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Original Article

Infants' Exposure to Aflatoxin M1 from Mother's Breast Milk in Iran

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Abstract

Background: The occurrence of aflatoxin M1 (AFM1) in milk, especially breast milk, is a valuable biomarker for exposure determination to aflatoxin B_1 (AFB_1). In the present study, the risk of exposure to AFM_1 in infants fed breast milk was investigated.

Methods: An enzyme-linked immunosorbent assay (ELISA) was used for the analysis of AFM_1 in breast milk samples from 132 lactating mothers referred to four urban Mothers and Babies Care Unit of Hamadan, western Iran.

Results: AFM_1 was detected in eight samples (6.06%) at mean concentration of 9.45 ng/L. The minimum and maximum of concentration was 7.1 to 10.8 ng/L, respectively. Although the concentration of AFM_1 in none of the samples was higher than the maximum tolerance limit accepted by USA and European Union (25 ng/kg) however, 25% had a level of AFM_1 above the allowable level of Australia and Switzerland legal limit (10 ng/L).

Conclusions: Lactating mothers and infants in western parts of Iran could be at risk for AFB_1 and AFM_1 exposure, respectively. Considering all this information, the investigation of AFM_1 in lactating mothers as a biomarker for postnatal exposure of infants to this carcinogen deserves further studies in various seasons and different parts of Iran.

Keywords: Aflatoxin M_1, ELISA, Human breast milk, Mycotoxin, Iran

Introduction

Mycotoxins are toxic metabolites produced by special fungal strains. Aflatoxins (AFs) are one of the first recognized and vastly researched mycotoxins in the world. They are one of the most potent toxic substances produced by the fungi *Aspergillus flavus* and *A. parasiticus*. Humans and animals are generally exposed to AFs via diet. It is proved that AFs are carcinogenic and may cause growth impairment and immune suppression in numerous animal species (1, 2). AFs have been established in human sera and cord blood of women promptly following birth. Therefore, the transplacental transfer of AF by the feto-placental unit has been established (3, 4). The high AF exposure of West African children and the effects of this exposure on children's growth have been demonstrated (5). Moreover, the higher level of AFB_1 has been correlated with reduced birth weight and jaundice in neonates (6). Immunity and different aspects of children's health may significantly be influenced by exposure to aflatoxins. Turner et al. reported a decrease in salivary IgA in Gambian children exposed to aflatoxin (7).

Aflatoxin M (AFM) is a hydrolyzed metabolite of AFB (8). When feed contaminated with AFB is ingested by dairy cattle, up to 0.3-6.2% will appear in the milk as AFM (9). AFM_1 is of special interest because it can be transmitted to a newborn offspring by the human's milk (10).

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Consumption of AFM_1-contaminated milk by human, especially neonates and children, is of substantial concern especially when considering that AFM_1 may be secreted in mother's breast milk (11). It has been recognized that children exposed to AFM_1 through milk or it's by products may become prone to infectious diseases, underweight, and stunted during infancy and for the rest of the life· (2).
In contrast to the infectious diseases, mycotoxins, because of their chronic effects on human being, have been neglected in most developing countries. However, only limited data are available on mycotoxin contamination of Iranian commodities. According to recent statistics issued by the Iranian Ministry of Health, cancer is the third most common known cause of death in Iran, after cardiovascular diseases and accidents (12). Moreover, there were insufficient data on the contamination of Iranian milk with AFM_1 and based on our knowledge there was limited information about the exposure of infants to aflatoxin from mothers' breast milk in Iran.
The aim of this study was to investigate the presence and extent of AFM_1 in mothers' breast milk samples by ELISA method.

