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Original Article

A Study on the Presence of Aflatoxin M_1 in Cow's Milk in Jiroft

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Background and Aims: Cow's milk is a daily staple food for many individuals that can be contaminated with many toxins such as aflatoxin M_1 (AFM_1). AFM_1 is a chemical form of the aflatoxin B_1 produced by some species of *Aspergillus* genus like *A. ochraceus*, *A. flavus*, *A. nomius*, *and A. parasiticus* that can contaminate feed and forage cattle. This toxin enters into the milk after eating contaminated feed by cows. AFM_1 can cause various dangerous diseases such as cancer and immune deficiency in humans. The present study is aimed to investigate the level of AFM_1 in cow's milk in Jiroft, Kerman Province, Iran.

Materials and Methods: A total of 90 cow's milk samples were collected in spring and summer 2019 from available stores in Jiroft city. Enzyme-linked immunosorbent assay was used to measure AFM_1 in all cow's milk samples.

Results: In the present study, AFM_1 was found in 88 (97.8%) milk samples with a range of 0.2-90.62 ppt (mean, 20.07±24.46 ppt). AFM_1 concentrations exceeded 50 ppt (maximum tolerance level of AFM_1 in the European Union) was seen in 12 (13.3%) samples.

Conclusions: The results of this study showed the presence of AFM_1 in cow's milk in Jiroft city. So, in this region, many people are exposed to dangerous diseases such as cancer due to the consumption of milk contaminated with AFM_1.

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Introduction

Cow's milk is one of the healthiest drinks in the world that has been associated with the growth and health of individuals [1]. This foodstuff contains various important nutrients including protein, choline, calcium, iodine, potassium, phosphorus, magnesium, zinc, iron copper, selenium, vitamins A, C, D, E, and vitamins B1, B2, B5, B6, and B12 [2]. Recent evidence suggests that cow's milk is associated with a reduction in the risk of type 2 diabetes, hypertension, stroke, heart disease, cancers, depression, osteoarthritis, chronic fatigue, and muscle pain [3]. Although cow's milk has marked effects on the growth and health of people, it can be contaminated with various toxins like bacterial toxins and mycotoxins [4]. Mycotoxins are highly toxic secondary metabolites produced by some fungal strains [5]. Mycotoxins can have carcinogenic, mutagenic, teratogenic, immunotoxic, and oestrogenic effects in humans and animals. Aflatoxins are the most common and important class of mycotoxins produced by four different species *of Aspergillus* genus like *A. ochraceus, A. flavus, A. nomius, and A. parasiticus.* Among aflatoxins (AFs), AFB_1 is the most hazardous, followed by AFB_2, AFG_1, and AFG_2 [6]. AFB_1 can be found in dairy cow feed such as alfalfa, hay, corn, barley, and legumes [7]. This toxin can be converted to aflatoxin M_1 (AFM_1) by cytochrome P450-associated enzymes in the liver and excreted into the cow's milk 12-24 hours after the first ingestion of AFB_1 contaminated feed [8]. It has been observed that the level of AFM_1 in milk is not affected by pasteurization and ultra-high-temperature treatments [9]. AFM_1 can cause various health problems like liver cancer, immune suppression, child growth impairment, neutro-penia, and acute hepatitis. Liver cancer is the most important disease caused by this toxin. So AFM_1 has been classified by the International Agency for Research on Cancer (IARC) as a group 1B carcinogen [10]. Many studies have been carried out in Iran and other countries on the concentration of AFM_1 in cow's milk [11-13]. In Hashemi study [11], AFM_1 was detected in 100 (55.56%) cow's milk samples in Fars province, Iran. In Dakhili et al. [12] study, AFM_1 was detected in sixty five (96.5%) samples in Qom province, Iran. In Camaj et al. [13] study, AFM_1 was detected in 80 (47.1%) cow's milk samples in Kosovo. Jiroft city in south Iran has a warm and humid climate and extensive vegetation that provides favorable conditions for the growth of aflatoxins-producing fungi. Furthermore, so far, no study has been done on the level of AFM_1 in cow's milk in this city. The purpose of this study was to examine the level of AFM_1 in cow's milk in the city of Jirof, Kerman Province, south of Iran.

Materials and Methods

Sampling

Jiroft is a small city in southern Iran. There are a few dairy shops in the city. This cross-sectional study was carried out during spring and summer 2019. A total of 90 cow's milk samples were collected. Thirty-six non-pasteurized traditional cow's milk samples

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were collected in the spring and forty-seven samples were collected in the summer. In addition, seven pasteurized commercial cow's milk samples from six various brands were collected. The samples were transported to the laboratory in a cold box and stored at -20°C until analysis.

Sample preparation

Each milk sample was centrifuged at 3500 rpm (2740 × g) at 10 °C for 10 min. After centrifugation, the upper creamy layer was removed, and then the lower phase was provided for the quantitative analysis.

