



## Incidence of Aflatoxin M<sub>1</sub> in Human and Cow Milk in Kashan, Iran

M.H. Movassaghghazani<sup>1\*</sup>, M. Ghorbani<sup>2</sup>

1. Department of Food Hygiene and Food Quality Control, Faculty of Veterinary Medicine, Shabestar Branch, Islamic Azad University, Shabestar, Iran

2. Faculty of Veterinary Medicine, Shabestar Branch, Islamic Azad University, Shabestar, Iran

### HIGHLIGHTS

- All human and cow milk samples from Kashan were contaminated with Aflatoxin M<sub>1</sub> (AFM<sub>1</sub>).
- AFM<sub>1</sub> concentration of human breast milk samples was less than standard limit of 25 ng/L.
- In 20.83% raw milk samples, the level of AFM<sub>1</sub> was greater than standard limit.
- Protective diet oriented approaches must be considered by the local authorities.
- The people must be educated by the government on public health risk of AFs.

### Article type

Original article

### Keywords

Aflatoxin M<sub>1</sub>  
Milk  
Enzyme-Linked Immunosorbent Assay  
Iran

### Article history

Received: 27 Jul 2017  
Revised: 3 Oct 2017  
Accepted: 26 Oct 2017

### Acronyms and abbreviations

AF=Aflatoxin  
EU=European Union

### ABSTRACT

**Background:** It is known that Aflatoxin M<sub>1</sub> (AFM<sub>1</sub>) contamination in milk can have carcinogenic, teratogenic, and mutagenic activities especially in liver for both children and adults. The main aim of this survey was to investigate the incidence of AFM<sub>1</sub> in human and cow milk in Kashan, Iran.

**Methods:** A total of 42 breast milk samples were collected from a central hospital in Kashan city during 3-month period from October to December 2012. In the same period of times, 48 cow raw milk samples were collected randomly from Kashan milk collection centers. Determination of AFM<sub>1</sub> in samples was carried out by competitive enzyme linked immunosorbent assay. Data were analyzed by SPSS software version 16.0.

**Results:** All human and cow milk samples were contaminated with AFM<sub>1</sub>. Concentrations of AFM<sub>1</sub> in all human breast milk samples were less than the maximum tolerance limit (25 ng/L) accepted by European Union (EU). In 20.83% raw milk samples, the level of AFM<sub>1</sub> was greater than the maximum tolerance limit (50 ng/L) according to EU. Mean AFM<sub>1</sub> concentration in cow milk samples was significantly ( $p<0.01$ ) higher than that of human milk samples.

**Conclusion:** Comparing to the previous Iranian studies, although the present status of AFM<sub>1</sub> in human and cow milk samples from Kashan city of Iran is not at high risk, but this finding dose not ignore the vital importance of exposure risk to this toxin in the consumers especially in children. So, protective diet oriented approaches must be considered by the local authorities. Also, the people must be educated by the government on public health risk of AFs.

### Introduction

Aflatoxin M<sub>1</sub> (AFM<sub>1</sub>) as a metabolite of AFB<sub>1</sub> is created in body of human and some mammalian animals. AFB<sub>1</sub> is produced by some molds, including *Aspergillus flavus*, *A. parasiticus*, and rarely *A. nomius* occurring in

some foodstuffs such as cereals, dried fruits, grains, nuts, etc. (Atasever et al., 2014; Iqbal et al., 2015; Mason et al., 2015).

It is known that AFM<sub>1</sub> contamination in milk can have

\* Corresponding author. ✉ movassagh2@yahoo.com

carcinogenic, teratogenic, and mutagenic activities especially in liver for both children and adults (Creppy, 2002; Duarte et al., 2013; Jalili and Scotter, 2015). On the other hand, children may be exposed to AFM<sub>1</sub> by consumption of contaminated cow milk as the powder milk or whole milk. Children who exposed to AFM<sub>1</sub> may be underweight and susceptible to infectious agents. Therefore, AFM<sub>1</sub> residue in human and animal milk is a global health problem (Darsanaki et al., 2013; Iqbal et al., 2015; Jalili and Scotter, 2015; Keskin et al., 2009; Obade et al., 2015).

