



Incidence of Aflatoxin M₁ in Human and Cow Milk in Kashan, Iran

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HIGHLIGHTS

- All human and cow milk samples from Kashan were contaminated with Aflatoxin M₁ (AFM₁).
- AFM₁ concentration of human breast milk samples was less than standard limit of 25 ng/L.
- In 20.83% raw milk samples, the level of AFM₁ 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.

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Acronyms and abbreviations

AF=Aflatoxin
EU=European Union

ABSTRACT

Background: It is known that Aflatoxin M₁ (AFM₁) 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 AFM₁ 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 AFM₁ 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 AFM₁. Concentrations of AFM₁ 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 AFM₁ was greater than the maximum tolerance limit (50 ng/L) according to EU. Mean AFM₁ 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 AFM₁ 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 M₁ (AFM₁) as a metabolite of AFB₁ is created in body of human and some mammalian animals. AFB₁ 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 AFM₁ contamination in milk can have

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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 AFM₁ by consumption of contaminated cow milk as the powder milk or whole milk. Children who exposed to AFM₁ may be underweight and susceptible to infectious agents. Therefore, AFM₁ 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 AFM₁ 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 AFM₁ 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 AFM₁ 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 AFM₁ 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 AFM₁ 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 AFM₁ (Tables 1 and 2). AFM₁ 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 AFM₁ was greater than the maximum tolerance limit (50 ng/L) according to EU. Mean AFM₁ 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 AFM₁, 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 AFM₁ 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 AFM₁ 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 AFM₁. There are also

Table 1: The incidence of aflatoxin M₁ 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 M₁ 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 AFM₁ in human milk in the other countries. For example, AFM₁ 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 AFM₁ was detected in only 4 out of 82 (5%) Italian human milk samples having mean level of 55.35 ng/L. Also, AFM₁ 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, laboratory 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 AFM₁ which their average toxin concentration was 27.08±3.95 ng/L; however, the AFM₁ 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 AFM₁ 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 AFM₁ 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, AFM₁ 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 AFM₁ 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 AFM₁ 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, AFM₁ concentration was lower than maximum tolerance level regulated by EU. In another work, it was declared that AFM₁ 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, AFM₁ 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 AFM₁ 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 AFM₁ 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.

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