Enzyme-linked immunosorbent assay (ELISA)

An ELISA test kit (Neogen Veratox ®, Lansing, USA) was used to measure the level of AFM_1 in cow's milk samples. All stages of the test were done by an ELISA automatic device (Add care 2000, China). Briefly, 100 µl of each standard, positive control, and the defatted milk samples were dispensed into appropriate wells of microplate and then shaken (600 rpm) for 20 min at room temperature. The solution in the microplate was discarded, and the plate was washed 5 times with wash buffer. 100 µl of the conjugate was added to each well. The microplate was shaken for 10 minutes at room temperature. The solution in the microplate was removed, and the plate was washed 5 times with wash buffer. One Hundred µl of substrate solution was added into each well and incubate for 15 minutes in the room. One Hundred µL of stopping solution was added to each well of the microplate. The plate was read at 650 nm. After that, the calibration curve was drawn and used to determine the level of AFM_1 in each cow's milk sample. According to the European Union (EU), the maximum level of AFM_1 in raw milk and heat-treated milk should not exceed 50 ppt and 25 ppt for adults and infants, respectively. This study was approved by the Ethics Committee of Jiroft University of Medical Sciences, Jiroft, Iran.

Statistical analysis

All the data were analyzed by SPSS version 23 software. Descriptive statistics was used for data classification and values were expressed as frequency and percentages. One-way ANOVA followed by Bonferroni's test was used for post hoc mean comparisons.

Results

In the present study, 90 cow's milk samples were analyzed for the presence of $AFM_{1.}$ AFM_1 was found in 88 (97.8%) milk samples with a range of 0.2-90.62 ppt (mean, 20.07±24.46 ppt). In our study, the mean of AFM_1 in pasteurized commercial cow's milk, milk samples from spring and milk samples from summer were 14.53 ± 15.8 ppt (range:1.36-44.71 ppt), 15.12 ± 22.02 ppt (range: 0.2-88.54 ppt), and 24.69 ± 26.66 ppt (range: 0.2-90.62 ppt), respectively. The level of AFM_1 in cow's milk samples is represented in Table 1. The post hoc Bonferroni's test revealed that the level of AFM_1 in milk samples from spring was significantly lower than milk samples from summer (p=0.005). There was no significant difference between the pasteurized commercial cow's milk samples and the samples collected in spring and summer (raw milk samples) (p> 0.05). AFM_1 concentrations exceeded 50 ppt (maximum tolerance level of AFM_1 in the EU) was seen in 12 (13.3%) samples.

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Table 1. The concentration of aflatoxinM_1 in cow's milk samples (ppt)

Sample	Number and percent of samples with aflatoxinM_1 in ppt range			
	0-25	26-50	>50	Total
pasteurized samples	5 (71.4)	2 (28.6)	0 (0)	7 (100)
Spring samples	31 (86.1)	2 (5.6)	3 (8.3)	36 (100)
Summer samples	30 (63.8)	8 (17.1)	9 (19.1)	47 (100)

Table 2. Limits of aflatoxin M1 in cow's milk samples for infants in different countries

Country	Limit (ppt)	Number and percent of exceeded limit	Number and percent of healthy milk samples
Romania	0	88 (97.8)	2 (2.2)
German	1	87 (96.7)	3 (3.3)
Austria and Belgium	10	45 (50)	45 (50)
Netherlands	20	27 (30)	63 (70)
European Union	25	24 (26.6)	66 (73.4)
France	30	20 (22.2)	70 (77.8)

AFM_1 concentrations exceeded 25 ppt (maximum tolerance level of AFM_1 in the EU for infants) was seen in 24 (26.6%) samples. Limits of aflatoxin M_1 in cow's milk samples for infants in different countries are represented in Table 2.