Materials and Methods

This study was of cross-sectional design. One hundred and thirty-two samples were randomly collected from lactating mothers, whose age range were 16-40 years and referred to one rural and three urban Mothers and Babies Care Units (MBCUs) of Hamadan, western Iran. The three urbans' MBCUs were categorized and selected based on their location in low, moderate and high socioeconomic areas and thirty three peoples were considered for each of the areas.
Samples were collected over the period from Nov. 2003 to Mar. 2004. Out of 132 lactating mothers surveyed, 118 who had full-term infants and fourteen (10.6%) who delivered premature infants (weight < 2500 g, ≤ 37 weeks gestational age) agreed to participate in the study voluntarily. This research project was approved by the Ethics Committee of Deputy of Research, Iranian Ministry of Health and Medical Education. All volunteers 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, cancer), infections, medication and smoking.
A questionnaire, administered by trained interviewers to the mothers of children recruited to the study, filled in at the time of sample donation, giving details of age, gestational age, stage of lactation, postnatal age, sex, birth weight, weight at the time of sampling, using formula and the component of mother's diet (including frequency of pistachio and groundnut consumption during 72 hours before milk sampling). Complementary data were gathered regarding the socioeconomic position of the mothers, namely, income, job and education level.
Ten mL of breast-milk samples were collected from each of the volunteers, who were all at different stages of lactation, by hand expression or manual breast pump during regular feeding of infants in the Mothers and Babies Care Unit into a sterile plastic container for analysis. All fresh milk samples were stored at -20ºC and protected against light until the day of analysis that was not longer than 60 days. To do the tests, samples were gently brought to the room temperature and then centrifuged at 3500 g for 10 min and defatted by removing upper cream layer. A competitive enzyme immunoassay kit (Ridascreen, Riedel-de Haen Art No. R1101; R-Biopharm GmbH, Darmstadt, Germany) was used for quantitative analysis of AFM_1. The standard solutions were provided in 0, 5, 10, 20, 40 and 80 ng/L concentrations. As per the manufacturer's instructions, 100 µl of the standard solutions or defatted milk was transferred directly to the appropriate microtiter wells and incubated for 60 min at room temperature in the dark. After washing three times, 100 µl of the properly di-

luted enzyme-conjugated antibody were added and incubated for 60 min at room temperature. Again wells were washed three times and then 50 µl of substrate and 50 µl of chromogen were added to the wells, mixed thoroughly and then incubated for 30 min at room temperature. In the last step 100 µl of the stop reagent was added to the wells, mixed well and the absorbance was measured at 450 nm against an air blank. All tests were done in duplicate and in some cases the milk sample diluted 1:10. The calibration curve was virtually linear in the 10-80 ng/L range. The AFM_1 concentration in ng/L corresponding to the extinction of each sample was read from the calibration curve. The detection limit of the method was 5 ng/L in the milk. Recoveries were determined in milk samples spiked at levels of 10–80 ng/L. The mean recovery and coefficient of variation were 90% and 15%, respectively. Analytical values were not corrected for recovery.

Statistical Analysis

Statistical analysis was performed using SPSS version 9.0 (SPSS Inc. Chicago, Illinois) software. The results were statistically analyzed using multiple linear regressions to evaluate the association between AFM_1 concentrations in breast milk and the potential factors as well as infants' anthropometric status. The Chi Square test and, if needed, Fisher's exact test was used to assess the possible differences in incidence of AFM_1 concentrations in different groups. P values of less than 0.05 were considered significant.

Results

The maternal and infantile descriptive data of the study were shown in Table 1 and 2. In the present study on 132 lactating mothers from four different parts of Hamadan province, western Iran, AFM_1 was found to be present in 8 samples (6.06%). The mean AFM_1 concentration in contaminated samples was 9.45 ng/L, and the minimum and maximum concentration was 7.1 to 10.8 ng/L, respectively (Table 3). Out of eight contaminated samples, 2 (25%) samples resulted above the allowable level of Australia and Switzerland (10 ng/L) and none of the samples displayed contamination higher than the maximum tolerance limit accepted by USA and European Union (25 ng/kg). Frequent nut consumption (pistachio and ground nuts) was declared by 11.36% of the mothers (Table 2).

Regarding the socioeconomic situation of the patients 50% of contaminated milk samples were associated with the persons who were resident in rural and moderate socioeconomic areas and the rest were related to the individuals living in low socioeconomic status areas (Table 2). However, no significant difference was found between the level of AFM1 and age, sex, postnatal age, gestational age, stage of lactation, birth weight, weight at the time of sampling, the component of mother's diet and the socioeconomic position of the mothers including income, job and education level.

Table 1: The descriptive data of the study

Variables	N	Range[a]	Mean ± SD
Mother age (yr)	132	16-40	25.16 ± 4.94
Infant age (mo)	132	0.1-24	7.44 ± 5.41
Height at birth (cm)	132	38-65	49 ± 3.50
Weight at birth (kg)	132	2-4.5	3.29 ± 0.51
Weight at the time of sampling (kg)	132	3.2-12.5	7.66 ± 2.32

[a] Min–max.