There are some reports about AFM<sub>1</sub> determination in human breast milk sampled from women resident in different regions of Iran such as Hamadan (Ghiasian and Maghsoud, 2012), Sari (Afshar et al., 2013), Khorrambid of Fars (Rafiei et al., 2014), Tabriz (Mahdavi et al., 2010), Tehran (Sadeghi et al., 2009), and Shahrekord (Jafari et al., 2017). Also, some Iranian researchers have evaluated AFM<sub>1</sub> residue in cow milk consumed in various areas of the country (Fallah, 2010a,b; Ghazani, 2009; Heshmati and Milani, 2010; Nemati et al., 2010; Oveisi et al., 2007; Tajik et al., 2016; Tajkarimi et al., 2008). However, according to our knowledge, there is no published study in this regards in Kashan city located in center of Iran. So, the main aim of this survey was to investigate the incidence of AFM<sub>1</sub> in human and cow milk in Kashan, Iran.

## Materials and methods

### Sample collection

This cross-sectional survey was carried out in Kashan city, Iran during 3-month period from October to December 2012. A total of 42 breast milk samples were collected from a central hospital in the city. Lactating women who had newborn aged less than one year old were included. Also, 48 cow raw milk samples were collected randomly from Kashan milk collection centers. All milk samples (5-10 ml) were collected into sterile glass bottle and were kept at -20 °C until the analysis time.

### Enzyme Linked Immunosorbent Assay (ELISA) test procedure

Determination of AFM<sub>1</sub> in samples was carried out by competitive ELISA test kit (R-Biopharm, Darmstadt, Germany). At first, each milk sample was centrifuged for 8-10 min at 3500 rpm and then preparation of the samples was done as described previously by Ghazani (2009). Briefly, after adding 100 µl diluted antibody solution inside each well, the plate was shaken gently and

incubated for 15 min at room temperature. Then, the liquid was poured out and each well was washed three times. About 100 µl standard solution or prepared sample was added and incubated for 30 min at room temperature. After pouring the liquid out of each well, washing process was carried out three times. Diluted enzyme conjugate solution was added and incubated for 15 min at room temperature. After washing process, 100 µl substrate/chromogen was added to each well and incubated for 15 min at room temperature. At the end stage, 100 µl stop solution was added and the absorbance was measured at 450 nm by ELISA reader (Sunrise, USA) for detection of AFM<sub>1</sub> in the milk samples. According to the manufacturer instruction, the limit of detection was 5 ng/L for fluid milk and the recovery rate in milk was 85%.

### Statistical analysis

Data were analyzed by SPSS version 16.0 software (SPSS Inc., Chicago, IL, USA). *P* value less than 0.01 was considered as significant.

## Results

All human and cow milk samples were contaminated with AFM<sub>1</sub> (Tables 1 and 2). AFM<sub>1</sub> concentration of all human breast milk samples was less than the maximum tolerance limit (25 ng/L) accepted by European Union (EU). In 20.83% raw milk samples, the level of AFM<sub>1</sub> was greater than the maximum tolerance limit (50 ng/L) according to EU. Mean AFM<sub>1</sub> concentration in cow milk samples was significantly (*p*<0.01) higher than that of human milk samples.

