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The Occurrence and Risk Assessment of Exposure to Aflatoxin M_1 in Ultra-High Temperature and Pasteurized Milk in Hamadan Province of Iran

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ABSTRACT

Objectives: Aflatoxins are a category of poisonous compounds found in most plants, milk and dairy products. The present research was carried out to detect the presence of aflatoxin M_1 (AFM_1) in samples of milk collected from Hamadan province, Iran.
Methods: Twenty five samples of ultra-high temperature (UHT) and 63 samples of pasteurized milk were collected and the amount of AFM_1 was measured by an Enzyme-Linked Immunosorbent Assay method. In addition, the estimated daily intake (EDI) and hazard index (HI) of AFM_1 was determined by the following equations:(EDI= mean concentration of AFM_1 × daily consumption of milk/body weight; HI= EDI/Tolerance Daily Intake).
Results: AFM_1 was detected in 21 (84%) UHT milk samples and in 55 (87.30%) pasteurized milk samples. Seven (28%) samples of UHT and 21 (33.33%) pasteurized milk samples had higher AFM_1 content than the limit allowed in the European Union and Iranian National Standard Limits (0.05 μg/kg). None of the samples exceeded the US Food and Drug Administration limit (0.5 μg/kg) for AFM_1. EDI and HI for AM_1 through milk were 0.107 ng/kg body weight/day, and 0.535, respectively.
Conclusion: A significant percentage of milk produced by different factories in Iran (84% of UHT and 87.3% of pasteurized milk) was contaminated with AFM_1. Therefore, more control and monitoring of livestock feeding in dairy companies may help reduce milk contamination with AFM_1. As the HI value was lower than 1, it can be assumed that there was no risk of developing liver cancer due to milk consumption.

Introduction

Aflatoxins are a primary class of mycotoxins that are poisonous and cause cancer. They are fungal secondary metabolites that are mainly produced by some *Aspergillus* species, especially *A. flavus*, *A. nomius* and *A. parasiticus* [1]. These molecules are produced under warm and humid conditions during pre- and post-harvest, during storage and transportation [2,3]. They can be found in cereal grains (particularly maize, rice, pearl millet, wheat, barley, oats, and sorghum), spices (red pepper, black pepper, turmeric cinnamon, ginger and cumin), oilseeds (sunflower, groundnut, cottonseed, soybean) tree nuts (such as almond, coconut, peanut), Brazil nuts, pistachios, milk (breast and animal), and dairy products [2-10].

The main aflatoxins are classified into 4 groups including aflatoxin B_1 ($AFB1_1$), B_2 (AFB_2), G_1 (AFG_1) and G_2 (AFG_2), that are classified according to their fluorescence under blue or green

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light. In addition, aflatoxin M1 (AFM_1) and M2 (AFM_2), are 2 more additional metabolic products that are derivatives found in the milk of lactating animals that have been fed on aflatoxin containing preparations (AFB_1 and AFB_2) [4,10,11]. According to the International Agency for Research on Cancer, classified AFM_1 as a carcinogen hence, modifying its classification from Group 2B to Group 1 [12].

Milk and dairy products are a source of many nutrients including proteins, fatty acids, calcium, vitamins, and minerals essential for human health, especially in infants and children [13]. Several studies have been performed on the incidence of AFM_1 in milk products and a permissible limit has been suggested. These regulations differ amongst countries and are usually concerned with economic considerations [3-5,9,10]. The Institute of Standards and Industrial Research of Iran, and the European Commission have set the maximum tolerable level of AFM_1 in milk as 0.05 μg/kg [14,15] however, the US Food and Drug Administration set the limit to 500 μg/kg [16].

Risk assessment of exposure to AFM_1 through the consumption of contaminated milk and dairy products is useful in the measurement of the risk of liver cancer. This is estimated by the determination of indices such as estimated daily intake [(EDI) expressed as ng/kg body weight (BW)/day] and hazard index (HI) to indicate risk for human [17,18]. A HI value > 1 indicates a risk to consumers [19].

At the present time, AFM_1 analysis is performed by different methods [thin-layer chromatography, high-performance liquid chromatography, and enzyme-linked immunosorbent assay (ELISA)]. However, the advantages of using the ELISA technique is that it is simple and less time consuming compared to other methods, and has high specificity and high sensitivity for the detection of aflatoxin groups and numerous samples [3-5,9,10].

The target of this research was to survey the presence of AFM_1 and the risk assessment of exposure to AFM_1 through the consumption of ultra-high temperature (UHT) and pasteurized milk in the Hamadan province of Iran.

