Progress in Nutrition 2018; Vol. 20, N. 4: 659-664 DOI: 10.23751/pn.v20i4.6889

Original article

A survey of aflatoxin M1 in different milk types in Turkey: risk assessment of children's exposure

Berna Madali, Atila Gulec, Aylin Ayaz

Department of Nutrition and Dietetics, Faculty of Health Sciences, Hacettepe University, TR-06100, Ankara,Turkey - E-mail: baylin.ayaz@gmail.com

Summary. *Background/Aims:* Aflatoxin M1 (AFM1) is a potent toxic compound frequently found in milk and dairy products. A high AFM1 incidence in milk and milk products creates an important public health risk due to the fact that milk and milk products are consumed widely by infants and children. The objective of this study was to evaluate children's exposure to Aflatoxin M1 via dietary milk consumption and to determine the AFM1 levels in different types of various heat treated milk which were sold in Turkey. *Methods*: In this study, 135 milk samples were analyzed to determine levels of AFM1 by enzyme linked immunosorbant assay (ELISA) test kit (Helica, USA) at 3 different periods of the years (February, May, July). While assessing the exposure levels of children to AFM1, Hazard Index was calculated by using to 80th percentiles of AFM1 levels in milk samples and according to the data of Turkey Nutrition Health Survey (TNHS)-2010 the body weights and roughly consumption levels of milk for different age groups. *Results*: The results of this study indicated that the mean concentration of AFM1 in milk samples was 8.6±4.57 ng/L. The AFM1 level of pasteurized milk samples was lower than UHT milks. The average AFM1 levels of whole, semi-skimmed and skimmed milk samples were found as 8.2±4.29ng/L, 10.5±5.01ng/L and 6.8±3.61ng/L, respectively. None of the samples exceeded the maximum limit of AFM1 which was established by Turkish Food Codex Regulation on Contaminants in foodstuffs. Hazard Index (HI) values were found to be higher in children under 9 years than in children above 9 years, which indicating a risk to infant health. *Conclusion*: These results indicated that it was essential to minimize health risk and to reduce AFM1 levels in milk. In addition, sustainable measures should be taken from farm to fork at all stages of the food chain system to prevent the formation of AFB1.

Key words: milk, aflatoxin M1, ELISA, exposure, children.

Introduction

Aflatoxins are a group of polyketide mycotoxins produced by *Aspergillus flavus (Asp.flavus)* and *Aspergillus parasiticus (Asp. parasiticus)* molds (1, 2). The main types of aflatoxins are B1, B2, G1 and G2 produced by herbal products. However, other bio-transformed aflatoxins such as aflatoxin M1 (AFM1) and aflatoxin M2 (AFM2) can occur in milk (3).

There are two main ways of aflatoxin contamination in the milk and dairy products. Firstly, when the animals in lactation period consume feeds contaminated with aflatoxin, aflatoxin B1 and B2 transform into aflatoxin M1 and M2 after metabolized in the animal body. These metabolized toxins pass to the milk produced from the animal and the contamination occurs. Secondly, the contamination occurs when the molds synthesizing aflatoxin pass to the milk and produce aflatoxin during transport, process and storage phases after milking (4).

Due to its high nutritional value, milk is an important natural food suggested for every age group (5). Although it is very important for nutrition, it has a high risk in terms of the AFM1 amount it can bring with (6). Because dairy products are the one of the most important exposure factors through diet for AFM1. A high AFM1 incidence in milk and milk products creates an important public health risk due to the fact that milk and milk products are consumed widely by all age groups and especially for infants and children.

Like aflatoxin B1, the target organ of aflatoxin M1 is the liver. According to International Agency for Research on Cancer (IARC), while aflatoxin B1 is classified in Group 1 due to its carcinogenic effect on humans and animals, aflatoxin M1 is classified in Group 2B (suspicious of developing cancer for humans) (7).

European Food Safety Authority (EFSA) emphasizes the principle of the lowest intake levels (ALARA/As Low As Reasonably Achievable) for toxins because aflatoxins cannot be completely destroyed from foods and feeds (8). According to Turkish Food Codex Contaminants Regulation (9), the maximum limit for aflatoxin M1 in milk used in the production of raw milk, heat-processed milk and milk products is 0.050 µg/kg. This study was planned and carried out in order to determine aflatoxin M1 levels in different types of different heat-processed milk sold in markets of Ankara city. In addition, exposure of children to AFM_1 through consumption of different heat-processed milk was assessed.

