarding their corn, peanut, and milk consumption amounts. Their results showed no significant difference between food consumption and excretion of AFM_1 that is in accordance with our findings.

Based on this study, there was not found any significant differences between the excretion level of AFM_1 with the taking some of foods such as milk, meat, rice and Halva Ardeh (a confection made from sesame). But, there was significant association between AFM_1 excretion and consumption of nuts. Several studies have reported that AFB_1 occurs in edible nuts of Iran as high as 77-99% (Cheraghali et al., 2007; Pour et al., 2010; Yazdanpanah et al., 2013) that confirm this finding observed in our survey.

It was observed that the urinary excretion level of AFM_1 in participants who had used traditional confection of Yazd, Iran was significantly higher than those who had not consumed. This matter could be due to considerable AFB_1 contamination rate of major ingredients used in this Iranian traditional product. Wheat flour, walnuts, pistachios and almonds are the main components which are usually used to produce traditional confection of this region of Iran. It seems that due to economic benefit, some Iranian confectioners generally use low quality ingredients which are cheaper than high quality ones. Based on present scientific databases, it has been indicated that low quality nuts and cereal are generally more contaminated to AFB_1 (Afshar et al., 2013; Cheraghali et al., 2007; Feizy et al., 2010; Montaseri et al., 2014; Pour et al., 2010; Sadeghi et al., 2009; Yazdanpanah et al., 2013).

Excretion of AFM_1 in this study was lower than in countries such as Brazil, Egypt (Iha et al., 2011; Polychronaki et al., 2008). It should be noted that minimum education level of all participants in this survey was BSc. It can be assumed that individuals with higher education level may be more awarded and knowledgeable about AF contamination and its implication for human health, and this is probably the main reason for the low excretion of AFM_1 the participants of this survey.

Previous studies have shown that probiotic bacteria could reduce the detrimental effects of AF (Byun and Yoon, 2003; Hernandez Mendoza et al., 2009; Montaseri et al., 2014). Therefore, the consumption of probiotic yogurt should be increased to minimize the adverse health effects of the toxin. Also, some researchers have indicated that using clay, as a good adsorbent for AF in intestinal, could effectively reduce human exposure to AF (Phillips et al., 2008). However, it seems that comprehensive training of the people is the most practical and useful method for this proposes.

Conclusion

Excretion level of AFM_1, as a biomarker of AFB_1 exposure, in urine samples of Iranian population was evaluated. Our findings indicate that exposure to the AF in staffs of Faculty of Health, Shahid Sadoughi University of Medical Sciences, Yazd, Iran was relatively notable. Considering severe adverse health effects of AF, the state of a comprehensive plan for training of the people to avoid eating high contaminated food, is one of the most practical and useful methods for reducing AF health risks.

Conflicts of interest

The authors declare that they had no conflict of interest.

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