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RESEARCH ARTICLE

Aflatoxin M1 in distributed milks in northwestern Iran: occurrence, seasonal variation, and risk assessment

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Abstract

Aflatoxin is one of the dangerous fungal toxins that is produced in the presence of humidity and heat and lack of proper storage conditions and is considered as a dangerous substance threatening human health. The work aimed to determination of aflatoxin M1 (AFM) level in raw, pasteurized, and sterilized milks offered in the study area and to evaluate the risk of its consumption. In this descriptive cross-sectional study, 60 samples of milk types supplied in the region were collected during two series of sampling (cold and hot seasons) and analyzed by ELISA method. LCR, MoE, and HI indices were used to assess the possible risk of consuming milk containing AFM. In all taken samples AFM was found, the toxin concentration range in the first and second series of sampling was 57.5–270.6 ng/L and 57–185.9 ng/L, respectively. The level of toxin in pasteurized and sterilized milks in both sampling series was higher than raw milks. Based on the obtained data and in order to assess the risk of milk consumption in the target population, associated values with EDI, LCR, MoE, and HI were also calculated and obtained in the range of 0.145–0.3 ng/k.d b.w, 0.0008–0.0017 additional case per one million population, 1892.9–3921.6 and 0.72–1.5 ng/kg b.w, respectively. According to the findings, all samples tested are contaminated and although it is within the range of the WHO recommendation, however, based on the calculated indicators, the risk of liver cancer threatens the consumers of these milks. Therefore, it is necessary to manage this issue through educational control and monitoring measures.

Keywords Aflatoxin · Traditional milk · Pasteurized milk · Risk assessment · Ardabil

Introduction

Milk is considered one of the most perfect food and that is effective in lowering blood pressure and increasing its beneficial fats, preventing colon cancer and osteoporosis, and providing many nutrients such as protein and calcium; therefore, the contamination of this valuable food and its products is considered a serious threat to the public health (Campone et al. 2018). It is also a staple food for infants, children, and other people growing up, which is used in various forms such as yogurt, cheese, and in applications such as confectionery, production of chocolates and cookies(Pour et al. 2020). Thus, as a valuable food, its absence from pathogens, toxins, and carcinogens should be among the health priorities of society (Khaneghahi Abyaneh et al. 2020).

Among the many factors that cause food spoilage, fungal toxins (mycotoxins) are very important. Fungi are present at high levels in the air and our environment, and if conditions of temperature and humidity be suitable, they

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can grow and proliferate (Ansari et al. 2019). *Aspergillus flavus* and *Aspergillus parasiticus* are the most important food contaminants that involved in the poisoning process, due to the secretion of the toxins (that are called aflatoxins) by these fungi; contamination of animals feed with these fungal agents results in contamination of milk and finally it is received by the consumers (De Roma et al. 2017). Aflatoxins are found in large amounts in food and moldy forage and are of primary importance among the present toxins (Patyal et al. 2020). This toxin has mutagenic, carcinogenic, and immunosuppressive effects (Sharma et al. 2020). Aflatoxins are acutely and chronically toxic to humans and animals and can cause dangerous diseases such as acute liver disease, liver cirrhosis, and tumors (Abyaneh et al. 2020; Fakhri et al. 2019). Of the more than 20 types of aflatoxins produced by fungal metabolites, the four main types of aflatoxins B1, B2, G1, and G2 are found in food; aflatoxins M1 and M2 are metabolites derived from the hydroxylation of aflatoxins B1 and B2, respectively (Ghaffarian Bahraman et al. 2019). They were isolated for the first time from the milk of animals fed contaminated feed. Aflatoxin is heat resistant and decomposes at 237 to 306 °C, so it resists common maintenance methods and thermal changes such as pasteurization, sterilization, autoclave, and other production methods in food processes, and these methods will not reduce the amount of toxin (Ismaiel et al. 2020).

The World Health Organization (WHO) has listed aflatoxin B1 in the first group of carcinogens and AFM (hydroxylated form of aflatoxin B1) in the second group of carcinogens for humans and animals (Xiong et al. 2020). According to epidemiological studies, the WHO has recommended the permissible level of AFM in milk and dairy products in the range of 50–500 ng/L (Hajmohammadi et al. 2020; Khaneghahi Abyaneh et al. 2020); in order to reduce the risks of consuming aflatoxin-contaminated feed, the permissible amount of AFM in milk has been set by the European Union at 0.05 µg/kg and in the US at 0.5 µg/kg (Authority 2005; Campone et al. 2018).