Materials and Methods

Collection of Samples

In this study, 135 milk samples of 20 different brands were randomly purchased from the market shelves of hypermarkets and supermarkets in Ankara, between February 2015 and July 2015. The analyzed milk samples were different milk types (goat, cow) with different heat processing (UHT, pasteurized) and different fat contents (skimmed-fat free, semi-skimmed and whole milk). The milk samples were purchased in different periods of the year (February, May and July, 2015) because of the possible effect of seasonal variations on AFM1 levels. Thirty of the milk samples included in the study were pasteurized milk (22.2 %). According to their fat contents, 55.5 % of them were whole milk, 28.9 % of them were semi-skimmed milk and 15.6 % of them were skimmed (fat-free) milk. The numbers of the analyzed cow and goat milk were 117 (86.7 %) and 18 (13.3 %), respectively. The distribution of different milk types included in the study based on their heat process types and fat contents are given in Table 1.

Pasteurized milk samples were transported to the laboratory with cooler bags and stored in refrigerator conditions (+4°C) until they were analyzed. All milk samples were analyzed before the expiration date.

The Determination of AFM1 Level

The AFM1 levels in different milk samples were determined by using ELISA and Helica Aflatoxin M1 test kit (Helica Biosystem Inc., San Diego, USA) in research laboratory of Hacettepe University, Department of Nutrition and Dietetics. According to the Helica test kit instruction, each sample was prepared with duplicates. All reagents were brought to room temperature before use. 200 microlitre of standard solution and prepared milk samples were added to the appropriate wells in the microtiter plate. After the incubation for 2 hours at room temperature, the wells were washed with PBS-Tween 20 three times. In the next step, 100 microlitre of the conjugate was added to each well and again incubated for 15 minutes at room temperature and washed three times. Afterwards, 100 microlitre of TMB was added and incubated for 15

Table 1. Characteristics of different milk types included in the study based on their heat process types and fat contents.

		Milk Type		
Heat process types	**Fat contents**	**Cow Milk**	**Goat Milk**	**Total**
UHT	Whole	45 *	6	51
	Semi-skimmed	36	3	39
	Skimmed	15 *	0	15
Pasteurized	Whole	18	6	24
	Semi-skimmed	0	0	0
	Skimmed	3	3	6
Total		117	18	135

* Three of the milk samples were organic milk.

minutes at room temperature in dark. Finally, 100 microlitre of stop solution was added and the absorbance values of samples were read with Cromate 4300 microplate reader (Awareness Technology Inc. / Florida) for 3 times at 450-630 nm.

The detection limit for the average AFM1 levels of milk samples is 2 ng/kg according to Helica test kit instruction. Recovery assays were carried out by spiking uncontaminated samples with 10 and 25 ng/kg AFM1 stock solution. The samples were analyzed using the test procedure. Method precision was evaluated through the analysis of samples and calculated the coefficient of variance (CV) (Table 2).

According to the International Programme on Chemical Safety/Global Environment Monitoring System (IPCS/GEMS) criteria, because fewer than 60% of results were less than the LOD, a reasonable estimate of the mean obtained by setting all not detected (ND) results to LOD/2 (10).

The Assessment of the Exposure Levels and Hazard Index of Individuals to AFM1

While assessing the exposure levels of children to AFM1, the calculations were made by taking the report published by WHO in 2005 (11). During the assessment of the exposure to aflatoxin M1; the formula (AFM1 levels in milk samples (ng/L) x consumed milk (L)/ body weight (kg) was used (12). In formula, it was used to 80th percentiles of AFM1 levels in milk samples. During the assessment of the exposure, the data of Turkey Nutrition Health Survey (TNHS)-2010 generated in 2010 for the body weights and consumed amount of milk (L) of different age groups was used (13). As the roughly consumption levels of milk in HI calculation was 500 ml for between the ages of 1-3, 4-6 and 10-12 girls/ boys and 400 ml for between the ages of 7-9 (14). The tolerable daily intake (TDI) for AFM1 is given as 0.2 ng.va.$(kg)^{-1}day^{-1}$ (15). The Hazard Index (HI) for aflatoxin M1 was calculated by dividing the EDI by TD_{50} (threshold dose per body weight which divided by 5000) (15).