Materials and Methods

1. Samples

From October 2017 to August 2018, a whole of 88 samples included UHT milk (n = 25) and pasteurized milk (n = 63) were collected and purchased from different markets in Hamadan province, Iran. Eventually, all samples were carried to the lab inside and kept according to the samples. So, for this purpose, the UHT and pasteurized milk samples were kept at room temperature and 4°C before analysis, respectively. All milk samples were analyzed for AFM_1 before the expiration date of the samples.

2. AFM_1 analysis

The quantitative determination of AFM_1 in milk samples were performed using an AFM_1 competitive ELISA kit (Ridascreen AFM_1 Art. No.: R1121, R-Biopharm, Darmstadt, Germany). Milk sample preparation was performed according to the instructions suggested by the ELISA kit (R-Biopharm, Darmstadt, Germany) [20]. The limit of detection and recovery rate of the ELISA kit was 0.005 μg/kg^{-1} and 100%, respectively.

3. Risk assessment of exposure to AFM_1

In this current study EDI and HI of AFM_1 were calculated to show the presence and concentration of AFM_1 and risk of liver cancer for this mycotoxin [17,18]. EDI was determined by the equation: EDI (ng/kg BW/day) = AFM_1 average in milk (μg/kg) × daily consumption of milk (kg/day)/BW (kg) × 1,000 (conversion of μg to ng). For the determination of EDI, AFM_1 concentrations in samples containing AFM_1 below the level of determination of ELISA kit (< 0.005 μg/kg) were considered 0.0025 μg/kg.

It has been reported that per capita consumption of milk in Iran is 70 kg a year, which equates to 0.192 kg/day [21]. In this current study, the average BW of an adult Iranian person was taken as 70 kg. The estimation of HI was carried out according to the method suggested by Kuiper-Goodman [22]. HI was determined by dividing the EDI with the tolerance daily intake (TDI). For AFM_1, TDI was 0.2 ng/kg/day, obtained by dividing TD50 (threshold dose per BW) with a variability factor of 5,000.

4. Statistical analysis

Data analysis was carried out using SPSS 16.0 for Windows (SPSS Inc., Chicago, IL, USA). The AFM_1 concentration was reported as the mean ± standard deviation. The one-sample T-test was applied to determine the difference between the mean concentration of AFM_1 in samples and the permitted amount of this mycotoxin according to the Institute of Standards and Industrial Research of Iran and European Union (EU) regulations which is 0.050 μg/kg. Differences between values were considered as significant at $p \leq 0.05$.

Results and Discussion

1. The occurrence of AFM_1 in milk

The occurrence and concentration of AFM_1 contamination in UHT and pasteurized milk samples are summarized in Table 1. Fifty-five (87.30%) pasteurized milk samples contained AFM_1 with a range of concentrations from < 0.005 μg/kg to 0.120 μg/kg. In addition, the AFM_1 concentration in 21 (33.33%) of pasteurized samples was higher than the maximum limit of 0.05 μg/kg set by EU regulations [14].

Table 1. The contamination of AFM_1 (μg/kg) in pasteurized and UHT milk samples.

Sample type	*N*	Positive (%)	Mean	SD	AFM_1 concentration (μg/kg)			
					Undetectable < 0.005 (%)	0.005-0.050 (%)	> 0.050 (%)	Range
Pasteurized milk	63	55 (87.30)	0.040	0.033	8 (12.70)	34 (53.97)	21 (33.33)	< 0.005-0.120
UHT milk	25	21 (84)	0.037	0.029	4 (16)	14 (56)	7 (28)	< 0.005-0.098
Total	88	76 (86.36)	0.039	0.032	12 (13.64)	48 (54.54)	28 (31.82)	< 0.005-0.120

AFM_1 = aflatoxin M_1; UHT = ultra-high temperature.

AFM_1 was detected in 21 (84%) UHT milk samples, with a range of concentrations from < 0.005 to 0.098 μg/kg. The AFM_1 concentration in 7 (28%) UHT samples was higher than 0.05 μg/kg. Collectively, AFM_1 contamination was detected in 86.36% of milk samples in the range of < 0.005 to 0.120 μg/kg. Furthermore, none of the milk samples had AFM_1 concentrations above the highest tolerance limit (0.5 μg/kg) set by the US Food and Drug Administration.

As referred to in previous studies, the occurrence of AFM_1 in milk and milk derivatives are due to the effects of feeding livestock with materials that contain aflatoxin B1. Several factors impact the level of aflatoxin B1 in cattle feed such as time and method of harvesting, temperature, and moisture content. Aflatoxin B1 swiftly grows in cattle feed that possess a moisture content of 13% to 18%, and an environmental humidity 50% to 60% [4].