Due to the lack of accurate statistics on the prevalence of food and waterborne diseases and related risk factors in the region has not been recorded, it is necessary to identify various risk factors and their adverse health effects such as aflatoxin that based on the available or obtained data from their incidence in different environments as well as the consumption of foods containing them should be evaluated and graded. Risk can be defined as "probability of occurring any adverse effect"; and risk assessment is considered as "a process of predicting whether there may be a risk of adverse effects on the health and environment caused by a chemical substance". In this regard, risk assessment as a useful and efficient tool can be used to identify hazards in the environment, including chemical agents and contaminants in food.

Based on available studies around the world, most dairy products including types of milks have varying degrees of contamination with a variety of aflatoxins, especially M1 (Akbar et al. 2019; Daou et al. 2020; Hajmohammadi et al. 2020; Min et al. 2020; Mohammedi-Ameur et al. 2020; Mozaffari Nejad et al. 2019, 2020; Patyal et al. 2020; Venâncio et al. 2019; Xiong et al. 2018); in some cases, studies have been conducted to evaluate the health risks of these toxins (Ahmadi 2020; Hooshfar et al. 2020; Madali et al. 2018; Marimón Sibaja et al. 2019; Milićević et al. 2017; Mozaffari Nejad et al. 2020; Nugraha et al. 2018; Pardakhti and Maleki 2019; Wang et al. 2018).

Due to the fact that a large amount of bread waste is collected daily in the community and consumed by livestock, especially dairy cows, this can indirectly cause contamination of food and dairy products, including milk and meat of these animals (Fakhri et al. 2019; Pardakhti and Maleki 2019). Considering the importance and also, because the physical as well as the mental health of society is closely related to the health of food products, and due to the fact that dairy products, especially milk, are one of the most consumed foods of the people, especially in Ardabil province with cold and humid climate, this food is exposed to a lot of contamination with AFM due to the use of bread waste in most livestock and cattle ranches as animal feed or through other means such as contaminated forage (Ahmadi 2020; Nugraha et al. 2018). Therefore, determining the level of AFM in milk and its products and determine the associated risk is very important to protect health of consumers in different age groups. Therefore, this study aimed to determine of AFM and its risk assessment in various dairy products (milk) offered in Ardabil city, including pasteurized, sterilized, and traditional types during hot and cold seasons (for exemplary summer and winter), as well as drawing an overview of the milk pollution status and provide solutions to reduce pollution, was done using ELISA method.

Material and methods

Collection of milk samples

In order to achieve the objectives of the present study, a total of 60 raw, pasteurized, and sterilized milk samples were randomly collected from different dairy farms, traditional raw, pasteurized, and sterilized milk supply points from Ardabil city and suburbs. Sampling was done twice during the hot and cold seasons of the year (winter and summer 2020). Before performing the test, the equipment and materials needed for sampling were washed with detergent to eliminate possible contamination and then sterilized by autoclave. Collected milk samples under sterile conditions and in the presence of ice were transferred to the laboratory.

Sample preparation

All cold milk samples were centrifuged in a refrigerated centrifuge at 3500 rpm for 10 min and the fat layers on them were removed. Then 100 µL was isolated from the rest milk sample for AFM evaluation and tested.

Measurement of AFM in milk

In this experiment, AFM ELISA kit (Germany, Biopharm-R) was used to measure AFM in milk. The test was performed according to the kit manufacturer's instructions. Fifty microliter of each standard was added to 50 µL of skim milk sample in the well. Then 50 µL conjugate and 50 µL antibody were added to each well, respectively. The kit was manually moved several times in different directions to thoroughly mix all the contents of each well. The kit was kept at room temperature for 10 min, and then all contents of the kit were removed and the kit was washed three times with distilled water. Then kit was hit in the inverted position, for draining and drying all distilled water in the wells. In the next step, 100 µL of chromogen solution was added to each well and then the kit was manually moved several times in different directions to mix all the contents of the well. The kit was then placed in the dark for 5 min. After 5 min, 100 µL of stopper solution was added to each well (Moradi et al. 2017; Venâncio et al. 2019). The kit is shaken several times, then the amount of light absorption was measured with ELISA reader (Bio-Tech, Germany) at a wavelength of 450 nm; after drawing the calibration curve and using it, the concentration of AFM was determined.