Table 2. Performance of ELISA Helica test kit for AFM1.

AFM1 spiked (ng/kg)	AFM1 found (ng/kg)	Recovery (%)	Standard Deviation (SD)	Coefficient of variation (%)
10	8.50	84.98	1.13	2.07
25	20.33	81.30	4.08	3.11

Statistical analyses were performed using the SPSS software version 23.0 (SPSS Inc., Chicago, USA). Descriptive analyses were expressed as mean with standard error and as minimum and maximum concentration of AFM1. Probability levels of $p < 0.05$ and $p < 0.01$ were adopted for the consideration of differences as statistically significant.

Results

The average AFM1 level of different types of different heat-processed milk included in the scope of this research was 8.6±4.57 ng/L. The average AFM1 levels in milk samples did not exceed the legal limit (50 ng/L), which was acceptable according to Turkish Food Codex Regulation on Contaminants in food stuffs. When the milk samples were analyzed by taking the nature of heat process into account; it was found that the average AFM1 level of UHT milk samples (9.3±4.58ng/L) was higher than the average AFM1 level of pasteurized milk samples (6.3±3.76ng/L) ($p<0.01$) (Table 3). According to the fat contents of milk samples, the highest average AFM1 level was found in the samples of semi-skimmed milk (10.5±5.01 ng/L) and the lowest average AFM1 level was found in the samples of skimmed milk (6.8±3.61 ng/L, $p=0.007$). According to animal species, the average AFM1 levels in cow (n=117) and goat (n=18) milk were identified as 9.0±4.68ng/L and 6.4±2.98ng/L ($p=0.032$), respectively. In the study, AFM1 level of goat milk samples was the lowest level.

The Hazard Index (HI) values were higher than 1.0 in until the age of 9, indicating a risk to infant health while the HI values for older than the age of 9 were less than 1.0 which the systemic effects were assumed not to be of concern (Fig. 1). The highest Hazard index values for all ages group were semi skimmed milk samples.

Discussion

In this study, it was determined that the average AFM1 level of pasteurized milk samples were lower

Table 3. Descriptive statistics of AFM1 residues in different types of milk samples.

Characteristics		N	Mean (ng/L)	Percentile				p value
				25	50	75	80	
Heat process type	UHT	105	9.3	6.0	8.3	12.3	12.9	0.000*
	Pasteurized	30	6.3	3.6	5.0	7.5	8.9	
Lipid content	Whole	75	8.2	4.8	7.0	11.8	12.3	0.007**
	Semi-skimmed	39	10.5	6.3	9.9	13.7	14.0	
	Skimmed	21	6.8	4.0	6.6	8.9	9.2	
Animal species	Cow	117	9.0	5.0	7.2	12.3	13.1	0.032***
	Goat	18	6.4	3.4	6.1	9.1	9.3	
Total		135	8.6	4.9	7.2	11.5	12.4	

*Mann Whitney U test ($p<0.01$).**Kruskal-Wallis test ($p<0.01$).
***Mann Whitney U test ($p<0.05$).

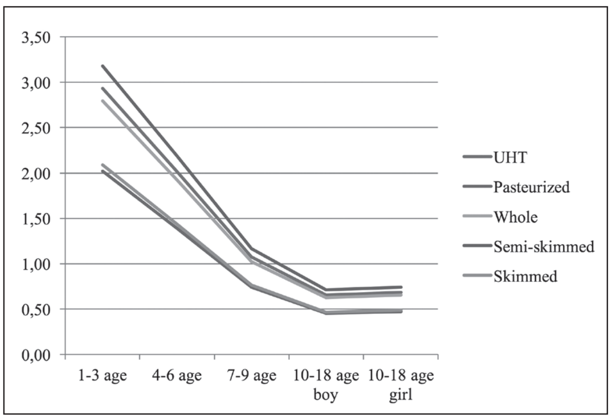

Figure 1. According to recommended milk amounts in different age groups, Hazard Index (HI) values for AFM1.

than UHT milk. However, many other studies showed that the average AFM1 level of pasteurized milk was higher than UHT milk (16, 17). In these studies, it was stated that this situation could be originated from geographical position and environmental factors. However, in our study, the higher level of AFM1 level in UHT milk sample compared with pasteurized milk could be explained as follows: In supermarkets and hypermarkets where milk were purchased, UHT milk were stored in room temperature and pasteurized milk were stored in refrigerator temperature (18). It is known that AFM1 level does not increase in 4°C and below temperatures but AFM1 level increases in optimum room temperatures (19).

