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ORIGINAL ARTICLE

Exposure of Infants to Aflatoxin M1 from Mother's Breast Milk in Ilam, Western Iran

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Abstract
Objectives: Aflatoxins as a highly toxic group of mycotoxins are present in the environment and foodstuff. These have been reported to cause serious health problems in humans. Since aflatoxin M1 (AFM1) is excreted into breast milk, investigating the exposure of infants to AFM1 is of special concern.
Methods: In the present study, breast milk samples were collected from 85 lactating mothers in Ilam province, Iran, and the levels of AFM1 were analyzed using the enzyme-linked immunosorbent assay-based technique. AFM1 was detected in breast milk of all lactating women. The mean contamination level was 5.91 $\pm$ 2.031 ng/L, ranging from 2 ng/L to 10 ng/L.
Results: Multiple regression analysis indicated no significant associations of consumption of milk and dairy products, meat, fish, legumes, grain products, fruits, and nuts with the concentration of AFM1 in breast milk. Furthermore, no significant association was observed between AFM1 concentration and anthropometric data of infants.
Conclusion: In western parts of Iran, lactating mothers and their infants could be at risk of aflatoxin B1 and AFM1 exposure, respectively. Therefore, in Iran, the evaluation of AFM1 in human breast milk as a biomarker for postnatal exposure of infants to this carcinogen requires more attention in different regions and various seasons.

1. Introduction

Aflatoxins are a highly toxic group of mycotoxins that are produced by certain species of the genus *Aspergillus*, especially *Aspergillus flavus*, *Aspergillus parasiticus*, and *Aspergillus nomius* [1]. These fungal metabolites are primarily found in different plant and plant products, especially nuts and grains [2]. Several experimental studies have shown that aflatoxins are highly toxic to the liver, and may cause hepatic cancer and acute liver

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cirrhosis [3,4]. Aflatoxins are also reported to be immunosuppressive, mutagenic, and teratogenic [5]. More than 18 aflatoxins have been identified, among which aflatoxin B1 (AFB1) is the most potent carcinogen. In animals fed with AFB1-contaminated food, this toxin is converted into a secondary hydroxylated metabolite named aflatoxin M1 (AFM1), which is subsequently secreted in milk and excreted in urine and feces [6,7]. In fact, AFM1 is detectable in mother's milk at 12−24 hours after the consumption of contaminated food. It rapidly decreases with time and is not detectable after 3 days of stopping the intake of contaminated foodstuff [8]. The transplacental transfer of AFM1 through the fetoplacental interface has been established. Milk and its products are one of the main staples of humans. However, consumption of contaminated milk and dairy products has been shown to cause serious health problems, and children as the major consumers of milk are at high risk for the life-threatening side effects of aflatoxins [6−8]. Recently, the International Agency for Research on Cancer has reconsidered AFM1 as a Group 1 carcinogen. Since AFM1 is resistant to high temperatures used in autoclaving and pasteurization processes [9], there is an urgent need for developing strategies to reduce the aflatoxin levels during the production of milk and milk products, especially during the storage phases. Many countries have regulations to control the levels of AFM1 in milk and milk products. The European Commission recommends that the maximum level of AFM1 in raw milk and dairy products should not exceed 50 ng/kg [10], and for infant food it is limited to 25 ng/kg. However, in China, the maximum permissible level of AFM1 is 500 mg/kg. In Austria and Switzerland, this limit is even lower at 10 ng/kg for baby food [11].

As AFM1 is secreted in human breast milk, infants and neonates are potentially exposed to the adverse effects of aflatoxins such as growth impairment, underweight, and infectious diseases [12,13]. Therefore, investigating the presence and level of AFM1 in human breast milk is of special concern. Several studies have been conducted in different countries to explore the AFM1 levels in breast milk of lactating mothers, and various results have been obtained. Data regarding the AFM1 levels in human breast milk in Iran are insufficient, and to our knowledge, no study has been conducted in Ilam province. Therefore, the purpose of the present study was to evaluate the presence and level of AFM1 in lactating mothers in Ilam, and investigate the possible correlations between AFM1 levels and anthropometric parameters of infants.

2. Material and methods

2.1. Participants and protocol

A total of 85 samples were collected from lactating mothers in Ilam province, west of Iran, during April−October 2014. All volunteers who agreed to participate in this study had full-term delivery. The research protocol was approved by the Ethics Committee of Deputy of Research, Ilam University of Medical Sciences, Ilam, Iran. The participants were informed about the study protocol, and if they agreed to contribute, a written informed-consent agreement was signed. The inclusion criterion was that the lactating women should be apparently healthy. The exclusion criteria were chronic diseases (e.g., diabetes mellitus and cancer), infections, use of medication, and smoking.

