Assessment of aflatoxin M1 in maternal breast milk in Eastern Turkey

Meryem Atasever a, Yeliz Yildirim b,*, Mustafa Atasever a, Ayhan Tastekin c

a Department of Food Hygiene and Technology, Faculty of Veterinary Medicine, Ataturk University, Erzurum, Turkey
b Department of Food Hygiene and Technology, Faculty of Veterinary Medicine, Erciyes University, Kayseri, Turkey
c Division of Neonatology, Department of Child Health, Faculty of Medicine, Istanbul Medipol University, Istanbul, Turkey

1. Introduction

Human milk is ultimately the best source of nutrition for infants. Breastfeeding offers many advantages to both babies and mothers from psychological, immunological and economic point of view. Although breast milk contains optimally balanced fats, carbohydrates and proteins, it may also contain toxic chemicals caused by pollution and other sources. People including 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 (Gürbay et al., 2010). It has been recognized that the children exposed to aflatoxin M1 (AFM1) through milk or it is by products may become prone to the infectious diseases, underweight, and stunted during infancy and for the rest of their life (IARC, 2002).

Aflatoxin M1 is usually considered to be a detoxification by product of aflatoxin B1 (AFB1), which may be found in milk products obtained from the livestock fed with AFB1-contaminated feed (Creppy, 2002). Among more than three hundred aflatoxins, AFB1 is the most prevalent and toxic one. AFB1 acute toxicity is less severe than that of AFB1 and it belongs to Group 2B as “a potentially carcinogenic agent for human” (IARC, 1993). Although AFM1, a genotoxic carcinogen, is less toxic (about 10 times) than AFB1, exposure of infants to AFM1 exhibits an alarming condition. High metabolic rate, lower detoxifying ability, low body weights and incomplete development of vital organs (especially the central nervous system) makes infants more susceptible than adults for the adverse effects of AFM1.

The occurrence of AFM1 in cheese may be due to AFM1 contamination of raw milk used in cheese manufacture or synthesis of aflatoxins by Aspergillus flavus and Aspergillus parasiticus growing on cheese (Zerfiridis, 1985). As moldy cheese is not generally produced by using starter cultures under controlled conditions in Turkey, the presence of AFM1 in this kind of cheese type is of concern.

In Turkey, there exist a few data (Keskin et al., 2009; Gürbay et al., 2010) about AFM1 incidence on human breast milk. The aim of this study was therefore; (i) to determine the presence and level of AFM1 in human milk in Eastern Turkey and (ii) to evaluate any possible correlation between moldy cheese consumption of mother and AFM1 exposure of their infants.

2. Materials and methods

2.1. Sample preparation

Milk samples were collected from women between December 2008 and April 2009. A total of 73 human breast milk samples were obtained from mothers whose infants were inpatient in Department of Pediatrics, Section of Neonatology, Ataturk University, Faculty of Medicine, Erzurum, Turkey.

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Moldy cheese consumption
Equal amounts of breast milk (20 ml) were obtained from a total of 73 volunteer lactating mothers who were asked to complete a questionnaire to provide information whether they had the habit to consume moldy cheese at least once a week. Seventy-three lactating mothers (44 had the habit of consuming moldy cheese at least once a week, 29 had no such habit) agreed to participate in the study. The collected samples were kept at 4 °C in sterile glass containers and were frozen within 24 h at −20 °C until the analysis. Immediately before the analysis, all the samples were thawed gradually at 4 °C, vigorously mixed and centrifuged at 10 °C. As aflatoxins are water soluble, the upper creamy layers were completely discarded and the lower phases were used for the quantitative test.

2.2. Quantitative analysis of AFM1 in samples by competitive ELISA

Competitive ELISA test kit: RIDASCREEN aflatoxin M1 (R-Biopharm GmbH) based on the antigen–antibody reaction was employed for the quantitative analysis of AFM1 in MBM samples. The kit was used according to the manufacturer’s instructions.

2.2.1. Preparation of the milk samples

Ten ml of milk samples were chilled to 10 °C and the centrifuged for 10 min at 3500 rpm. The upper layer of fat was completely discarded. An aliquot (100 μl per well) of the lower fat-free phase was used in the test.

