SİDAS MEDYA
Akademik Gıda®
ISSN Online: 2148-015X
http://dergipark.gov.tr/akademik-gida

Akademik Gıda 18(3) (2020) 228-232, DOI: 10.24323/akademik-gida.818066

Research Paper / Araştırma Makalesi

Exposure Assessment and Risk Characterization of Aflatoxin M1 Intake Through Consumption of Milk by General Population in Bosnia and Herzegovina: Preliminary Study

Elma Omeragic ✉, Aleksandra Marjanovic, Jasmina Djedjibegovic, Amila Turalic, Aida Lugusic, Miroslav Sober

University of Sarajevo, Faculty of Pharmacy, Zmaja od Bosne 8, 71000 Sarajevo, Bosnia and Herzegovina

Received (Geliş Tarihi): 05.09.2019, Accepted (Kabul Tarihi): 17.08.2020
✉ *Corresponding author (Yazışmalardan Sorumlu Yazar): elma.omeragic@ffsa.unsa.ba (E. Omeragic)*
+90 387 61 989 267 +90 387 33 586 178

ABSTRACT

Aflatoxins M1 and M2 are the hydroxylated metabolites of aflatoxins B1 and B2 and can be found in milk or milk products. The aim of this study was to obtain the exposure and health risk assessment of this toxin in raw milk using a selection of existing test results of different periods in a different region of Bosnia and Herzegovina. For AFM1 intake assessment, the data available from the report of Pre-Accession Assistance To Agriculture And Rural Development Sector Analyses in Bosnia and Herzegovina was used. For the concentration of the aflatoxin M1 in raw milk, the data of the previous studies and unpublished data from the Laboratory of Toxicological Chemistry Faculty of the Pharmacy University of Sarajevo were used. Risk characterization was performed by calculating the risk of hepatocellular carcinoma (HCC) incidence per year for four exposure scenario. The results indicated that the average estimated daily intake of aflatoxin M1 was 0.049 ng/kg body weight (bw) per day. The risk of hepatocellular carcinoma (cases/year/100 000 individuals), depending on the HBsAg prevalence, was relatively low (average 0.000064-0.000074 cases/year/100 000). It is concluded that in recent years, the carcinogenic risk of aflatoxin M1 has been low and not critical in Bosnia and Herzegovina.

Keywords: Aflatoxin M1, Milk, Exposure assessment, Risk characterization

Bosna-Hersek'te Genel Nüfus Bazında Süt Tüketimi Yoluyla Aflatoksin M1 Alımına Ait Maruziyet Değerlendirmesi ve Risk Karakterizasyonu: Ön Çalışma

ÖZ

Aflatoksin M1 ve M2, aflatoksin B1 ve B2'nin hidroksile metabolitleri olup, süt ve süt ürünlerinde bulunabilir. Bu çalışmanın amacı, Bosna-Hersek'in farklı bölgelerinden farklı dönemlere ait test sonuçlarını kullanarak çiğ sütteki bu toksine maruz kalma ve sağlık riski değerlendirmesi yapmaktır. AFM1 alım değerlendirmesi için, Bosna-Hersek'te Tarım ve Kırsal Kalkınmaya Yönelik Katılım Öncesi Yardım Sektör Analizleri raporundaki veriler kullanılmıştır. Aflatoksin M1'in çiğ sütteki konsantrasyonu için, Saraybosna Eczacılık Üniversitesi Toksikolojik Kimya Fakültesi Laboratuvarı'ndan alınan önceki çalışmaların verileri ile yayınlanmamış veriler kullanılmıştır. Dört maruziyet senaryosu için yıllık hepatoselüler karsinom riski (HCC) insidansı hesaplanarak risk karakterizasyonu yapılmıştır. Sonuçlar, ortalama tahmini günlük aflatoksin M1 alımının günde 0.049 ng/kg vücut ağırlığı olduğunu göstermiştir. Hepatoselüler karsinom riski (vaka/yıl/100.000 kişi), HBsAg prevalansına bağlı olarak nispeten düşük bulunmuştur (ortalama 0.000064-0.000074 vaka/yıl/100.000). Son yıllarda, aflatoksin M1'in kanserojen riskinin düşük olduğu ve Bosna Hersek'te kritik olmadığı sonucuna varılmıştır.