Risk assessment

To assess the risks of exposure to AFM through daily consumption of milk, initially, the daily intake of AFM in ng/d.kg body weight was estimated using the following equation (Hooshfar et al. 2020; Sharma et al. 2020):

$$EDI = \frac{C \times I \times E}{B.W} \tag{1}$$

Which in the above equation, C is the concentration of the contaminant (AFM) in the tested milks in ng/L, I is the daily milk intake in L/d, E is the exposure duration in day, and $B.W$ is average weight of individuals in the test population in kilogram. According to the report of the Agriculture Ministry of Iran, the approximate daily milk intake for the general population was 0.25 L on average (Fooladi Moghaddam et al. 2019; Mozaffari Nejad et al. 2020; Sharma et al. 2020; Wang et al. 2018). The general population weight was estimated at 70 kg according to the *WHO Exposure Handbook* (2011 Edition). Then, for risk characterization MoE[1] (EFSA 2005; Authority 2005), cancer risk (Organization 2017), and HI[2] (Ishikawa et al. 2016) approaches were used. These three indicators calculated using the average and median daily intake of AFM and average milk consumption in the community. The risk of AFM-induced liver cancer was calculated by multiplying the probability of cancer with the average and median AFM exposure.

$$\text{Cancer risk} = P \times EDI \tag{2}$$

Which, P is probability of cancer and EDI is AFM exposure as ng/kg.d b.w. In this formula, P was calculated using Eq. (3).

$$P_{\text{Cancer}} = \left(P_{\text{HBsAg}^+} \times \%Pop_{\text{HBsAg}^+}\right) + \left(P_{\text{HBsAg}^-} \times \%Pop_{\text{HBsAg}^-}\right) \tag{3}$$

$$P_{\text{Cancer}} = (0.0562 \times 0.015) + (0.0049 \times 0.985) \tag{4}$$

Which, P_{cancer} is cancer potency, and according to IR Iran CDC, $HBsAg^+$ prevalence in Iran is 1.5%, so in the Eq. 3, the Pop[3] for both carriers ($HBsAg^+ = 0.015$) and non-carriers ($HBsAg^- = 0.985$) of HBV[4] infection in the population was put in Eq. 4; also with considering the potency of carcinogenicity for AFB1 (P) equivalent 0.0049 extra cancer cases per 100,000 for chronic HBV for antigen negative ($HBsAg^-$) populations and its value equivalent 0.0562 extra cancer cases per 100,000 for $HBsAg^+$ populations, the value of P_{cancer} was calculated equivalent 0.00567 and then was placed in Eq. (2) (Organization 2017).

To calculate the MOE for average and median exposures to AFM, BMD[5] was used as the dose with the least measurable response (570 ng/d.kg b.w: as AFM potency for hepatocellular carcinoma based on 2-year study in male Fischer rats) (Udovicki et al, 2019; Serraino et al. 2019; Udovicki et al. 2019), and its value was obtained with dividing this reference value by the EDI in consumers (EFSA 2005; Udovicki et al. 2019). Its value above 10,000 was considered a low-level public health concern.

HI, was calculated using EDI as ng/kg b.w and TD_{50}[6] to evaluate carcinogenic and non-carcinogenic effects of AFM caused by milk consumption. This index was determined by dividing the EDI with TDI (Milićević et al. 2017; Udovicki et al. 2019). For AFM, TDI was 0.2 ng/kg.d, obtained by dividing TD_{50} (threshold dose per BW) with a variability factor of 50,000 (Hooshfar et al. 2020). As a criterion, HI

[1] Margin of exposure.

[2] Hazard index.

[3] Percentage of population.

[4] Hepatitis B virus.

[5] Benchmark dose.

[6] Threshold dose per B.W which divided by 5000.