## Discussion

In this study, although 100% human breast milk samples were contaminated with AFM<sub>1</sub>, the toxin levels of all samples were below maximum tolerance limit according to EU (25 ng/L). Similar to our findings, Sadeghi et al. (2009) stated that out of 160 human milk samples belonged to women in Tehran, capital of Iran, 157 samples (98.12%) were contaminated with AFM<sub>1</sub> and the toxin level in only one sample was higher than the maximum tolerance limit. In contrast, another survey in Khorrambid city, Iran revealed that out of 87 breast milk samples, 24 (27.6%) samples were contaminated with AFM<sub>1</sub> with an average value of 0.56±1.23 pg/ml (Rafiei et al., 2014) which was lower than what found in the present research. Also, Afshar et al. (2013) analyzed 136 human milk samples in Sari, Northern Iran and only one sample was contaminated with AFM<sub>1</sub>. There are also

**Table 1:** The incidence of aflatoxin M<sub>1</sub> in human milk samples from Kashan, Iran

Number of human milk samples	Number of contaminated samples	Mean±SD (ng/L)	Range		
			<5 ng/L	5-10 ng/L	10 ng/L<
42	42 (100%)	3.34±0.20	36 (85.71%)	6 (14.28%)	0 (0%)

**Table 2:** The incidence of aflatoxin M<sub>1</sub> in cow milk samples from Kashan, Iran

Number of cow milk samples	Number of contaminated samples	Mean±SD (ng/L)	Range		
			<50 ng/L	50-100 ng/L	100 ng/L<
48	48 (100%)	27.08±3.95	38 (79.16%)	10 (20.83%)	0 (0%)

some reports about occurrence of AFM<sub>1</sub> in human milk in the other countries. For example, AFM<sub>1</sub> was found in 92% of samples collected from resident women in United Arab Emirates which was close to the results of the current survey (Abdulrazzaq et al., 2003). In contrast, Galvano et al. (2008) announced that AFM<sub>1</sub> was detected in only 4 out of 82 (5%) Italian human milk samples having mean level of 55.35 ng/L. Also, AFM<sub>1</sub> was found in 98.7 and 90% of human milk samples from Serbia (Kos et al., 2014) and Colombia (Diaz and Sánchez, 2015), respectively. The controversies which were observed the results of the previous studies could be attributed to some factors like seasonal variation, laboratorial methods, demographic characteristics, including education level, clinical condition, diet type, etc.

Similar human milk samples, all cow milk samples of this survey were contaminated with AFM<sub>1</sub> which their average toxin concentration was 27.08±3.95 ng/L; however, the AFM<sub>1</sub> levels in 20.83% samples were greater than tolerance level regulated by EU (50 ng/L). Our results are comparable with some previous investigations done in Iran and the other countries. Heshmati and Milani (2010) reported that AFM<sub>1</sub> was found in 55.2% of 210 ultra-high-temperature milk samples in Tehran, Iran, and the toxin concentration in 33.3% samples was higher than the maximum accepted limit by EU. Fallah (2010a) detected AFM<sub>1</sub> in 66 out of 91 (72.5%) pasteurized milk samples collected from four large Iranian cities (mean: 0.052 µg/L; range: 0.013-0.250 µg/L). Also, AFM<sub>1</sub> was detected in 67.1% cow milk samples of central part of Iran with mean value of 52.8 and 46.4 ng/L in pasteurized and ultra-high-temperature milk, respectively (Fallah, 2010b). Ghazani (2009) reported that 100% pasteurized milk samples in Tabriz were contaminated to AFM<sub>1</sub> and 62% samples had contamination higher than 50 ng/L with a mean value of 50.55±3.73 ng/L. Bilandžić et al. (2010) reported that the level of AFM<sub>1</sub> in 61 cow milk samples of Croatia was in the range of 11.6 to 58.6

ng/L; they also showed that in 98.4% milk samples in Croatia, AFM<sub>1</sub> concentration was lower than maximum tolerance level regulated by EU. In another work, it was declared that AFM<sub>1</sub> level in 5% out of 40 milk samples in Portugal was higher than the maximum accepted limit by EU (Duarte et al., 2013). Also, investigation on raw milk obtained from Morocco, AFM<sub>1</sub> was detected in 27% samples with range of 10-100 ng/L and the level of toxin in 8% samples was higher than EU standard (El Marnissi et al., 2012). Although most previous researches were similar to our findings, but some others were controversial. It should be considered that the differences seen in AFM<sub>1</sub> levels in cow milk samples in all over the world can be influenced by many parameters especially sampling season, climate condition, hygienic situation of animal housing, and types of feed consumed by the lactating animals.