At the time of sampling, a questionnaire was filled in giving details of age, gestational age, stage of lactation, postnatal age, sex, birth weight, weight at the time of sampling, and mother's diet (including frequency of consumption of milk and milk products, pistachios, nuts, rice, fruits, vegetables, and meat within 72 hours before milk sampling). Complementary data regarding the socioeconomic position of the mothers, namely, their income, job, and education level, were gathered.

2.2. Sample preparation

Ten milliliters of breast-milk samples were collected by hand expression or manual breast pump during regular feeding of infants into sterile plastic containers. The samples were transported to the laboratory in refrigerated containers (4°C) and stored at −20°C until use. Five milliliters of each milk sample were incubated for 30 minutes at 4°C and centrifuged at 16,100 g for 5 minutes. The skimmed milk below the fat layer was sampled, transferred to a new test tube, and stored at −20°C until use. A competitive enzyme-linked immunosorbent assay (ELISA)-based kit (Beacon Analytical Systems Inc., Portland, ME, USA) was used to determine the AFM1 levels in the skimmed milk sample obtained.

2.3. Analysis of AFM1 in samples by competitive ELISA

The obtained skimmed milk samples and kit contents were left outside for 30 minutes to reach the room temperature. The AFM1 levels were determined according to the manufacturer's instructions. Briefly, 100 μL of the standards and milk sera were transferred to a microtiter plate precoated with AFM1 antibody (supplied with the kit). All samples and standards were assayed in duplicate. The plate was gently shaken and incubated at room temperature for 30 minutes. After the incubation time, the AFM1 horseradish peroxidase (HRP)-conjugated antibody was added to the plate and incubated at room temperature for further 15 minutes. The plate was then washed four times with the washing solution in order to remove the unbound conjugated antibody. The substrate solution (100 μL) was added into the wells, and the reaction was allowed to proceed in the dark for 30 minutes at room temperature resulting in the formation of a blue color. The reaction was then stopped by adding 100 μL of stop solution, which changed the color to yellow. Finally, the absorbance was

Table 1. Descriptive data of the study.

Variables	*N*	Range	Mean ± SD
Mother age (y)	85	19–40	26.31 ± 5.12
Mother weight (kg)	85	46–71	57.94 ± 10.38
Mother height (cm)	85	140	158.1 ± 12.55
Mother BMI	85	18–28	24.61 ± 3.44
Infant age (mo)	85	0.2–21	7.68 ± 2.21
Infant height at birth (cm)	85	41–67	60.21 ± 5.12
Infant weight at birth (kg)	85	1.97–4.1	3.18 ± 0.75
Infant weight at the time of sampling	85	3.11–8.16	5.90 ± 1.12
Infant head circumference (cm)	85	32–44	39 ± 2.01

BMI = body mass index; *N* = number; SD = standard deviation.

read at 450 nm using an ELISA Plate Reader (Tecan, Durham, NC, USA). A standard curve was drawn by plotting absorbance values against AFM1 concentrations. The absorption intensity was inversely proportional to AFM1 concentration in milk samples.

2.4. Statistical analysis

Statistical analysis was performed using SPSS version 16 software. The data were analyzed using multiple linear regressions to evaluate the association between AFM1 concentrations in breast milk and the potential factors as well as infants' anthropometric status. A p value of < 0.05 was considered statistically significant [14].

3. Results

Descriptive data of 85 mothers and their infants are presented in Table 1. AFM1 was detected in breast milk of all lactating women. The mean contamination level was 5.91 ± 2.031 ng/L, ranging between 2 ng/L and 10 ng/L (Table 2).

Multiple regression analysis indicated no significant associations of consumption of milk and dairy products, meat, fish, legumes, grain products, fruits, and nuts with the concentration of AFM1 in breast milk. In addition, no significant association was observed between AFM1 concentration and anthropometric data of infants.