Several studies have reported that milk is a food with a high occurrence of AFM_1 contamination although the concentration levels differ from one study to another. It should be noted that the concentration of AFM_1 changes according to the season, milk produced in Spring and Summer are contaminated with lower levels of this mycotoxin mainly due to availability of fresh feed [4,5,23,24].

This study was performed in the west of Iran, Hamadan province. In this district, a considerable amount of milk is produced and consumed, although there are few reports about AFM_1 in milk samples. The detection of AFM_1 contamination in samples in this current study (86.36%) was higher than a previous study (63.97%) by Ghiasian et al [25] carried out in Hamadan province although the mean AFM_1 concentration in the samples in this current study was lower (0.039 μg/kg vs 0.043 μg/kg). These results are reflected by other studies across Iran that indicated that the prevalence of aflatoxin in milk samples was still high, although its average concentration decreased [4,26].

In some studies, the prevalence of AFM_1 in UHT or pasteurized milk samples was higher than in this study [27-33]. However, a study performed in Kosovo using the ELISA method, reported that 70 (83.3%) samples out of 84 samples of pasteurized milk had AFM_1 contamination, and in 18 (21.4%) contaminated samples had concentrations of AFM_1 higher than the EU permitted level, similar to this current study [34]. Furthermore, a study performed in Italy showed that of the 43 samples of pasteurized milk that were screened for AFM_1, 11 (25.58%) samples were contaminated and 8 (72.7%) of those samples had a concentration of AFM_1 above the European Union limit for milk [24]. In other studies conducted in Iran, pasteurized milk samples were identified as being contaminated with AFM_1 and in one study where 76 pasteurized milk samples were collected in Sari, 100% were positive for AFM_1 and had concentrations of 0.0117 to 0.1066 μg/kg [35]. However, the incidence of AFM_1 in milk samples in some previous studies performed in Iran and other countries, was lower than observed in this current study [29,32,36-39]. In a previous study conducted in Pakistan using high-performance liquid chromatography to detect AFM_1, Iqbal et al [39] reported that 41.6%, 35 out of 84 UHT milk samples had AFM_1 contamination and 23.8% of those had concentrations above the EU recommended limit, but in this current study 21 (84%) samples of UHT milk were contaminated with AFM_1, and 7 (28%) of those samples were observed to have AFM_1 concentrations above the EU recommended maximum limit. Similarly, in a study conducted in Iran, Fallah [23] reported that contamination with AFM_1 in UHT milk (0.000 to 0.516 μg/kg) as detected by ELISA method showed 68 (62.3%) out of 109 samples were contaminated with AFM_1 and 19 (17.4%) samples were above the acceptable level.

Similar results reported in China showed 84 (~55%) out of 153 UHT milk samples had contamination with AFM_1, and 20.3% of those were above the EU permitted level [40]. A Sicilian study by Santini et al [41] using the ELISA technique observed that 5 (41.7%) out of 12 UHT milk samples were contaminated with AFM_1 but no samples above the EU regulations for AFM_1 concentration in milk. A Turkish study by

Table 2. Occurrence and levels of AFM1 in pasteurized and UHT milk samples reported in previous studies.