In this study, the average AFM1 levels of whole, semi-skimmed and skimmed milk samples were found as 8.2±4.29ng/L, 10.5±5.01ng/L and 6.8±3.61ng/L, respectively (Table 3). Different results were found to be related with the fat contents of milk samples (20, 21). As there are many factors affecting AFM1 level in the milk, the relation of milk with the fat could not be explained completely in such studies (20, 21).

Many previous studies showed that AFM1 levels in the milk of different animals were different and AFM1 level of cow milk was higher than goat milk (1, 22). The AFM1 level in the milk can change due to the different metabolisms of animals during lactation period. However, in the studies, this difference was associated with the feeds that animals consume, not different metabolisms of animals. It is stated that while cows are mostly living in farms and they are fed with grains, goats are living in higher places and reach fresh feeds. On the other hand, it is also reported that as the grains given to the goats are stored for maximum 2-3 months, AFM1 level of grains can be lower compared with feeds stored for a longer time (22).

Especially in the studies made in Turkey after 2010, it is found that the number of samples exceeding the legal limit in terms of AFM1 level in UHT milk samples is very few. These studies resemble the results of this study (23, 24). Before 2010, when studies on the detection of AFM1 level were reviewed, it was stated that the number of samples exceeding the legal limit was much more (25). This discrepancy can be explained by the increased quality of feeds given to the animals and more favorable storage conditions in line with advanced technology in milk factories. When the results of the studies made in different countries are compared, it is found that the number of samples exceeding the legal limit accepted by European countries has lower rates (26, 27).

The average AFM1 levels of milk samples included in the study was found as 8.6±4.57 ng/L. Compared to the other studies (25, 26, 28), the detected average AFM1 levels in different milk types were lower in this study. Furthermore, the exposure levels of for children to AFM1 were lower than the other studies, only with the consumption of milk (25, 26, 28). The reason is that in the other studies, the average body weight of children and the amounts of milk consumption were different. The highest values of using estimated daily intake levels calculated HI were found between the ages of one and three due to be highly susceptible to toxins (Figure 1).

Conclusion

At the end of this study, it is worth to note that if the milk is consumed in recommended amounts, AFM1 can create risk, especially for infants. For this reason, it should be necessary to attributed to a single nutrient but to multiple nutrients and foods to fulfill nutritional requirements for optimal health of children. Besides, good agricultural practices towards the prevention of AFB1 contamination in every stage, from the production of milk to its consumption, including feeds given to the animals, should be adopted and official control and sanction mechanisms should be applied in a healthy and efficient way.

Acknowledgment

This study was funded by Council of Higher Education. The authors declare no potential conflict of interest.