Anahtar Kelimeler: Aflatoksin M1, Süt, Maruziyet değerlendirmesi, Risk karakterizasyonu

INTRODUCTION

Milk is a major source of dietary energy, high-quality proteins and fat. It contains the water-soluble vitamins B1, B2, B3, B5, B6, B12, vitamin C, folate and the fat-soluble vitamins A, D, E, and K. Milk is a good source of calcium, magnesium, phosphorus, potassium, selenium, and zinc. Bioavailability of some nutrients in milk, for example, calcium, is high compared with that in other foods in the diet [1].

This important food source can be contaminated with some different unwanted chemicals. Veterinary drugs, heavy metals, radionuclides, mycotoxins (aflatoxins, ochratoxin A, patulin, trichothecenes such as deoxynivalenol, nivalenol and T-2 and HT-2 toxins, as well as zearalenone and fumonisins) and pesticides are chemical contaminants that can enter to animal feed and they have some residues in milk [2].

Aflatoxins are amongst the most poisonous mycotoxins. They can be produced by three species of *Aspergillus- A. flavus, A. parasiticus,* and *A. nomius. Aspergillus flavus* produces only B aflatoxins (B1 and B2), while *Aspergilus parasiticus* and *Aspergilus nominus* produce both B and G aflatoxins (G1 and G2) [3]. Aflatoxin B1 (AFB1) present in feed of lactating animals gets transformed to 4-hydroxylated metabolite in the liver and is excreted in milk as aflatoxin M1 (AFM1) [1]. Milk and milk products including breast milk, are more or less exclusively the source of AFM1 exposure for human. Aflatoxin M1 is clasificated as B2 category (possible carcinogen for human) by International Agency for Research on Cancer (IARC) and causes liver, kidney and digestive problems. AFM1 has liver carcinogenicity effects and genetic destruction effects in human [3]. The amount of aflatoxin M1 in milk is in relation to the consumed aflatoxin B1. Approximately 1-3% of ingested AFB1 is converted into AFM1 [4]. Aflatoxins contamination of feed fluctuates seasonally. The contamination is generally higher in summer (high temperature) and autumn (high humidity). The concentration of aflatoxins can be measured by gas chromatography (GC), high-performance liquid chromatography (HPLC), thinly layer chromatography (TLC) and enzyme-linked immunosorbent assay (ELISA) [3]. Destruction of AFM1 in milk depends on time and temperature combination of the heat treatment applied. Some investigation was observed that pasteurization caused a decrease in the level of AFM1 at the rate of 7.62% or can partially reduce the amount of AFM1 in milk. However, some reports showing that aflatoxins M1 are stable during heat-treatments such as pasteurization and sterilization and is not destroyed in yoghurt and cheese making [1, 5].

Different studies also show a great synergic effect between aflatoxin M1 and hepatitis B virus in a way that hepatitis B patients ($HBsAg^+$) exposed to this toxin are 30 time more prone to liver damages and cancer [3,6]. The maximum allowable concentration of aflatoxin M1 in raw milk, heat-treated milk and milk for the manufacture of milk-based products is 50 ng/L according to the European Commission regulation [7] and to the Ordinance on the maximum allowable concentration of certain contaminants in foods in Bosnia and Herzegovina [8].

Dominant milk production in BiH is based on the small farms, of which only 3.5% are farms with more than 20 dairy cows. The annual milk purchases by registered dairy plants are almost 136 000 tonnes. Marketed milk, (i.e. pasteurized and UHT) accounts for more than half of all milk products in processing [9].

The aim of this study was to do exposure and health risk assessment of this toxin in raw milk using a selection of existing test results of different periods in a different region of Bosnia and Herzegovina.