2.2.2. ELISA test procedure

A sufficient number of microtiter wells were inserted into the microwell holder for all standards and samples. One hundred μl standard solution and the samples prepared in separate wells were added and incubated for 30 min at 20–25 °C in the dark. The liquid was eliminated and the microwell holder was tapped upside down on an absorbent paper to remove the remainder of the liquid. The wells were washed twice with 250 μl washing buffer. Subsequently, 100 μl of the enzyme conjugate (peroxidase-conjugated AFM1) was added into each well and incubated for 15 min at 20–25 °C in the dark. The wells were washed three times with 250 μl washing buffer again. Consequently, 100 μl substrate/chromogen were added into each well and incubated for 15 min at room temperature in the dark. Finally, 100 μl of the stop reagent was added into each well and kindly shaken. The absorbance was measured at 450 nm in ELISA plate reader (ELX-800, Bio-Tek Instruments, USA) against an air blank within 15 min after stop solution addition.

2.2.3. Evaluation

According to the test protocol, the lower detection limit was 10 ng/l (10 ppt) for milk and 50 ng/l (50 ppt) for cheese. The recovery rate in milk was 95% with a mean coefficient variation of 15%. The samples were evaluated according to the RIDAVIN computer programme, prepared by R-Biopharm, and the statistical analyses were performed by the Mstat Statistical Programme.

2.3. Questionnaire

A questionnaire was used for collecting data from lactating mothers about the frequency of moldy cheese consumption in order to identify the potential sources of AFM1.

3. Results

Among the examined MBM of volunteer lactating mothers for their containment of AFM1, 18 out of 73 samples (24.6%) were found to be positive with the range of 1.3 and 6.0 ng/l (Table 1). AFM1 was determined from 12 MBM of 44 lactating mothers with moldy cheese consumption habit (27.2%) and 6 MBM of 29 mothers with no such habit (20.6%) ranging from 1.3 to 6.0 ng/l and 2 to 4.5 ng/l, respectively. No significant difference was observed between the groups for their AFM1 contents (p > 0.05) (Table 1).

As there seems no limit value for AFM1 in mothers’ breast milk neither in Turkey nor in European Union, we considered the EC limit value of AFM1 for infant formula (25 ng/l) (European Commission, 2010). Within this frame, although approximately one fourth of MBM samples tested were positive for AFM1 (24.6%), none of the samples exceeded the limit set by EC and Turkish legislations. Besides, the results indicated that there was no significant correlation between the moldy cheese consumption and the presence of AFM1 in MBM (p > 0.05).

4. Discussion

Mycotoxins are one of the most critical naturally occurring toxins in various foods under proper conditions. Review of literature shows that, because of the immunologic and nutritional effects of aflatoxin, there is a reasonable probability that the 6 top WHO risk factors for short lifespan, as well as the risks of liver cancer, are modulated by aflatoxin (Williams et al., 2004). Indeed, even small amounts of this metabolite in milk are of importance for the consumers of large quantities of milk, like children, for whom proportionally, milk can represent an important portion of aflatoxin intake (González-Osnaya et al., 2008).

El-Tras et al. (2011) indicated that the relative risk of exposure to AFM1 via the consumption of MBM was higher than that of the infant formula. It has been reported that children exposed to aflatoxins may become stunted, underweight and more susceptible to infectious diseases in childhood and the later life (Bhat and Vasanthi, 2003). The capability of carcinogens biotransformation is slower in infants than in adults, consequently the circulation and exposure time to toxins is increased in infants’ tissues (WHO, 1986). Therefore, the determination of aflatoxin levels in breast milk appears critical considering the vulnerability of infants.

From the results of current study, MBM could be regarded as a source of AFM1 for infants even the toxin is occurred at low levels. The low levels of aflatoxins do not guarantee the safety of mothers, since it has been reported that maternal consumption of aflatoxin-contaminated foods during breastfeeding could result in the accumulation of aflatoxins and their toxic metabolites in breast milk (Polychronaki et al., 2006; Gürbay et al., 2010).