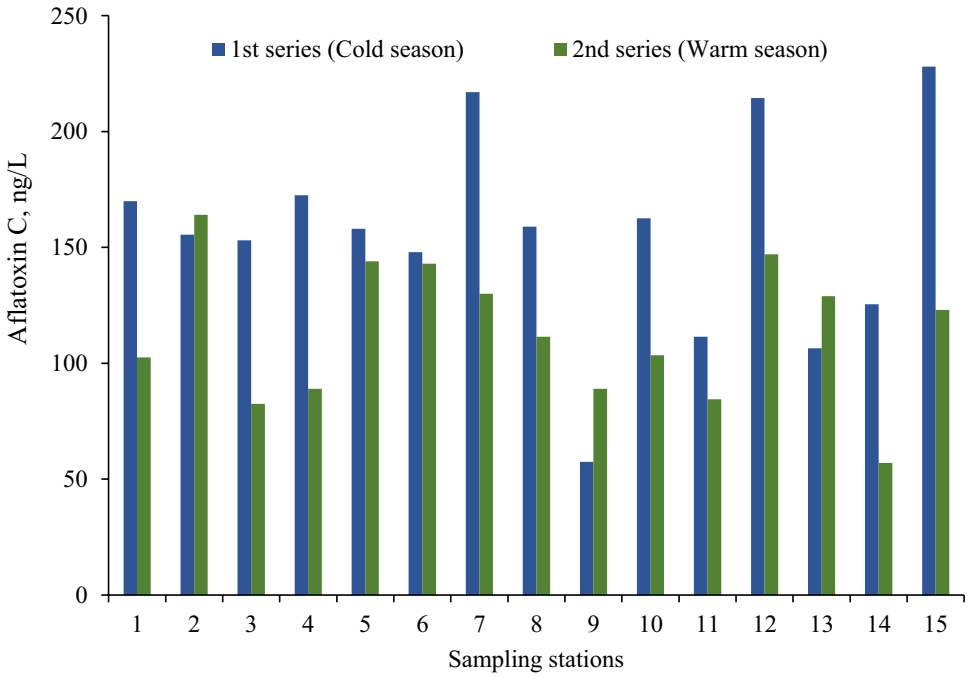

Fig. 1 Comparison of AFM concentrations in traditional milk samples in two sampling series

index of AFM greater than 1 ng/kg bw value indicates that consumers are in the significant risk (Ishikawa et al. 2016; Kuiper-Goodman 1990).

Statistical analysis

SPSS software version 23 was used to analyze the results. Kolmogorov–Smirnov and Lilliefors tests were used to determine the normality of the data. Two-way ANOVA was used to investigate the relationship between aflatoxin concentration and climatic conditions as well as milk types. One-way *t*-test was used to compare the contamination of milk with aflatoxin in two seasons as well as recommended guideline and standards.

Results and discussion

Evaluation of AFM in traditional and pasteurized milk

Aflatoxin contamination can be determined by examining the food intake, especially milk as one of the most consumed foods; and aflatoxin exposure to milk should be minimized. In order to determine of AFM levels in the types of milk supplied in the city, during two stages (the cold and warm seasons of the year, as a representative: winter and summer) and in each stage, 30 samples of milk (15 traditional and raw samples + 15 pasteurized and sterilized samples) and in total 60 samples were collected from available milks and analyzed by ELISA method. Based on the results shown in Figs. 1, 2, and 3, the amounts of toxin in the 1st and 2nd sampling series in traditional and raw milk samples were in the range of 57.5–228 ng/L and 57–164 ng/L, with standard deviation 44.81 and 29.87 ng/L, respectively. The average level of the toxin during the two sample stages in raw milks were 155.93 and 113.3 ng/L, respectively. Also, in pasteurized and sterilized samples AFM levels in the 1st and 2nd sampling series had ranges of 123.75–270.6 ng/L and 113.3–185.9 ng/L, with standard deviation 43.88 and 18.48 ng/L, respectively. The mean of the toxin during the two sample stages in pasteurized and sterilized milk samples were 219.08 and 136.4 ng/L, respectively. Figures 1, 2, and 3 also provide a comparison of AFM concentrations in different types of milk in two sampling steps.

As shown in the Figs. 1, 2, and 3, the amount of AFM in the tested samples in the first series (as cold season) and the second series (as warm season) was different quantitatively. So that its average level in traditional and pasteurized milk in the second series has almost 28% and 38% decrease compared to the first series, respectively. Also, pasteurized milk had an average of 29% more contamination than the traditional one in the first series and 17% more in the second series of sampling; and in general, the level of toxin contamination in the second series has decreased by about 33.5% compared to the first series.

Kolmogorov–Smirnov and Shapiro–Wilk tests showed that the obtained data are normal (Sig. < 0.05). Two-way ANOVA comparison of the mean results of milks contamination with AFM in two sampling series showed that there is a significant difference between its level in cold and hot seasons ($P_{value} < 0.0001$). Also, comparison of the mean results