## Conclusion

Comparing to the previous Iranian studies, although the present status of AFM<sub>1</sub> in human and cow milk samples from Kashan, Iran is not at high risk, but this finding dose not ignore the vital importance of exposure risk to this toxin in the consumers especially children. So, protective diet oriented approaches must be considered by local authorities. Also, the people must be educated by the government on public health risk of AFs.

## Conflicts of interest

The authors declare that there is no conflict of interest.

## Acknowledgments

This study was self-funded. This research was ethically approved by the local institutional review board.

## References

- Abdulrazzaq Y.M., Osman N., Yousif Z.M., Al-Falahi S. (2003). Aflatoxin M<sub>1</sub> in breast-milk of UAE women. *Annals of Tropical Paediatrics*. 23: 173-179.
- Afshar P., Shokrzadeh M., Kalhori S., Babae Z., Saeedi Saravi S.S. (2013). Occurrence of ochratoxin A and Aflatoxin M<sub>1</sub> in human breast milk in Sari, Iran. *Food Control*. 31: 525-529.
- Atasever M., Yildirim Y., Atasever M., Tastekin A. (2014). Assessment of aflatoxin M<sub>1</sub> in maternal breast milk in Eastern Turkey. *Food and Chemical Toxicology*. 66: 147-149.
- Bilandžić N., Varenina I., Solomun B. (2010). Aflatoxin M<sub>1</sub> in raw milk in Croatia. *Food Control*. 21: 1279-1281.
- Creppy E.E. (2002). Update of survey, regulation and toxic effects of mycotoxins in Europe. *Toxicology Letters*. 127: 19-28.
- Darsanaki R.K., Chakoosari M.M.D., Aliabadi M.A. (2013). Aflatoxin M<sub>1</sub> contamination in milk and milk products in Iran: a review. *Journal of Chemical Health Risks*. 3: 13-20.
- Diaz G.J., Sánchez M.P. (2015). Determination of aflatoxin M<sub>1</sub> in breast milk as a biomarker of maternal and infant exposure in Colombia. *Food Additives and Contaminants: Part A*. 32: 1192-1198.
- Duarte S.C., Almeida A.M., Teixeira A.S., Pereira A.L., Falcao A.C., Pena A., Lino C.M. (2013). Aflatoxin M<sub>1</sub> in marketed milk in Portugal: assessment of human and animal exposure. *Food Control*. 30: 411-417.
- El Marnissi B., Belkhou R., Morgavi D.P., Bennani L., Boudra H. (2012). Occurrence of aflatoxin M<sub>1</sub> in raw milk collected from traditional dairies in Morocco. *Food and Chemical Toxicology*. 50: 2819-2821.
- Fallah A.A. (2010a). Aflatoxin M<sub>1</sub> contamination in dairy products marketed in Iran during winter and summer. *Food Control*. 21: 1478-1481.
- Fallah A.A. (2010b). Assessment of aflatoxin M<sub>1</sub> contamination in pasteurized and UHT milk marketed in central part of Iran. *Food and Chemical Toxicology*. 48: 988-991.
- Galvano F., Pietri A., Bertuzzi T., Gagliardi L., Ciotti S., Luisi S., Gazzolo D. (2008). Maternal dietary habits and mycotoxin occurrence in human mature milk. *Molecular Nutrition and Food Research*. 52: 496-501.
- Ghazani M.H.M. (2009). Aflatoxin M<sub>1</sub> contamination in pasteurized milk in Tabriz (Northwest of Iran). *Food and Chemical Toxicology*. 47: 1624-1625.
