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Original article

Infants breastfed: a require or a potential risk

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Summary. The Ochratoxin A(OTA) is a nephrotoxic and carcinogenic mycotoxin which generates important risks for human health worldwide owing to food contamination merchandise, environment. Dietary contaminants ingested by nursing mothers can be found in their breast milk. Nevertheless, the rate of OTA lactation transfer has not been investigated so far at various stages of breastfeeding. The study aimed at assessing the presence and values of the mycotoxin Ochratoxin A in human milk in Isfahan, Iran, and sought to evaluate the potential risk for the newborn babies based on the mycotoxin taking. 41 lactating women were randomly selected and breast milk samples after delivery were taken to determine the prevalence of Ochratoxin A value by enzyme linked immunosorbent assay and high performance liquid chromatography technique. Considering the total samples analyzed, only one case was contaminated with Ochratoxin A at 45 ng/l. Accordingly, the results indicated the exposure of mothers and neonates to Ochratoxin A and although the observed incidence was low, it is recommended that further investigations be conducted on mycotoxin contamination in large quantity both in food and biological fluids in addition to protection strategies to decrease the risk in Isfahan and other parts of the country.

Key words: mycotoxin, risk assessment, breast milk, infant foods

Introduction

Breast milk is considered as the best source of nutrients for infants, providing a unique blend of nutritional and non-nutritional benefits (1) but, there is the mycotoxins entrance through utilization of the contaminated foods with molds and their metabolites (2). Ochratoxin A (3), a mycotoxin produced by some species of the genera Aspergillus and Penicillium, established fact for the widespread occurrence in food commodities and it is found in cereals, legumes, coffee, peanuts, meat transport from feed and other plant substrate (4-6). Other foodstuffs may also be contaminated such as cows and human's milk (7, 8). The foodstuffs contaminated by OTA represents an essential supply of exposure for the whole population (9). OTA has been demonstrated to possess many toxic effects on animals and human(10).The most crucial points are nephrotoxicity, immunotoxicity, and carcinogenicity which classified OTA as a possible human carcinogen

(group 2B) (10-14). This compound has been shown to have nephrotoxic effects on all mammalian species and has been related to fatal human kidney disease, referred to as Balkan Endemic Nephropathy and by having an increased incidence of tumors of the top of urinary effect (15).

OTA might also be detected in animal derived products, human blood, breast milk and infant formulae. The European Union (EU) has set a legal limit of 0.50 μg kg–1 for OTA in processed cereal-based foods and baby foods for infants and young children (16).

Presents' blood and/or urine OTA were analyzed as biomarkers of exposure. In this context, it is essential to take into account the kinetics, namely the long half-life of OTA in human blood (17). The prevailing database for OTA blood and urine levels in the adult population of different countries shows notable geographical, regional, and inter-individual variability in OTA intake (18-21). Human milk which is considered as a potential supply of Ochratoxin A documented by its detection in samples was collected in several countries (21-25) reflecting a variable dietary mycotoxin exposure of nursing mothers in numerous countries. A young child may be more sensitive than adults to harmful environmental chemicals as a result of differences in absorption, distribution, excretion, and metabolism (26, 27).

The OTA intake of nursing mothers will largely determine the exposure of the breastfed infants. Although it is known that OTA may be excreted in milk, empirical data on the extent of mycotoxin transfer from blood to breast milk are scarce and contradictory (22). lactation transfer of xenobiotic is a complicated process (28), and it can vary greatly with breastfeeding stage and with the nutritional status of the mother (29, 30). OTA was found more regularly in milk samples from individuals with a higher intake of liver paste and cakes, bread, bakery products and cured pork meat and the risk of OTA contamination was also increased by the intake of juices (31, 32).Therefore, this indicated an effective transfer of the toxin to the sucking. If the same trend is true in humans, the exposure of the breastfed infant to the toxin might be a major matter of concern for human health (33).

The purpose of the present survey was to determine a level of OTA exposure of breastfed infants and characterize the lactation transfer of mycotoxin. Consequently, there is actually a probable correlation between this mycotoxin values (in positive samples) and potential factors such as the kind of job, dietary pattern and personal habits which were evaluated.