Location	Samples	No. of samples	Detection method	Positive samples (%)	Range (μg/kg)	Contaminated milk sample (%) > 0.05 μg/kg	Reference
Kenya	PM	53	ELISA	NR	0.0076 - 0.21	26 (49.1%)	Lindahl et al [50]
	UHT	55		NR	< LOD - 0.47	16 (29.1%)	
China	PM	131	ELISA	120 (91.6)	< 0.005 - 0.3523	78 (59.5%)	Xiong et al [29]
	UHT	111		58 (52.3)	< 0.005 - 0.0725	2 (1.80%)	
China	PM	38	ELISA	18 (47.4)	0.005 - 0.263	0	Li et al [36]
	UHT	193		138 (71.5)	0.007 - 0.040	23 (11.9%)	
Pakistan	UHT	60	HPLC	42 (70)	LOD - 0.3029	21 (35%)	Iqbal et al [1]
Iran	PM	220	ELISA	187 (85)	0.0054 - 0.5122	154 (70%)	Tajic et al [28]
	UHT	140		93 (66.4)	0.0058 - 0.5084	76 (54.2%)	
Brazil	PM	30	ELISA	16	ND - 0.064	NR	dos Santos et al [51]
Italy	UHT	31	HPLC	18 (58.1)	0.009 - 0.026	0	Armorini et al [38]
Iran	PM	20	HPLC	NR	0.008 - 0.231	1 (5%)	Taherabadi et al [52]
Jordan	PM	30	ELISA	30 (100)	0.0146 - 0.21678	12 (40%)	Omar [31]
Iran	PM	30	ELISA	4 (13.3)	NR	4 (13.3%)	Rouhi et al [32]
Brazil	PM	7	ELISA	7 (100.0)	0.01 - 0.03	2 (28.6%)	Sifuentes dos Santos et al [27]
	UHT	28		28 (100.0)	0.01 - 0.08	5 (71.4%)	
Kosovo	UHT	94	ELISA	74 (78.7)	0.00502 - 0.06226	4 (4.2%)	Rama et al [34]
Croatia	UHT	706	ELISA	706 (100)	0.00398 - 0.1835	(9.64%)	Bilandzic et al [30]
Iran	PM	80	ELISA	77 (96.25)	NR	20 (16%)	Moosavy et al [33]
Pakistan	UHT	84	HPLC	35 (41.6)	LOD – 0.88	20 (23.8%)	Iqbal et al [39]
India	PM	7	HPLC	3 (42.9)	0.0018-0.0038	3 (42.9%)	Siddappa et al [37]

ELISA = enzyme-linked immunosorbent assay; HPLC = high-performance liquid chromatography; LOD = limit of detection; ND = not detected; NR = not reported; PM = pasteurized milk; UHT = ultra high temperature milk.

Atasever et al [42] reported that 89 (59%) out of 150 UHT milk samples contained AFM_1. Another study carried out in Turkey by Kabak and Ozbey [43] reported less contamination with AFM_1 (20%) and where contamination was observed it was at low concentrations in UHT milk samples. However, in a recent survey by Temamogullari and Kanici [44] AFM_1 was observed in 100% of the surveyed UHT milk samples from Turkey.

In this current study a high occurrence of AFM_1 contamination was observed in UHT and pasteurized milk samples. Table 2 show the compilation of data for the detection of AFM_1 from previous studies in several countries that were measured by ELISA and high-performance liquid chromatography methods. Although the presence of aflatoxin in milk and dairy products may endanger human health, this risk may be reduced by implementing 1) Education for producers about planting, harvesting, preserving and transportation, 2) Teaching the principles of good industrial livestock husbandry, 3) Prevention of milk and dairy product contamination during processing and packaging, 4) further studies into the field of detoxification methods to reduce contamination with AFM_1 in dairy products [4].

2. The exposure to AFM_1 through milk consumption

EDI of AFM_1 through milk was 0.107 ng/kg BW/day in this current study. This value was lower than those reported by Zinedine et al [45] in Morocco (3.26 ng/kg BW/day), Cano-Sancho et al [46] in Spain (0.305 ng/kg BW/day) and Bahrami et al [21] in the west of Iran (0.17 and 0.242 ng/kg BW/day during summer and winter, and higher than that observed by Leblanc et al [47] in France (0.01 ng/kg BW/day), Shundo et al [48] in Brazil (0.08 ng/kg BW/day) and Duarte et al [49] in Portugal (0.08 ng/kg BW/day).

In the current study, the HI value was 0.535. As the HI value was lower than 1, it can be assumed that there was not a potential risk for liver cancer among Iranian consumers in Hamadan province due to the consumption of milk [17-19]. In a study performed by Milićević et al [19] in Serbia, HI values were calculated for infants aged 1–4 years which were 11.78 and 11.52 for males and females respectively, values which were higher than the observations in this current study.

Conclusion

The results of the current study indicated that a significant percentage of pasteurized and UHT milk samples (86.36%) produced by different factories, are contaminated with AFM_1. In addition, 31.82% of contaminated samples had concentrations of AFM_1 higher than the limit allowed in the European Union and by the Iranian National Standards Limits (0.05 μg/kg). In this study the EDI of AFM_1 through milk for an adult with a BW of 70 kg, was 0.107 ng/kg BW/day. Although this indicated a high incidence of AFM_1 in milk samples, HI (0.535) was lower than 1, and it showed milk intake in the west of Iran did not have any potential risk for liver cancer in consumers. However, concerns about the presence and the concentration of AFM_1 in milk is common for some consumers especially infants and children. To help reduce AFM_1 contamination of dairy products, more control and monitoring of livestock feeding, and the companies that produce these products can be carried out.

Conflicts of Interest

Authors have no conflicts of interest to declare.

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