Table 2: Maternal descriptive data

Variables	Rural (n= 33)	Urban (socioeconomic areas) (n= 99)			Total (n=132)
		Low (n=33)	Moderate (n= 33)	High (n=33)	
Employed	0	2	4	22	28
Not employed	33	31	29	11	104
Uneducated	16	1	0	0	17
Under university-educated	15	27	33	16	91
University-educated	0	0	0	14	14
Nut [Ψ] consumption *	2	3	0	10	15
<1 glass dairy [£] consumption *	21	27	21	9	78
1-2 glass dairy consumption *	9	4	8	15	36
> 2 glass dairy consumption *	3	2	4	9	18

[Ψ] Pistachio and ground nuts
*< 48 hrs.
[£] Milk and yogurt

Table 3: Aflatoxin M_1 concentration (ng/L) from contaminated breast milk samples from Iran

Sample	n	Positive samples	AFM_1 contamination of positive samples (ng/L)		
			Range[a]	Median	Mean ± SD
Human breast milk	132	8 (6%)	7.1 - 10.8	9.95	9.45 ± 1.50

[a] Min–max.

Table 4: Summary of selected reports of occurrence of aflatoxin M_1 from mothers' breast milk in different countries

Location	Number of samples	Incidence of contaminated samples (%)	Range (ng/L)	Method	Reference
Zimbabwe	54	11	2–50	ELISA	30
France	42	0	NA	ELISA	30
Australia	73	11	28–1031	HPLC	26
Thailand	11	45	39–1739	ELISA	26
Abu Dhabi	445	99.5	2–3000	ELISA	11
UAE	140	92	NA	HPLC	21
Turkey	75	75	61–300	HPLC	27
Iran	160	98.1	0.3–26.7	ELISA	32
Iran	91	22	5.1– 8.1	ELISA	31
Iran	132	6	7.1–10.8	ELISA	Current study

NA= Not available

Discussion

Human milk is an ideal and most bio-available source of calcium and protein for infants. It also contains suitable amounts of carbohydrate, and fat. Many persons in developing countries are chronically encountered to high levels of mycotoxins in their life and because of this chronicity, the vast induced diseases are still remain neglected. Aflatoxin production is the problem of improper post-harvest handling. During storage of the crops, the high humidity and temperature promote mold growth (2). Although human exposure to high levels of aflatoxins commonly occurs through consumption of maize and peanuts, which are dietary staples in several countries, in Iran however, maize mostly used for animal feed and is not major in human diet. The trivial levels of AFM_1 in breast milk from Iranian women mostly could be related to low use of this cereal. Furthermore, the nuts such as pistachio, peanuts, almond kernels, which are suitable for contamination with AFB_1, are often consumed and could be the risk food items for AFM_1 contamination in mothers in Iran (13). In the current study, pistachio and ground nuts were consumed by 15 (11.36%) subjects of the total population studied (Table 2). However, only limited data are available on fungal and aflatoxin contamination in general food commodities for human consumption in Iran (14-16).

In most developing countries including Iran, children are often breastfed until 1-2 years of age or even more. In addition, infants and children living in developing countries have many other problems compromising health, such as general food shortages, malaria, diarrhea, measles and protein energy malnutrition that may make them more susceptible to AFM_1 detrimental effects. The results of relatively high AFM_1 levels in few mothers in our study imply individual dietary habits that may possibly cause the exposure of their children even after weaning. Some data on occurrence of AFM_1 in cow milk in the first 1970s (17) as well as in recent years (14, 18, 19) in Iran have been reported and high proportions of positive samples were found in most surveys. Furthermore, the mean concentration of AFM_1 in raw milk of Hamadan was shown to be 18 ng/L, so with attention to daily per person milk consumption in this area, the intake of AFM_1 from milk has estimated to be 1.06 ng/person/day (20).

Based on a literature review, there is little information on the AFM_1 intake by breast feeding (21, 22). According to our results, at a mean contamination level of 9.5 ng AFM_1/L, a baby at one week old will be exposed to 0.57-0.86 pg AFM_1/feeding based on milk consumption of 60–90 mL/feeding (23) and for average of 8 feedings/24hr, daily intake of AFM_1 would be 4.56–6.88 ng/24hr. Although, the Joint FAO/WHO Expert Committee on Food Additives has not ascertained any tolerable daily intake for AFM_1, but forcefully advised that the level of this toxin should be kept as low as possible (2, 22). In the present study, only six mothers because of lacking enough milk used infant formula for feeding their babies 3-4 times per day. Nevertheless, despite the hazards of AFM_1, the stress should be on the benefits and advantages of breast feeding in comparison to artificial lactation.