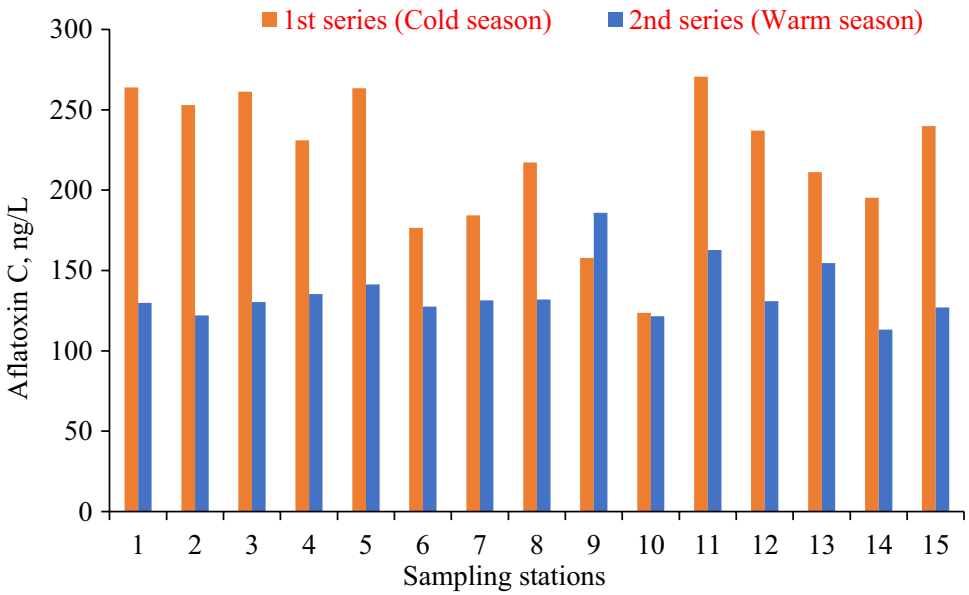

Fig. 2 Comparison of AFM concentrations in pasteurized and sterilized milk samples in two sampling series

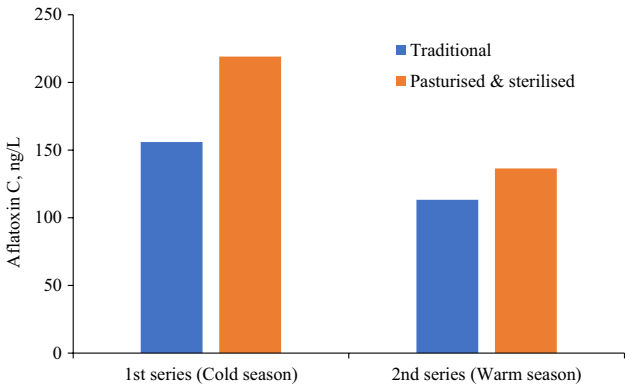

Fig. 3 Comparison of AFM concentration results in different types of milk in two sampling series

of AFM contamination in traditional and pasteurized milks showed a significant difference ($P_{value} = 0.004$). Finally, the interaction between milk and season at the level of 0.05 did not have a significant effect ($P_{value} = 0.073$).

According to WHO guidelines, the acceptable level of milk's AFM is 50–500 ng/L and also based on European standards its value set to 50 ng/L (35). By comparing the results with these recommendations, it can be seen almost all samples of milk in both series and types of milk exceeded the recommended value as the European standard but were lower than the maximum recommended level by the WHO. Also, One-way *t*-test confirmed mentioned hypotheses (Sig. < 0.0001). Therefore, this is an important issue and it is necessary to take measures to prevent and control of milk contamination with this toxin.

Air temperature of 27 °C and relative humidity of 85% provide suitable conditions for the *Aspergillus* growth (Salari et al. 2020; Xiong et al. 2020). Storage of livestock forage in unfavorable environmental conditions causes the growth of *Aspergillus* mold and subsequent formation of aflatoxin B1 in animal feed and its transfer in the form of AFM, as a product of hydroxylation in the animal body, to animal milk (Mohammedi-Ameur et al. 2020). *Aspergillus flavus* and *Aspergillus parasiticus* can easily grow and produce toxin in forage that has a moisture content of 13 to 18% and also in ambient humidity of 50 to 60% (Hooshfar et al. 2020). In addition to humidity and temperature, other parameters such as feed pH and mechanical destroying also play a role in fungal growth (Daou et al. 2020). Therefore, aflatoxin production can be prevented by controlling the environmental conditions of the feed storage site and making it unfavorable for fungi growth (Ahmadi 2020). In addition to possible problems in livestock feed, another issue that can be problematic in some areas, is using of some food wastes such as bread crumbs that are usually used in some areas to feed livestock (Abyaneh et al. 2020). This is especially common in traditional societies, where bread crumbs are used as complementary feed along with fodder for livestock (Abyaneh et al. 2020; Venâncio et al. 2019). Among the necessities that should be considered in this regard and to prevent the contamination of fodder and livestock feed, it can be mentioned education, culture, supervision, guidance, and law enforcement (Serraino et al. 2019). There is another point about AFM that, it is relatively stable and resistance to drying and thermal processes, because aflatoxins decomposition temperature is in range 237–306 °C. So, if raw milk is contaminated, this toxin may be present in the final product, including pasteurized milk, cheese, butter, and other dairy products (Campone et al. 2018; De Roma et al. 2017).