Material and methods

Sample collection

In during 3-month period of 2015, 41 breast milk samples were collected from lactating women with newborn aged less than 7 months accidentally in Navab -Safavi health center, Isfahan, Iran. After making the necessary arrangements, the mothers were referred to the health centers in the morning and were informed about the research objectives. Written informed consents were signed by participants and Information on personal characteristics, dietary habits, were obtained by interview and were recorded. About 5 to 10 mL of breast milk sample was collected in the sterile bottles. The samples were kept at 4°C were kept frozen at under -20°C in Research Laboratory of School of Food Security Research Center, Isfahan University of Medical Sciences until measurement processes.

Exclusion criteria for the subjects were infections or metabolic disease; diseases of the breast or central nervous system, malnutrition, maternal allergy, alcohol, and addiction. The mothers' age was between 18 to 35 years. Other exclusion criteria were newborns with any malformation, cardiac or hemolytic disease. The diseases were diagnosed by history, physical examination, etc. This study was approved by the local ethics committee of the Isfahan University of Medical Science, and informed consent was obtained from each patient participant prior to enrolment.

Based on data collected by the dietary questionnaire, we classified the subjects according to the frequency of consumption into moderate consumers group (up to 7 times a week) and habitual consumers group (more than 7 times a week) (24, 34)

Extraction procedure

Before starting the experiments, the samples were thawed. Ochratoxin A is a water soluble toxin who used for determined in breast milks the quantitative

test, in the beginning with enzyme-linked immunosorbent assay (19) method for the screening test, then the all positive samples with HPLC method.

ELISA test procedures were performed according to the manufacturer's instruction of the test kit (Helica Company, Santa Ana, California, USA).

Analysis OTA in human breast milk samples by ELISA and HPLC technique

According to the kit procedure, 1 ml of breast milk drawn in a polypropylene tube. Then 5 ml dichloromethane (CH2Cl2) were added and mixed head overhead 5-10 minutes (Rotor) was allowed the mixture to separate into two layers for 10 minutes. Afterward was removed the upper layer and take 1 ml volume of the layer underneath. This final sample volume aporates to dryness under a mild stream of nitrogen at 50°C, was solved the residue in 0.2 ml dilution buffer (supplied in the kit) then pipette 50 µl into the wells of the ELISA plate. The standard solutions and the breast milk samples were added to the wells, and washed after each incubation session. The optical absorption of the samples was measured through the photometric method using ELISA reader at the wavelength of 450 nm. Afterward the calibration curve was drawn and used in order to determine the OTA concentration considering the samples' absorption rat. To ensure the accuracy of the whole process, all positive samples were repeated once again. ELISA standard curve values were obtained with a blank and 6 Standards concentration 0.025, 0.05, 0.1, 0.2, 0.5 and 2 ng/ml, in duplicated style. Acceptable range (OD) were >1.609 for lowest and <0.314 for highest standard concentration. The limit of detection (LOD) was 0.25 ng/ml.

In this survey used Waters HPLC system (Waters 2690, USA) equipped with a fluorescence detector 474 and an auto sampler; excitation and emission wavelengths were 365 and 430 nm, respectively. The HPLC column and guard were Capital HPLC 150×4.6 mm, 3mm, 3mm (Capital HPLC Ltd) and 0.5 cm chromolith, respectively. The mobile phase prepared by methanol, acetonitrile, water (3:2:6, v/v/v), KBr (0.12 g) and HNO3 4 N (0.35 ml), in isocratic mode with a flow rate of 2 ml/min. The temperature in column oven was 40 C. Nine Ochratoxin A standards of between 0.5 and 15 ng/ml and 0.1 e 10 ng/ml were obtained from Sigma Aldrich (St. Louis, MO, USA) and injected, respectively.

Statistical analysis

Statistical analysis was performed by the SPSS version 18 software by student t-test for comparison of OTA concentrations in breast milk samples. Also, variable inter groups comparison was analyzed by means of non-parametric test Manne Whitney. $P < 0.05$ were considered significant.

Results

The age ranges of 41 volunteers were 18-35 years (mean 27.5 years). 61% of the mothers' delivery modes were vaginal and 39% were cesarean. 19 cases (46.3%) of these mothers lived in rural areas and 22 cases in cities. 21 cases of infants were female and 19 cases (48.8%) were male. 53.7% and 46.3% of infants' age were below 3 months and above 3 months, respectively.

Table 1 presented the Specification data of statistical population (41 mothers and their infants).