- Ghiasian S.A., Maghsood A.H. (2012). Infants' exposure to aflatoxin M<sub>1</sub> from mother's breast milk in Iran. *Iranian Journal of Public Health*. 41: 119-126.
- Heshmati A., Milani J.M. (2010). Contamination of UHT milk by aflatoxin M<sub>1</sub> in Iran. *Food Control*. 21: 19-22.
- Iqbal S.Z., Jinap S., Pirouz A.A., Faizal A.A. (2015). Aflatoxin M<sub>1</sub> in milk and dairy products, occurrence and recent challenges: a review. *Trends in Food Science and Technology*. 46: 110-119.
- Jafari T., Fallah A.A., Kheiri S., Fadaei A., Amini S.A. (2017). Aflatoxin M<sub>1</sub> in human breast milk in Shahrekord, Iran and association with dietary factors. *Food Additives and Contaminants: Part B*. 10: 128-136.
- Jalili M., Scotter M. (2015). A review of aflatoxin M<sub>1</sub> in liquid milk. *Iranian Journal of Health, Safety and Environment*. 2: 283-295.
- Keskin Y., Başkaya R., Karsli S., Yurdun T., Ozyaral O. (2009). Detection of aflatoxin M<sub>1</sub> in human breast milk and raw cow's milk in Istanbul, Turkey. *Journal of Food Protection*. 72: 885-889.
- Kos J., Lević J., Duragić O., Kokić B., Miladinović I. (2014). Occurrence and estimation of aflatoxin M<sub>1</sub> exposure in milk in Serbia. *Food Control*. 38: 41-46.
- Mahdavi R., Nikniaz L., Arefhosseini S.R., Jabbari M.V. (2010). Determination of aflatoxin M<sub>1</sub> in breast milk samples in Tabriz-Iran. *Maternal and Child Health Journal*. 14: 141-145.
- Mason S., Arjmandtalab S., Hajimohammadi B., Khosravi Arsanjani A., Karami S., Sayadi M., Oryan A. (2015). Aflatoxin M<sub>1</sub> contamination in industrial and traditional yogurts produced in Iran. *Journal of Food Quality and Hazards Control*. 2: 11-14.
- Nemati M., Mehran M.A., Hamed P.K., Masoud A. (2010). A survey on the occurrence of aflatoxin M<sub>1</sub> in milk samples in Ardabil, Iran. *Food Control*. 21: 1022-1024.
- Obade M., Andang'o P., Obonyo C., Lusweti F. (2015). Exposure of children 4 to 6 months of age to aflatoxin in Kisumu County, Kenya. *African Journal of Food, Agriculture, Nutrition, and Development*. 15: 9949-9963.
- Oveisi M.R., Jannat B., Sadeghi N., Hajimahmoodi M., Nikzad A. (2007). Presence of aflatoxin M<sub>1</sub> in milk and infant milk products in Tehran, Iran. *Food Control*. 18: 1216-1218.
- Rafiei H., Dehghan P., Pakshir K., Pour M.C., Akbari M. (2014). The concentration of aflatoxin M<sub>1</sub> in the mothers' milk in Khorrambid city, Fars, Iran. *Advanced Biomedical Research*. Published online 2014 July 31. doi: 10.4103/2277-9175.137859.
- Sadeghi N., Oveisi M.R., Jannat B., Hajimahmoodi M., Bonyani H., Jannat F. (2009). Incidence of aflatoxin M<sub>1</sub> in human breast milk in Tehran, Iran. *Food Control*. 20: 75-78.
- Tajik H., Moradi M., Razavi Rohani S.M., Hadian M. (2016). Determination of aflatoxin M<sub>1</sub> in pasteurized and UHT milk in West-Azerbaijan province of Iran. *Journal of Food Quality and Hazards Control*. 3: 37-40.
- Tajkarimi M., Aliabadi-Sh F., Salah Nejad A., Poursoltani H., Motallebi A.A., Mahdavi H. (2008). Aflatoxin M<sub>1</sub> contamination in winter and summer milk in 14 states in Iran. *Food Control*. 19: 1033-1036.