Table 1 Calculation of EDI of AFM in the milks consumed in Ardabil city

Milk type	Mean concentration of AFM in milk (ng/L)		Median concentration of AFM in milk (ng/L)		AFM EDI (ng/d.kg B.W.)			
					Mean		Median	
	S1	S2	S1	S2	S1	S2	S1	S2
Traditional	0.156	0.113	0.158	0.111	0.203	0.147	0.206	0.145
Pasteurized and sterilized	0.219	0.136	0.231	0.131	0.285	0.178	0.301	0.17

Several studies have investigated the relationship between season and AFM levels in milk and have achieved different results. In most cases, the rate of aflatoxin contamination in winter was higher than other months. A 2019 study by Himani Sharma and et al. in India (Hisar city, Haryana) on varieties of milk over a 4-month period showed the average concentration of AFM in pasteurized milk was 397 ng/L (range: 222–2281) and in local and traditional milks was 216 ng/L (range: 10–1016) (Sharma et al. 2020). The results of their study are consistent with the results of the present study and traditional milks had less contamination than pasteurized milks. Other studies by Akbar et al. in Punjab, Pakistan (Akbar et al. 2019), Ansari et al. in northwestern of Iran (Ansari et al. 2019), Becker-Algeri, T. A. et al. in Brazil (Becker-Algeri et al. 2020), Çetin, B.et al. in Turkey (Çetin et al. 2019), De Roma, Antonella et al. in Southern Italy (De Roma et al. 2017), Ismaiel, A. A. et al. in Egypt (Ismaiel et al. 2020), Mohammedi-Ameur, S. et al. in Algeria (Mohammedi-Ameur et al. 2020), and Venâncio, R. L. et al. in Brazil (Venâncio et al. 2019) confirm variations of AFM contamination of milks in cold and hot seasons and their results were similar to the present study.

According to the results of this study, it was found that the average contamination of AFM in pasteurized and sterilized milks (177.74 ng/L) was higher than raw and traditional milks (131.69 ng/L). One-way *t*-test showed that this difference was significant and the contamination rate was lower in traditional milks. The reason for this can be attributed to the proper nutrition of livestock in traditional livestock farms and mainly the storage of fodder and food (Bahrami et al. 2016). So that on most days of the year, livestock feeding in traditional farms is done on pastures outside the farm and using fresh forage, and in the cold days of year, due to the smaller area and the possibility of better management of these traditional farms, the used forage has better storage conditions and the possibility of less pollution than industrial and larger livestock centers, which are usually fed in a fixed place throughout the year (Ismaiel et al. 2020). Of course, as is clear from the results, during the cold seasons of the year due to the high percentage of humidity and the possibility of wet being of forage storage areas, as a result, the possibility of growing a variety of fungi, including *Aspergillus flavus* and *Aspergillus parasiticus*, as well as the rate of contamination will increase (Mohammedi-Ameur et al. 2020). A study conducted by Ansari et al. in 2019 on pasteurized cow milk showed that during the cold seasons of the year compared to the warm seasons pasteurized milk samples were more contaminated (Ansari et al. 2019). Ahmed A. Ismaiel and et al. in 2020 studied the seasonal variations of the AFM in traditional milks; his findings showed most milk samples were AFM contaminated during the 2-year study, and the level of toxin contamination was in the range of 50–660 ng/L in the first year and 50–510 ng/L in the second year in the positive samples (Ismaiel et al. 2020). A study in 2019 by Akbar, N. and et al. in Punjab, Pakistan, on raw and traditional milk supplied in the region demonstrated that overall, about 53% raw milk samples from dairy farms were contaminated beyond the US MRL (0.50 µg/L) for AFM with than average level of 0.59 µg/L (Akbar et al. 2020). These studies, along with other studies conducted by Ahmad, M. in Lahore, Pakistan (Ahmad et al. 2019), Kuboka, Maureen M in Nairobi (Kuboka et al. 2019), Mohammedi-Ameur, S. in Algeria (Mohammedi-Ameur et al. 2020), Patyal, A. in Punjab, India (Patyal et al. 2020), Xiong, Jianglin in central-south China (Xiong et al. 2020), and numerous other studies confirm the results of present study about contamination of raw milk with AFM.

Health risk assessment

Based on the available information, this study is the first study in the field of AFM risk assessment in the study area. In this work, in order to risk characterization, after determining EDI, "LCR[7]", "MoE" and "HI" indices were calculated and estimated.

