Original Article

The concentration of aflatoxin M_1 in the mothers' milk in Khorrambid City, Fars, Iran

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Abstract

Introduction: Aflatoxins are secondary toxic metabolites produced by certain group of *Aspergillus* species in suitable conditions. These toxins are highly toxic, immunosuppressive, mutagenic, teratogenic and carcinogenic metabolites. The purpose of this study was to detection aflatoxin M_1 concentration in mother's milk from rural area of Khorrambid town of Fars Province.

Materials and Methods: In this study, 87 milk samples of mothers were collected by cluster sampling methods in the period between June and July 2011 and the amount of aflatoxin M_1 was measured by a competitive ELISA method.

Results: From 87 mother's milk, 24 (27.6%) samples were contaminated with aflatoxin M_1 with mean concentration of 0.56 ± 1.23 pg/ml (range 0.13-4.91 pg/ml).

Conclusion: The amount of aflatoxin M_1 in mothers' milk was lower than 50 ng/l (Europe Union and Iranian standard). Detection of Aflatoxin M_1 in mothers' milk is due to consuming contaminated food. This contamination not only threatens the health of the mothers but also has irreversible effects on the growth and health of their babies.

Key Words: Aflatoxin M_1, enzyme-linked immunosorbent assay, milk, mothers, mycotoxins

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INTRODUCTION

Breastfeeding is useful for the health of both mothers and infants. Infants, who are exclusively breastfed, are protected from many diseases.[1] Sometimes some unwanted or unfavorable contaminants such as mycotoxins, which are associated with maternal diet, are secreted in milk.[2] Although nutrition is something vital for human survival, however consumption of contaminated foodstuffs is one of the most important ways for toxic and carcinogenic materials entering the body. In early life, breast-feeding is usually the main method of feeding. Because children, particularly newborns, are more susceptible to various diseases, therefore breast milk should be free from harmful substances and toxins. Mycotoxins are secondary toxic metabolites of filamentous fungi, which are produced under favorable conditions on variety of food and feedstuffs.[3-5] Several studies have proved the

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presence of aflatoxins in human body fluids including milk.[6-8] Preparation, processing and consumption of foods in a safe manner are very important. Aflatoxins are highly toxic, immunosuppressive, mutagenic, teratogenic and carcinogenic metabolites. Different species of *Aspergillus*, such as *A. flavus, A. parasiticus and A. nomius* can grow on foodstuffs under appropriate conditions, and their toxins are usually found in human and animal body fluids including milk.[5,6,8-10] Aflatoxins are found in nuts, corn, cotton seed and crops before harvest.[11] Aflatoxin M_1 (AFM_1) is the main secondary metabolites found in milk secreted by animals and nursing mothers who have consumed food contaminated with aflatoxin B_1 (AFB_1).[12] International Agency of Research on Cancer (IARC) has classified aflatoxin M_1 and B_1 in terms of carcinogenesis in human beings in class 1.[10] Children are considered to be more susceptible to mycotoxins than adults because of their lower body weight, higher metabolic rate, lower ability to detoxify and incomplete development of some organs and tissues, notably the central nervous system.[13-14] In our country, few studies have been conducted about the concentration of aflatoxin M_1 in human breast milk. In the villages of Khorrambid Town, the possibility of fungal infections is higher because most people are employed in farm and livestock business and store most of the food and feedstuffs in traditional methods. Furthermore, fungal toxins in mother's milk can threaten the health of mother and her infant and consequently society's health. Therefore, this study was designed in order to determine the concentration of aflatoxin M_1 in mothers' milk samples.

MATERIALS AND METHODS

Informed written consent was obtained from all the women before inclusion in this study. A total of 87 mother breast milk samples (5-10 ml) collected into sterile glass bottles by self-extraction from 7 rural health centers in Khorrambid-Iran, during June-July 2011. The criteria for inclusion was the health of nursing mothers who had healthy infants who were full term and breastfed, aged less than one year with normal birth weight, and with no chronic disease. All samples were kept at –20°C until the time of analysis. The samples were thawed gradually at 4°C and then vigorously mixed and were centrifuged for 15 min at 3500 g/10°C. Then, the upper creamy layer was completely removed by using a Pasteur pipette and the skimmed milk (lower phases) was used directly for testing AFM_1.

The quantitative detection of AFM_1 in the samples was performed according to the instructions of the competitive enzyme immunoassay using Euroclone kits (Italy). The test is based on the antigen-antibody reaction. The wells in the microtiter strips were coated with specific antibodies to AFM_1. 200 µl of samples (standard solutions and milk samples) were added to the wells to occupy the binding sites proportionately then mixed gently and incubated for 30 min at room temperature in the dark. Then the liquid was poured out of the wells and the wells were filled with 300 µl washing buffer and poured out the liquid. Then other steps were done by the kit instruction. The mean lower detection limit of the Euroclone AFM_1 test was 5 ppt. Data were analysed using the software PASW-18, with descriptive statistical methods (calculation of the pollution, the mean and Standard Deviation) and tests of Mann-Whitney, Kruskal-Wallis and the correlation coefficient.

RESULTS

Our results showed out of 87 breast milk samples, 24 were contaminated by aflatoxin M_1 that is, the mean concentration levels of aflatoxin M_1 were 0.56 ± 1.23 and ranging from 0.13 to 4.91 pg/ml [Table 1].