4. Discussion

Infancy is regarded as a unique period in human life, mainly due to the dependence of infants on mother's breast milk as the only nutritional source [15]. Human breast milk is the most bioavailable source of nutrients for infants, which also provides the hormones and immunological factors that protect the infants from potential disease-causing agents [16]. Several factors have been reported to affect the quality of human breast milk, among which maternal consumption of contaminated food is considered to be the main cause of breast milk contamination. Aflatoxins as a highly carcinogenic, mutagenic, and teratogenic group of mycotoxins [17] are regarded as one of the most naturally occurring toxins threatening the human health. Since the rate of biotransformation is lower in children compared to that in adults and due to a higher growth rate along with more consumption of food and water/kg weight [18], children are considered to be highly susceptible to the detrimental effects of environmental toxins, specially aflatoxins. Early infant exposure to aflatoxins has shown to result in immunosuppression, growth impairment, and underweight. Several studies have reported the presence of AFM1 in infant formula as well as in human breast milk in different countries. Therefore, AFM1 in breast milk of lactating mothers can be considered as a potential health-threatening issue for infants.

In the present study, the concentration of AFM1 in the breast milk of 85 lactating women of Ilam province was measured. AFM1 was found in all samples with a mean contamination level of 5.91 ± 2.031 ng/L, ranging from 2 ng/L to 10 ng/L.

Data regarding the frequency and mean concentration of AFM1 in breast milk samples vary in different countries, indicating that the contamination rate is lower in the developed countries compared to that in developing/undeveloped countries. Studies from Germany, France, and England reported no contamination in breast milk samples [19,20]. However, the results of a study in

Table 2. Occurrence of AFM1 in human breast milk.

			AFM1 contamination (ng/L)	
Sample	*N*	Positive samples (%)	Range	Mean ± SD
Human breast milk	85	85 (100)	2–10	5.91 ± 2.031

AFM1 = aflatoxin M1; *N* = number; SD = standard deviation.

Australia showed that 26% of the milk samples of 73 lactating women were contaminated with AFM1, which can be attributed to the warm and humid climate of this country [21].

The presence of AFM1 in breast milk samples of lactating women in different provinces of Iran has been evaluated, and various results have been obtained. The frequency of contamination of samples in our study (100%) was similar to that previously reported for Tehran province of Iran (98%) [22]. However, in that study, the mean concentration of AFM1 was 8.2 ± 5.1 ng/L. Mahdavi et al [23] have also recently evaluated the presence of AFM1 in breast milk of lactating mothers in urban and rural areas in Tabriz (Iran). In their study, the frequency of contamination in rural areas was 22%, while there was no AFM1-positive sample in urban areas. In a recent study in Isfahan (Iran), AFM1 was found in only one out of 80 breast milk samples (1.25%), with a mean concentration of 6.88 ng/L.

The mean concentration of AFM1 in our study (5.91 ng/L) was lower than the reported values for Australia (560 ng/L) [24], United Arab Emirates (664 ng/L) [25], Egypt (13.5 ng/L) [26], Tehran (8.2 ± 5.1 ng/L) [22], and Sudan (400 ng/L) [27].

Since diet is regarded as the main source of human exposure to aflatoxins, consumption of AFB1-contaminated food might result in the secretion and presence of AFM1 in breast milk. Polychronaki et al [26] have found a relationship between the occurrence of AFM1 in breast milk of lactating women, and consumption of ground nuts and corn. Other studies in different countries as well as in different parts of Iran have also related consumption of milk and dairy products, peanuts, pistachios, nuts, corn, and certain food products with the presence and level of AFM1 in breast milk samples [22,24,28]. However, in the present study, multiple linear regression analysis failed to show any significant correlation of AFM1 concentration with infant anthropometric parameters or the type and amount of food consumed.

According to the European Commission and US regulation, the maximum level of AFM1 for infant food commodities should not exceed 25 ng/kg. However, Austria and Switzerland have fixed the limit to 10 ng/kg for infant milk. In the present study, the mean concentration of AFM1 in human breast milk (5.91 ± 2.031 ng/L) was lower than the maximum tolerable limit accepted by the European Union and US regulation ($p < 0.01$). It was also lower than the maximum limit accepted by Austria and Switzerland ($p < 0.01$).

In conclusion, an analysis of the breast milk of Iranian women indicated exposure of mothers to aflatoxins through their normal diet. Based on our results, the calculated average infant exposure to AFM1 showed that infants are exposed to an average of 2−5.6 mg/kg AFM1 daily from breastfeeding in Ilam, Iran, which is still lower than the accepted limit [29]. However, due to the low biotransformation rate of infants, attempts should be directed toward finding ways to reduce the contamination of foods with aflatoxins. Therefore, in order to reduce the presence of aflatoxins in breast milk and infant exposure, people, especially mothers, should be educated about the ways of conveyance of aflatoxin into foods, and associated hazards following unsuitable food storage and ingestion of contaminated foods. Finally, this warrants the need for monitoring the AFM1 level in human breast milk samples over the breastfeeding period.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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