Equation 1 was used to calculate EDI and considering the average and median values of AFM in different types of milk as well as setting the values of parameters "I, E, and B.W" equal 0.25 L/d, 365 d, and 70 kg, respectively, and its values were obtained according to the Table 1. As can be seen in this table, the EDI value obtained for different types of milks is in the range of 0.145 to 0.301 mg/kg.d. And in both cases, its amount is more for pasteurized and

[7] Liver Cancer Risk.

Table 2 Estimation of LCR of AFM in consumers of milks in Ardabil city

Milk type	LCR (Additional cancer cases/year/10^5 population)			
	Mean		Median	
	S1	S2	S1	S2
Traditional	0.00115	0.000824	0.00117	0.00082
Pasteurized and sterilized	0.00162	0.00101	0.00171	0.00097

Table 3 Calculation of MoE for AFM in consumers of milks in Ardabil city

Milk type	MoE			
	Mean		Median	
	S1	S2	S1	S2
Traditional	2804.15	3859.31	2767.47	3921.617
Pasteurized and sterilized	1995.86	3205.72	1892.9	3340.4

sterilized milks than traditional milks. In traditional milks, the minimum EDI level was related to the second series and its maximum amount was related to the first series of sampling which was calculated on median mode. However, in pasteurized and sterilized milk, the minimum and maximum values were related to the second and first series of sampling, respectively, and they obtained in the median state calculation. The estimated EDI values in this study can actually be underestimated because it only includes milk consumption and will not include other dairy products as well as other possible sources of AFM. Internationally, AFM EDI was calculated as 0.11 ng/k.d b.w through milk consumption for the European diet, which is several times less than gained in this work. The findings of this study generally indicate the high-level exposure with types of milk consumption, which is comparable to studies conducted by other researchers such as Torovic (2015) with 0.06 ng/k.d b.w (Torović 2015), Škrbić et al. with 1.42 ng.k.d b.w (2014) (Škrbić et al. 2014), Rozhin Bahrami et al. (2016) with 0.242 ng/k.d b.w (Bahrami et al. 2016), Dragan R. Milićević et al. (2017) with 0.18–0.4 ng/k.d b.w range (Milićević et al. 2017), Rafael Luiz Venâncio et al. (2019) with 0.2 ng/k.d b.w (Venâncio et al. 2019), Shirin Hooshfar et al. (2020) with 0.42 ng/k.d b.w (Hooshfar et al. 2020), Mozaffari Nejad et al. (2020) with 0.07 ng/k.d b.w (Mozaffari Nejad et al. 2020).

For characterizing of AFM risk cancer risk (Organization 2017), MoE (EFSA, 2005; Authority 2005), and Hazard Index (HI) (Ishikawa et al. 2016) approaches was used; all three parameters above have been estimated using the mean and median daily intake of AFM and the average milk

Table 4 Estimation of Hazard Index of AFM in consumers of milks in Ardabil city

Milk type	Hazard index			
	Mean		Median	
	S1	S2	S1	S2
Traditional	1.016	0.74	1.03	0.727
Pasteurized and sterilized	1.428	0.89	1.51	0.85

consumption in the community. Relevant results are presented in Tables 3 and 4. According to Table 2, the risk of liver cancer in the milk supplied in the city based on carried calculations was in the range of 0.0008 to 0.0017, which is related to second (traditional) and first (pasteurized) sampling series, respectively. These values represent the approximate likelihood of additional cases of liver cancer during the year per one million population; with this explanation and obtained results, it can be concluded that there is no serious concern in this regard and cancer risk is very low. In the study of Hooshfar et al. (2020) in Iran, the results of the risk assessment and analysis study of primary liver cancer due to exposure to AFM in milk showed that there is the risk of cancer for a more realistic scenario calculated with the average incidence and average consumption in milk consumers, as 0.0001 additional cancer case in 10^5 people, which is comparable to the obtained value in this study (0.0007–0.0016 more cases per year 10^6 in this work) (Hooshfar et al. 2020). Other studies by researchers have reported values for LCR as follows, with different ranges, including AF Moghaddam et al. (2019), 0.08–0.72 (Fooladi Moghaddam et al. 2019), Bozidar Udovicki et al. (2019) 0.0036–0.0047, and 0.0007–0.0009 for Serbia and Greece, respectively (Udovicki et al. 2019), Daou, Rouaa et al. (2020), showing 0.0041 additional cancer case (Daou et al. 2020), whose values are comparable to the results of the present work.