Mean and standard deviation of data related to anthropometric characteristics of 87 mothers and their infants and their correlation with aflatoxin M_1 concentration are presented in Table 2. In this study, 11.5%, 28.7% and 5.7% of the mothers were respectively underweight, overweight and obese and 54% of mothers had a normal BMI. Also, 55 (63.2%) and 52 (59.8%) of mothers worked on farms and livestock, respectively. Twenty (23%) mothers also had

Table 1: Occurrence of AFM_1 in mother's breast milk

Variables	*n*	Positive samples	AFM_1 concentration (pg/ml)	
			Range	Mean±SD
Aflatoxin M_1	87	24 (27.6%)	0.13-4.91	0.56±1.23

Table 2: Maternal and infant's descriptive data and their correlation with the Aflatoxin M_1 Concentration in breast milk samples

Variables	Mean±SD	Aflatoxin M_1	
		R	*P* value
Mothers' age (Years)	27.4±5.18	-0.07	0.47*
Mothers' weight (kg)	59.4±10.34	-0.02	0.81*
Mothers' height (cm)	158.9±5.4	0.18	0.07*
Mothers' BMI	23.5±4.04	-0.09	0.37*
Infants'age (Months)	5.26±2.78	-0.02	0.86*
Infants' weight at birth (kg)	3.17±0.52	-0.02	0.83*
Infants' height at birth (cm)	49.21±3.46	-0.04	0.67*
Infants' head circumference (cm) at birth	34.73±1.66	-0.25	0.016*

*Spearman's correlation coefficient

natural miscarriage. Besides, in terms of education, 57 (65.5%), 24 (27.6%) and 6 (6.9%) were high school drop-outs, had high school diploma, and had higher education, respectively.

In the present study, the relationship between the concentration of aflatoxin M_1 and the villages being sampled, was significant (P = 0.005). As shown in Table 2, Spearman's correlation test showed that there is a significant reverse linear relationship between aflatoxin M_1 and infants' head circumference (r = –0.25 and P = 0.016). However, based on this test, no significant relationship was observed between aflatoxin M_1 concentration with other variables related to mother and child [Table 2]. Non-parametric Mann-Whitney test showed that in the studied mothers, the relationship between the concentration of aflatoxin M_1 with livestock, farming activities and abortion was not significant (P > 0.05) and non-parametric Kruskal-Wallis test showed that no relationship was observed between the level of mothers' education with the concentrations of aflatoxin M_1 (P = 0.78).

DISCUSSION

Spore of different fungi including *Aspergillus, Penicillium* and *Fusarium* easily contaminate agricultural products under appropriate conditions during various stages of planting, growing and harvesting. Humans and animals are exposed to receiving fungal toxins due to consumption of contaminated foods.[15] Exposure of human to aflatoxins can be determined by a combination of food analysis and food intake studies or by monitoring levels of aflatoxins in biological fluids. Monitoring the levels of aflatoxins in biological fluids is more reliable indicator of actual exposure.[6] Aflatoxin M_1 is considerably toxic and carcinogenic. However, the health risk of this toxin has not been well assessed in breast-fed infants.[16] The presence of aflatoxin M_1 in the breast milk indicates that mother has consumed foods contaminated with Aflatoxin B_1, and her embryo or her infant exposed with the contaminated milk.[5,17] Considering the fact that mother milk with natural and healthful properties is unique for nutrition of an infant, breast feeding is encouraging all over the world yet. People including nursing mothers in lactating period, are exposed with different naturally occurring and/or synthetic contaminants, and nearly all nutrients are also polluted with these types of contaminants in different degrees. Therefore, importance of feeding with natural and safe foods including milk is becoming popular day by day.[18]

In our study, aflatoxin M_1 has a significant reverse relationship with infants' head circumference at birth (P = 0.016). However, in previous studies from Iran, like those by Mahdavi[19] in Tabriz and Sadeghi[20] in Tehran, the relationship between the infants' height and this toxin was reported significant (P < 0.05). In our study, the relationship between mother's height and aflatoxin M_1 was not significant (P = 0.07), however, this relationship might become significant in a study with more samples.

As our results demonstrated, out of 87 samples, 24 samples (27.6%) were positive for AFM_1. Aflatoxin M_1 concentration in this study was less than that found in a study in Egypt with ranges of 6.3-497 pg/ml and 4.2-108 pg/ml.[21] The contamination rate and range in the present study were less than those in Egypt (36% of contamination with range of (10.27-21.43 pg/ml).[22] The mean of aflatoxin M_1 concentration in this study was reported lower than that in other studies such as 71 pg/ml in Victoria,[6] 560 pg/ml in the United Arab Emirates,[9] 664 pg/ml in Thailand,[6] 2100-9200 pg/ml in Gambia,[5] 20-1816 ng/l in Liverpool, Britain[23] and 6.96 pg/ml in Tabriz.[19] Besides, the frequency of aflatoxin M_1 contamination in this study was reported lower than that in Ahwaz with 1300-12500 pg/ml.[24] A reason may be the hot and humid climate of Khuzestan, which provides appropriate conditions for growing fungi on the food and agricultural products and also fungal contaminations. However, in the present study, the studied region has a temperate cold and dry weather which may provide more restricted conditions for the growth of aflatoxigenic species of *Aspergillus* and the reason for the low incidence of AFM_1 contamination in milk of mothers in this region may contribute to this issue. The number and prevalence percentage of AFM_1 contamination of mothers' milk in a study in Tabriz consisted of 20 samples and 22%, respectively which to a great extent is consistent with the present study and perhaps, it is due to similarities of climatic conditions of both the regions (cold weather).

CONCLUSION

Based on the results of this study, the milk samples were contaminated with aflatoxin M_1 which can have some adverse effects on infants' growth parameters. However restriction of breast feeding is not an acceptable advice for mothers. Specific regulations for control of AFB_1 in feeds and a systematic program monitoring of Aflatoxin M_1 in milks is emphasized to reduce the level of such toxins. This requires a regular program to prevent the growth of the toxigenic fungi in all agricultural and food products. More investigations are necessary to determine the level of this toxin in human body fluids and nutrition diets to

find the relationship between this toxin and health parameters.

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