Totally Ochratoxin A was detected in 8 samples (19.5%) of 41 human breast milk by ELISA method (as the primary screening test) ranging from <5 to 0.258 ng/l. whereas estimating this mycotoxin by HPLC method were shown only in one breast milk sample (2.4%) with 45 ng/l.

As a result, differences were observed in Ochratoxin A in the samples which were evaluated via the ELISA and HPLC methods (Table 2).

No differences were observed between potential factors of the job, dietary pattern and personal habits with Ochratoxin A values (in positive and negative samples).

Discussion

Mothers in lactation period are exposed to different naturally occurring and/or synthetic contaminants, and nearly all nutrients are also polluted with these kinds of contaminants in different degrees. In different systems, the determination of their levels in biological fluids, for example, breast milk, is important

Table 1. Specification data of statistical population (mothers and their infants)

	Maternal age (years)	Mode of delivery		Gender of infants		Area		Baby age	
		Vaginal	Cesarean	Female	Male	Urban	Rural	Below 3-month	Above 3-month
Total 41 case	18-35[a] 27.5[b]	25[c] 61%[d]	16[c] 39%[d]	21[c] 51.2%[d]	20[c] 48. 8%[d]	19[c] 46.3%[d]	22[c] 53.7%[d]	22[c] 53.7%[d]	19[c] 46.3%[d]
Positive (8 case by ELISA method)	21-32 26.5	4 50%	4 50%	6 75%	2 25%	3 37.5%	5 62.5%	4 50%	4 50%
Positive (1 case by HPLC method)	27[a,b]	1 100%	-	-	1 100%	-	1 100%	1 100%	-

[a]Indicates mothers age range. [b]Indicates mothers age mean. [c]Indicates sample count. [d]Indicates sample percentage

Table 2. Determination of Ochratoxin A in 41 human breast milk specimens by ELISA and HPLC methods

Method	Positive samples (%)[a]	<5b (%)[a]	≥5-10 b (%)[a]	≥10-50b (%)[a]	<50b (%)[a]
ELISA	8(19.5%)	33(80.5%)	5(12.2%)	2(4.9%)	1(204%)
HPLC	1(2.4%)-	-	-	1(2.4%)	

[a]Indicates percentage of total samples. [b]ng/l

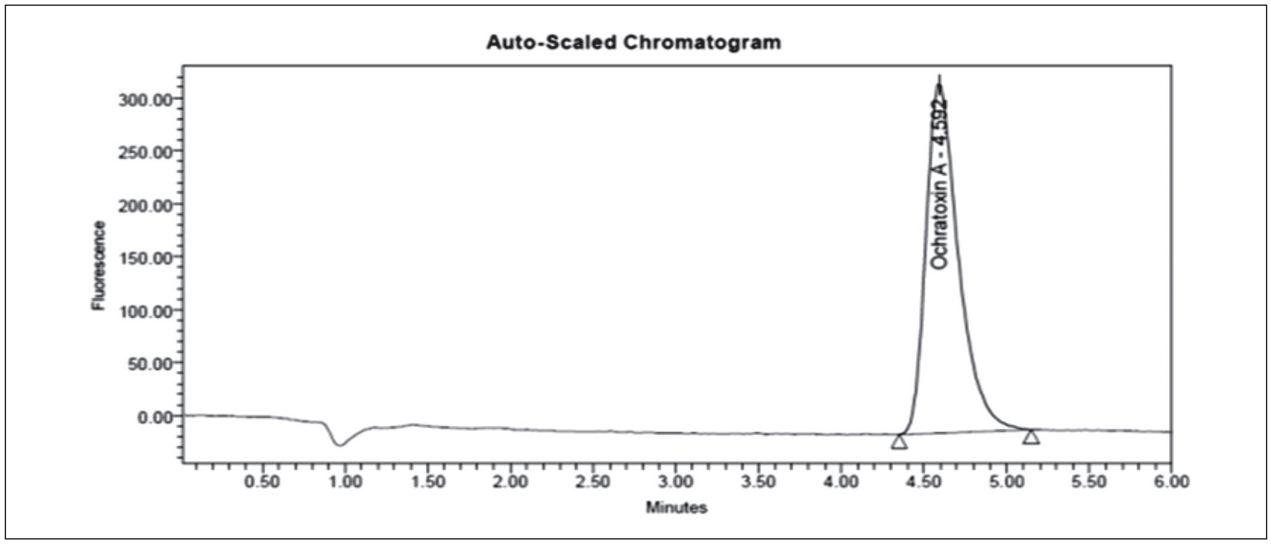

Figure 1. Ochratoxin A chromatogram of a naturally contaminated human milk sample by HPLC technique

when considering the vulnerability of infants (34).The study confirms the presence of Ochratoxin A in human milk and it gives an update on the actual situation in Isfahan (Central Iran). In the present study, although 8 (%19.5) out of the 41 breast milk samples were screened by ELISA method, just contamination in one case (2.4%) with 45 ng/l level was confirmed via HPLC method.