Also, the data in Table 3 show that the MoE range calculated for the consumed milks during the two series of sampling was 1892.9 to 3921.6. According to the guidelines of the EFSA[8] Scientific Committee, if the MoE is based on BMDL10[9] of 10,000 or more in animal studies, it is not a public health concern and may be considered a low priority for risk management (Authority 2005). Our obtained results for both of milk types showed that MoE values for mean and median AFM exposure in consumers were less than 10,000,

[8] European Food Safety Authority.

[9] Benchmark dose level 10 (associated with a 10% extra risk of adverse effect in the exposed test animals, as compared to the background levels of risk).

indicating a health concern arising from AFM exposure through all consumers. A study was conducted by Bozidar Udovicki et al. (2019) in Serbia and Greece for exposure assessment and risk characterization of AFM intake in consumers of milk and yoghurt which based on results, MoE values were reported for Serbia 213.2–460.4 and Greece 1142.3–1628.6 ranges (Udovicki et al. 2019). The results of mentioned study along with studies by Wang et al. (2018) with MoE level lower than 10,000 and A Ismail et al. (2020) with MoE range 1156.5–1545.5 (Ismail et al. 2020) are in line with the results of the present study.

Table 4 also shows the calculated hazard index for the population consuming milk containing the AFM. Based on the data in the table, the lower and upper limits of the mentioned index are equal to 0.727 and 1.51, respectively. Also, using the above information and existing criteria, it can be seen that in the first series of sampling, almost all samples had a higher and in the second series had a lower value of hazard index. Also, the index value for traditional milk was lower than pasteurized and sterilized milk in both series of sampling. Previous studies have shown a relationship between the risk of primary liver cancer and exposure to AFM due to milk and dairy consumption; accordingly, Kuiper-Goodman (1990) recommended that the risk index (HI) must be determined at the risk of AFM liver cancer for consumers (Tsakiris et al. 2013). A value higher than 1 was determined for the index as a sign of danger and in other studies it was used to determine the risk of exposure to the AFM. In a study in 2016, Bahrami et al. reported the above index in traditional milks of western regions of Iran in the range of 0.54–1.46 (Bahrami et al. 2016). In the study of Dragan R. Milićević et al. in 2017, the index values for raw and heated-treated milks in both groups of male and female were reported to be 1.83–2.01 and 0.885–0.985, respectively (Milićević et al. 2017). Another study in 2018 by Rahmani et al., showed HI values of AFM exposure for children consuming raw and pasteurized milk in Iran about 1.03 and 1.3, respectively (Rahmani et al. 2018). The results of the above studies, along with (Fakhri et al. 2019; Hooshfar et al. 2020; Mozaffari Nejad et al. 2019; Sakin et al. 2018) studies, confirm the results of the present study and are also comparable.

Conclusion

According to the results obtained from the data of this study, it is clear that milks supplied in the city have relatively high levels of AFM contamination. It was also found that in the cold season it is higher than in the warm season. Although, the level of contamination in the analyzed milk samples is within the range recommended by the WHO, but by calculating indicators such as LCR, MoE, and HI, the risk of milk consumption in the community (in general for all age groups), it was found that consumption of these milks will have potential adverse effects on consumers.

Since thermal processes do not guarantee the absence of AFM in milk and milk products, the presence of this dangerous and very toxic substance in milk is extremely important. Existence of aflatoxin-free milk is desirable for communities, but achieving this ideal will not be easy. Despite setting standard values as well as control measures to ensure and promote milk hygiene, these measures do not seem to be sufficient. In order to achieve milk without AFM, it must be animal feed without AFB1 contamination. Improper storage conditions as one of the main reasons for the growth and spreading of *Aspergillus* on animal feed must be optimized with simple procedures including not feeding livestock with dry bread and moldy fodder, creating suitable environmental conditions in animal feed storage warehouses, and continuous and accurate monitoring of fodder and animal feed maintaining by experienced experts. Although environmental and nutritional factors are important parameters in the amount of pollution, but the extent and how these factors affect is not clear. Therefore, it is necessary to conduct extensive studies in this regard.

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Author contribution Ahmad Mokhtari, Mehdi Fazlzadeh, and Ali Nemati participated in the conceptualization and design of the research and supervised the work. Eslam Moradi-asl and Vahid Taefi Ardabili are responsible for experimental analysis and interpretation of data. Anoshirvan Seddigh contributed to literature search and quality assessment. All authors have read and approved the final paper as submitted.

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Data and materials availability The data used and analyzed during the current study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval The study was approved by the Ethical Committee of Ardabil University of Medical Sciences, Iran (Code of ethics: IR.ARUMS.REC.1398.213).

Consent to participate Not applicable.

Consent for publication All the authors agreed to publish the data in this journal.

Conflict of interest The authors declare no competing interests.

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