In this study, 24.65% of MBM samples were found positive for AFM1 with the range of 1.3–6.0 ng/l. Considering the two previous studies in Turkey, AFM1 was found in low levels but the contamination frequency in our samples was higher than that of the results reported by Keskin et al. (2009) who found that the AFM1 levels were within the concentration range of 5.10–6.90 ng/l in 13.1% of the AFM1-positive samples. Also, both contamination levels and frequencies were lower than that reported by Gürbay et al. (2010) who indicated that all the breast milk samples were AFM1-positive ranging from 60.90 to 299.99 ng/l in Turkey. Herein, the low level of contamination could be due to the low level of AFB1 consumed by lactating mothers, such that the 75.35% of the milk samples have no detectable level of the AFM1 and none of the samples exceeded Turkish and European legislations for infant formula (25 ng/l).

Likewise, a recent study conducted in Turkey by Ertas et al. (2011), reports that 7% of milk products (milk, cheese, yogurt

<table>
<thead>
<tr>
<th>Sample</th>
<th>Tested (n)</th>
<th>Positive samples, n (%)</th>
<th>AFM1 contamination of positives samples (ng/l)</th>
<th>AFM1 contamination of all samples (ng/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Range</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>MBM</td>
<td>73</td>
<td>18 (24.65%)</td>
<td>1.3–6.0</td>
<td>3.01 ± 1.42</td>
</tr>
<tr>
<td>Mothers with no habit of moldy cheese</td>
<td>29</td>
<td>6/29 (20)</td>
<td>2.0–4.5</td>
<td>3.06 ± 1.63</td>
</tr>
<tr>
<td>Mothers with the habit of moldy cheese</td>
<td>44</td>
<td>12/44 (27)</td>
<td>1.3–6.0</td>
<td>2.97 ± 0.98</td>
</tr>
</tbody>
</table>

Table 1: The occurrence of AFM1 in human breast milk and the statistical correlation between AFM1 content and moldy cheese consumption of mother.
and dairy dessert) and 5% of cheese samples were contaminated with AFM1 above the limits of Turkish Food Codex.

Numerous researchers from different countries have carried out studies about the incidence of AFM1 in human breast milk and human milk bank. Among these, Afshar et al. (2013) found only one MBM sample positive for the AFM1 among 136 samples analysed (0.73%) with the contamination level of 20 ng/l. Mahdavi et al. (2010), detected AFM1 in 22% of the MBM samples in concentrations of 6.96 ± 0.94 ng/l. Sadeghi et al. (2009) reported 157 of 160 MBM samples to be contaminated with AFM1 ranging from 0.3 to 26.7 ng/l in Iran. Adejumo et al. (2013) reported that 52% of the MBM was contaminated with AFM1 ranging from 3.49 to 35 ng/l and 16% exceeded the EU limit of 25 ng/l in Nigeria. Two surveys from Egypt by Polychronaki et al. (2006) and (2007) indicated that the incidence of AFM1 in MBM was 56% and 36% and ranged from 5.6 to 5131 pg/ml and 4.2 to 889 pg/ml, respectively. El-Tras et al. (2011) also in Egypt, issued that 69.6% of the MBM samples were positive ranging from 7.3 to 328.6 ng/l. Abdulrazzaq et al. (2003) indicated that overall 92% of the breast-milk samples contained AFM1 in the United Arab Emirates. Navas et al. (2005) in Brazil reported that, only one (2%) of 50 human milk bank samples analysed was contaminated with AFM1. Different contamination levels and incidences found in various studies may be attributed to many internal and external factors including analytical methods, and several environmental, storage and climatic conditions that affect the aflatoxin production (Cotty and Jaime-Garcia, 2007).

The mothers’ questionnaire analyses did not reveal any relationship between the AFM1 contamination of MBM and mothers moldy cheese consumption. Sadeghi et al. (2009) indicated a marked correlation between the cereal consumption and AFM1 in breast milk. Besides, El-Tras et al. (2011) reported that the presence of AFM1 in MBM was significantly associated with mothers’ raw milk consumption.

5. Conclusion

These results pointed out the exposure of mothers and neonates to AFM1. Low amount of AFM1 found in this study supports the need for continuous breastfeeding of infants from the food safety point of view. It is recommended that further studies be extended to large quantity of human milk samples, infant formulas, milk-based products and other edible products destined to children. There is need to continue to monitor the level of contamination both in food and biological fluids to ensure infant protection and to develop protection strategies.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Transparency Document

The Transparency document associated with this article can be found in the online version.

References


World Health Organization (WHO), 1986. Principals for evaluating health risk from environmental, storage and climatic conditions that affect the aflatoxin production (Cotty and Jaime-Garcia, 2007).

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