Discussion

Cow's milk has many nutrients, minerals, and vitamins that are vital for the body [1]. However, milk can become contaminated with AFM_1 and cause very dangerous diseases in humans [8]. So, throughout the world, there is great attention focused on the measurement and control of AFM_1 contamination in milk [11-13]. This study aimed to investigate the level of AFM_1 in cow's milk in Jiroft city, Kerman Province, Iran. In the present study, AFM_1 was detected in 88 (97.8%) milk samples. Jiroft has a warm and humid climate that can increase the growth of toxin-producing fungi. Therefore, forage and other cow's feed can easily contaminate by these toxins. Feeding cows with this forage can contaminate their milk. In the study by Anyango et al. [14], AFM_1 was detected in all (100%) milk samples. In the study by Dakhili et al. [12], AFM_1 was detected in Sixty five (96.5%) samples. In the study by Goncalves et al. [15], AFM_1 was detected in 52 (100%) samples. In the study by Shuib et al. [10], AFM_1 was found in 4 (4%) samples. In the study by Bokhari et al. [16], AFM_1 was found in 24 (75%) cow's milk samples. The result of the present study was consistent with the findings of Anyango et al. [14], Dakhili et al. [12], and Goncalves et al. [15] studies but was inconsistent with the findings of Shuib et al. [10], and Bokhari et al. [16] studies. In the current study, 13.3% of the milk samples had AFM_1 more than the maximum tolerance limit. In the study by Anyango et al. [14], AFM_1 was found in 26.4% of samples with concentrations above tolerance levels accepted by the EU. In Dakhili et al.'s study [12], about 15% of the milk samples had AFM_1 greater than the maximum tolerance limit. In Goncalves et al.'s study [15], 31 samples (59.6%) showed levels higher than the maximum tolerance limit. In the study by Shuib et al. [10], only three samples (3%) exceed the EU legal limit. In the study by Bokhari et al. [16], 50% of samples exceed the EU legal limit. The result of the present study was consistent with the findings of Anyango et al. [14], study but was inconsistent with the findings of Shuib et al. [10], Dakhili et al. [12], Goncalves et al. [15], and Bokhari et al. [16] studies. Differences in the results of various studies may be related to several environmental, nutritional, and physiological factors including animal breed, hepatic biotransformation capacity, mammary infections, AFB_1 level in feed, daily intake of AFB_1, temperature, and humidity of air, amount and type of plants in each region, food preservation and consumption methods, methods of measuring the toxin, and technician's skill in measuring the toxin [6, 17, 18]. In addition, the lactation period is very important in the amount of toxin in the milk. During the lactation peak (weeks 25 & 26), the amount of toxin is reduced due to the dilution of the toxin in the high volume of milk produced [17]. In the present study, the level of AFM_1 in summer samples was significantly higher than in spring. Hot and humid weather in summer can increase the growth of fungi and produce more aflatoxins in cow's feed that is kept in poor conditions. Although the maximum tolerance limit recommended by EU is more useful, it is varied in different countries depend on the availability of toxicological data of AFM_1, availability of exposure data of the toxin, data on the level of AFM_1 in various commodities, availability of sampling and analytical methods, commercial laws in different countries, and the amount of food available [19]. The maximum tolerance limit in infants and children are lower than in adults due to their higher metabolic capacities, lower body weight, inability to sufficient detoxification, and incomplete development of the immune system and some tissues and organs [6, 19]. In the present study according to the mycotoxin regulations in countries such as Romania (maximum level of AFM_1=0 ppt), German (maximum level of AFM_1=1 ppt), Austria (maximum level of AFM_1=10 ppt), and Netherlands (maximum level of AFM_1=20 ppt) [19], 97.8%, 96.7%, 50% and 30% of cow's milk samples, respectively, were not suitable for use by infants. In addition to raw milk, we assayed for AFM_1 in pasteurized milk from all brands available in Jiroft city. There was no significant difference between the pasteurized commercial cow's milk and raw milk. According to the EU mycotoxin regulations, a total of 50% of pasteurized milk weren't suitable for use by infants and children, however, all pasteurized milk was suitable for use by adults. In Shaker et al.'s study [18], all pasteurized milk samples had AFM_1 greater than the maximum tolerance limit recommended by EU. In Skrbic et al.'s study, 50% of pasteurized milk samples had AFM_1 lower than the maximum tolerance limit. This result of the present study was consistent with the findings of Skrbic et al. study [20] but was inconsistent with the findings of Shaker et al. study [18]. AFB_1 enters the cow's body through various contaminated feeds and is secreted into milk after conversion to AFM_1 by the liver. In Iran, many studies have shown that corn is an important feed in the entry of AFB_1 into the cow's body [21-23]. In the studies by Ghiasian et al. [21], Messripour

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et al. [22], and Karami-Osboo et al. [23], 69.15%, 38%, 43.6% of corn samples were contaminated with AFB_1, respectively. Temperature and humidity are the most important factors that can increase the growth of toxin-producing fungi and corn contamination.

Conclusion

The results of this study showed the presence of AFM_1 in cow's milk in Jiroft city, South of Iran. In addition, a large number of milk samples showed levels higher than the maximum tolerance limit proposed by EU, Romania, Austria, Belgium, France, and the Netherlands. So, in Jiroft city, many people are exposed to dangerous diseases such as cancer due to consumption of milk contaminated with AFM_1. Our study showed that infants are more at risk. Therefore, the level of AFM_1 in cow's milk should be measured, and in case of high cow's milk contamination, lactation should be avoided. Future studies are recommended to examine the presence of AFM_1 in various cow feed. Cow's feed storage should be standardized and ventilated to control its temperature and humidity to prevent fungal growth.

Conflict of Interest

The authors declare that there is no conflict of interest.

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