The toxin concentration measurement varied widely in different studies reported by different scale

levels ng/ml which in our study it was converted to ng/l.

Present results are comparable with those obtained in other similar studies performed in recent years in various Asian and non-Asian countries. In our survey, the total quantity samples were 41 cases which match the studies with 40-50 samples conducted in Italy (35), France (36), Brazil (37) and Chile (25). Although the number of the samples was fewer than the reported studies by El Sayed et al in Egypt with 120 samples (38), Andrade et al in Brazil with 224 cases (39), a study in Sweden with 92 cases (8) , Sierra Leone with 113 cases (40) and Italy with 231 cases (41), it was higher than the countries like Egypt (42), Italy (43) and Poland (44) with 10, 31 and 13 samples, respectively.

One case with OTA positive level (2.4%) determined in our study was consistent with a study in Iran with 2 (2.74%) (24) and a study in Brazil with 2(4%) (37) but less than the study in Chile with 11(100%) (45) and in Brazil with 66(66%) (46), Hungary with 22(20%)(47), Sweden with 38(41%) (8) and was higher than that study reported by Andrade et al., in Brazil(39) who did not determine any OTA level.

According to the European Union Standard, 14 (16%) positive samples revealed more than the maximum limit of 40 ng/l for Ochratoxin (ranging from 1.6 to 60 ng/l) (48).

The frequent presence of measurable OTA levels in nursing Iranian women documents their dietary mycotoxin exposure. The average OTA concentration in this cross sectional study during the 3month study period was 45 ng/l compared with toxin levels of human breast milk reported in Egypt with 5.07-45.01 ng/l (38), Italy (35), Switzerland (49), Italy (41), Slovakia (50) and Italy (51) with values of 10-40 ng/l, 10-130 ng/l, 1-57 ng/l, 2.3-60.3 ng/l and 1.7 to 75 ng/l, respectively, and it corresponds to Norway (52) with 10-182 ng/l. Furthermore, our results were less than the results of the studies in Turkey (34), Miraglia, Marina, et al., in Italy (43), in Sweden (8), Brazil (37) and Iran (24) with values of 620.87-13111.3 ng/l, 0.08-0.54 ng/ml, 0.22-7.63 ng/l, 0.011-0.0024 ng/ml and 90&140 ng/l, respectively, but were more than the results of the studies in Poland (53)with 0.00053-0.017 and Brazil (46) with 0.3-21 ng/l. In all aforementioned survey used HPLC techniques.

While the study in Khorrambid, Fars Province, Southern of Iran (54) and Norway (55) confirmed with ELISA techniques matched with the results of the screening section of the present study. Although OTA level results determined in Iran ranged from 1.6 to 60 ng/l in 87 human milk samples with 96.6% positive samples (24) but in Norway, it ranged from 22 to 26.4 ng/l in 78 sample with 51% positive cases (55). But from 8 positive cases of the primary screening test with ELISA method, only one case was confirmed by HPLC technique corresponding to the survey in Sari, Mazandaran Province, Northeast of Iran (24). Therefore, despite the acceptable sensitivity of ELISA technique, it is recommended that all positive samples in this method must be confirmed by HPLC technique.

Conclusion

Comprehensive programs should be developed in order to regularly investigate and control these toxins in both humans' and animals' food chains so that the amount of these toxins can be reduced and their side effects can be prevented. Moreover, conducting further studies on a lot of human milk samples in Isfahan and other parts of Iran are recommended in order to identify the status of the society members' exposure to these toxins. Monitoring foods for the presence of mycotoxins like OTA and the disposal of contaminated products should decrease the risk to the human and animal health. Therefore, regular monitoring of foods for the presence of mycotoxins for lactating mothers seems necessary.

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