This is the first report of AFM_1 in breast milk of women from Western Iran. Investigation of the breast milk of mothers from four different regions in Hamadan, Iran has revealed unexpected levels of AFM_1 indicating insignificant exposure of mothers to aflatoxins. This is surprising in a country where AFM_1 in milk and its by-product is considered endemic (17-20). In spite of several studies carried out on AFM_1 in some parts of Iran, AF contamination of diet is not still well known, thus the potential risk of contamination by this toxin in biological fluids is a necessity that should be determined.

Based on Lamplugh *et al.*, the frequency of AFM_1 detection in breast milk during summer times were higher than those in winter times (24). According to our previous study on occurrence of AFM_1 in raw milk during the summer and winter seasons in Hamadan district, the significant difference in the level of AFM_1 contamination was observed between two seasons. Hamadan province has a cold and mountainous climate and in the current study milk samples were collected in the cold season. Therefore, if the sampling had been carried out in warmer season in summer, we could expect much more significant amounts of AFM_1 in lactating mothers. According to Polychronaki *et al.,* (22) more than 80% of breast milk samples collected from Egyptian mothers have been contaminated with AFM_1. Studies carried out by other investigators have demonstrated highly variation in contamination (11, 21, 25-27) (Table 4). Out of 445 and 140 breast-milk samples of United Arab Emirates (UAE) women, 99.5% and 92% contained AFM_1, respectively (11, 21). In contrast, no positive samples were detected in breast milk samples obtained from women in France, United Kingdom and Bangkok (28-30). Irrespective of the knowledge of the mothers about this problem, a possible explanation for this high variation in contamination levels may be due to the differences in geographical situation between studied areas.

According to two Iranian studies recently carried out on human milk samples from East Azerbaijan Province as well as Capital, Tehran, the levels of AFM_1 were significantly higher than those in breast milk samples from Hamadan (31, 32). The frequency of detection (6.06%) of AFM_1 in women in this investigation has been higher than some studies in Turkey, France, Bangkok and United Kingdom (25, 28-30) and lower than those reported in two studies from Iran, Zimbabwe, Ghana, Egypt, Thailand and one study in Abu Dhabi with 99.5% contamination (11, 22, 24, 30-33) (Table 4). Furthermore, the range of AFM_1 in breast milk of the lactating women in the current study was rather low compared with those found in breast milk in Sudan, Zimbabwe, Ghana, Egypt, Thailand and two studies from Iran (22, 24, 26, 30-33) and higher than those reported in Turkey and Abu Dhabi, UAE (11, 25). Of 54 samples collected from women in rural villages in Zimbabwe, 6 (11%) were found to be positive with levels up to 50 pg/ml (30). The frequency of detection of AFM_1 in our study is comparable with the latter study.

It is documented that mycotoxins may occur as combinations and aflatoxin would be expected to co-occur with ochratoxin A (OTA) in milk (2, 34). Although a study on corn samples from Iran has shown contamination with OTA (15), its presence in milk and the health risk of this mycotoxin to neonates and infants has not yet been evaluated in Iran.

Out of eight contaminated samples, four contaminated milk samples were associated with the individuals who were resident in rural and moderate socioeconomic areas (each two samples) and four were related to the mothers of low socioeconomic area of the city. No breast milk sample contaminated with AFM_1 was seen in mothers who were resident in high socioeconomic areas of Hamadan. However, because of low frequency of contaminated samples, statistical analysis failed to show significant difference between four surveyed areas.

No statistical significant differences were observed between AFM_1 and age, sex, postnatal age, gestational age, stage of lactation, birth weight, weight at the time of sampling, the component of mother's diet and the socioeconomic position of the mothers including income, job and education level. These results are comparable with those of other Iranian studies (31, 32, 35).

Generally, the analysis of the breast milk of Iranian women, indicating the exposure of mothers to aflatoxins in their normal diet. Therefore, in order to reduce the presence of aflatoxins in breast milk and infant exposure, people especially mothers should be educated about the conveyance ways of aflatoxin into foods, its hazards following unsuitable food storage and ingestion of contaminated foods. Concerning this problem, attention should be paid to control measures, such as nutrition education, food safety, food hygiene, good agricultural practice, and good quality control to limit mother and infant exposure to aflatoxins.

Ethical Considerations

Ethical issues (Including plagiarism, Informed Consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc) have been completely observed by the authors.

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