References

1. Bahrami R, Shahbazi Y, Nikousefat Z. Aflatoxin M 1 in milk and traditional dairy products from west part of Iran: occurrence and seasonal variation with an emphasis on risk assessment of human exposure. Food Control 2016; 62:250-6.
2. Iqbal SZ, Asi MR, Ariño A. Aflatoxins - Maloy, Stanley. In: Hughes K, editor. Brenner's Encyclopedia of Genetics (Second Edition). San Diego: Academic Press; 2013. p. 43-7.
3. Kamkar A, Fallah AA, Mozaffari Nejad AS. The review of aflatoxin M1 contamination in milk and dairy products produced in Iran. Toxin Reviews 2014; 33(4):160-8.
4. Çelik TH, Sarimehmetoglu B, Kuplulu O. Aflatoxin M1 contamination in pasteurised milk. Veterinarski Arhiv 2005; 75(1):57-65.
5. Škrbić B, Živančev J, Antić I, Godula M. Levels of aflatoxin M1 in different types of milk collected in Serbia: Assessment of human and animal exposure. Food Control 2014; 40:113-9.
6. Van Egmond H, Svensson U, Fremy J. Mycotoxins. In: Residues and contaminants in milk and milk products. Brussels: International Dairy Federation.; 1997. 17-88 p.
7. International Agency for Research on Cancer (IARC), IARC Monographs on the Evaluation of Carcinogenic Risks to Humans (2002).
8. European Food Safety Authority (EFSA) Opinion of the Scientific Panel on Contaminants in the Food Chain on a request from the Commission related to Aflatoxin B1 as undesirable substance in animal feed. EFSA-Q-2003-035. (2004).
9. Republic of Turkey Ministry of Food Agriculture and Livestock, Turkish Food Codex Regulation on Contaminants, 28157/2011 (2011).
10. World Health Organization (WHO), GEMS/Food-EURO Second Workshop on Reliable Evaluation of Low-level Contamination of Food–Report of a Workshop in the Frame of GEMS/Food-EURO (1995).
11. World Health Organization (WHO), Dietary Exposure Assessment of Chemicals in Food. Report of a Joint FAO/WHO Consultation Annapolis (2005).
12. Shephard G, Van der Westhuizen L, Sewram V. Biomarkers of exposure to fumonisin mycotoxins: a review. Food Addit Contam 2007; 24(10):1196-201.
13. Ministry of Health of Turkey General Directorate of Primary Health Care, Obesity Prevention and Control Program of Turkey (2010-2014) (2010).
14. Besler HT, Rakıcıoğlu N, Ayaz A, Demirel ZB, Özel HG, Samur GE, et al. Türkiye'ye ÖzgüBesin ve Beslenme Rehberi. 1. ed. Ankara2015.
15. Kuiper-Goodman T. Uncertainties in the risk assessment of three mycotoxins: aflatoxin, ochratoxin, and zearalenone. Can J Physiol Pharm 1990; 68(7):1017-24.
16. Rama A, Latifi F, Bajraktari D, Ramadani N. Assessment of aflatoxin M1 levels in pasteurized and UHT milk consumed in Prishtina, Kosovo. Food Control 2015; 57:351-4.
17. Zheng N, Sun P, Wang J, Zhen Y, Han R, Xu X. Occurrence of aflatoxin M1 in UHT milk and pasteurized milk in China market. Food Control 2013; 29(1):198-201.
18. Kanungo L, Bhand S. A survey of Aflatoxin M1 in some commercial milk samples and infant formula milk samples in Goa, India. Food Agric Immunol 2014; 25(4):467-76.
19. Galvano F, Galofaro V, Galvano G. Occurrence and stabil-

ity of aflatoxin M1 in milk and milk products: a worldwide review. J Food Protect 1996; 59(10):1079-90.
20. Panariti E. Seasonal variations of aflatoxin M1 in the farm milk in Albania. Arh Hig Rada Toksikol 2001; 52(1):37-41.
21. Tsakiris IN, Tzatzarakis MN, Alegakis AK, Vlachou MI, Renieri EA, Tsatsakis AM. Risk assessment scenarios of children's exposure to aflatoxin M1 residues in different milk types from the Greek market. Food Chem Toxicol 2013; 56:261-5.
22. Hassan HF, Kassaify Z. The risks associated with aflatoxins M1 occurrence in Lebanese dairy products. Food Control 2014; 37:68-72.
23. Temamogullari F, Kanici A. Short communication: Aflatoxin M 1 in dairy products sold in Şanlıurfa, Turkey. Journal of Dairy Science 2014; 97(1):162-5.
24. Kocasari FS. Occurrence of aflatoxin M1 in UHT milk and infant formula samples consumed in Burdur, Turkey. Environmental monitoring and assessment 2014; 186(10):6363-8.
25. Unusan N. Occurrence of aflatoxin M1 in UHT milk in Turkey. Food and Chemical Toxicology 2006; 44(11):1897-900.
26. Tekinşen KK, Eken HS. Aflatoxin M1 levels in UHT milk and kashar cheese consumed in Turkey. Food Chem Toxicol 2008; 46(10):3287-9.
27. Cano-Sancho G, Marin S, Ramos AJ, Peris-Vicente J, Sanchis V. Occurrence of aflatoxin M 1 and exposure assessment in Catalonia (Spain). Rev Iberoam Micol 2010; 27(3):130-5.
28. Sefidgar S, Mirzae M, Assmar M, Naddaf S. Aflatoxin M1 in pasteurized milk in Babol city, Mazandaran Province, Iran. Iranian journal of public health 2011; 40(1):115.

Correspondence:
Aylin Ayaz
Department of Nutrition and Dietetics, Faculty of Health Sciences, Hacettepe University, 06100, Sıhhiye Ankara, Turkey.
Tel: +90.312.3051094-126
Fax: + 90.312.3091310
E-mail: baylin@hacettepe.edu.tr