MATERIALS AND METHODS

Dietary Exposure Assessment

Estimated daily intake (EDI) through milk consumption was an assessment by combining consumption data of milk with contamination data for AFM1 and divided by the individual body weight (Eq. 1).

$$EDI\left[\frac{ng}{kg\ b.w.day}\right] = \frac{Daily\ consumption\left[\frac{l}{day}\right] concentration\ of\ contaminant\left[\frac{ng}{l}\right]}{Body\ weight\ [kg]} \quad (1)$$

The total diet study was not being undertaken in Bosnia and Herzegovina, so far. For that reason, for intake assessment, we used the data available from report of IPARD (Pre-Accession Assistance To Agriculture And Rural Development) Sector Analyses in Bosnia and Herzegovina [9] according to which consumption of milk per capita in Bosnia and Herzegovina was approximately 172 litres (471 mL/day) in 2010 year.

The bodyweight of 70 kg was used as the average body weight for the European adult population (aged above 18 years).

Concentration of Aflatoxin M1 in Raw Milk in Bosnia and Herzegovina

This study was performed by analyzing published articles. Reviewing published articles dealing with the concentration of aflatoxin M1 in milk samples origin from Bosnia and Herzegovina, we found a just one published study about this topic. In the study conducted from Bilandžić et al. [10] a total of 285 samples of raw cow's milk were sampled from small and large dairy farms in the period October-November 2014 in the following cantons of Bosnia and Herzegovina: Una Sana, Banja Luka region, Canton 10, Central Bosnia, Zenica Doboj, Tuzla, West Herzegovina, Herzegovina-Neretva, Romanija and Eastern Herzegovina was analyzed.

Additionally, for this study more recent results of 53 milk samples that are analyzed in the Laboratory of toxicological chemistry Faculty of pharmacy University of Sarajevo (Unpublished data) on the AFM1 concentration with enzyme-linked immunosorbent assay (Aflatoxin M1 sensitive ELISA test (5121AFMS), EuroProxima) have been used in a way to cover major regions in milk production of whole county at the period from April to June in 2014. The average concentration of AFM1 used in calculations average exposure were calculated as Pooled Means of reported means as follows (Eq. 2):

$$c_{average} = \frac{N1 \cdot M1 * N2 * M2}{N1 + N2} \quad (2)$$

Where N is reported number of samples and M is reported mean of AFM1 concentration in milk [11].

The result of AFM1 concentrations used for exposure assessment and risk characterisation were presented in Table 1.

Risk Characterization

Five different exposure scenarios were used: (a) *"mean exposure in spring sampling period"*, calculated from milk consumption according to FAO data (2012) and mean AFM1 concentrations during spring sampling period, (b) *"mean exposure in autumn sampling period"*, calculated from milk consumption according to FAO data (2012) and mean AFM1 concentration over the autumn sampling period, (c) *"worst-case exposure scenario in spring sampling period"* calculated from milk consumption according to FAO data (2012) and maximum concentrations of AFM1 in the spring sampling period, (d) "*worst-case exposure scenario in autumn sampling period"*, calculated from milk consumption according to FAO data [8] and maximum AFM1 concentrations in autumn sampling period and the *„average exposure scenario for both sampling period"* calculated from milk consumption according to FAO data (2012) and average AFM1 concentration calculated according Eq. 2.

Risk characterization was performed by calculating the Risk of hepatocellular carcinoma (HCC) incidence per year for each exposure scenario.

Risk of hepatocellular carcinoma (HCC) incidence per year, was calculated from the estimated dietary exposure to AFM1 multiplied by the AFM1 cancer potency (*Pcancer*) as follows:

$$HCC = EDI \cdot Pcancer \quad (3)$$

Where EDI is estimated dietary exposure to AFM1 and *Pcancer* the AFM1 cancer potency.

AFM1 cancer potency was calculated as follows:

$$Pcancer = 0.001 \cdot \%HbsAg^{-} + 0.03\%HbsAg^{+} \quad (4)$$

Where, $\%HbsAg^{-}$ and $HbsAg^{+}$ are the percentage of Hepatitis B virus surface antigen-negative and Hepatitis B virus surface antigen-positive individuals, respectively. In this calculation percentage of $HBsAg^{+}$ individuals of 1.057% (low estimate) and 1.535% (high estimate) reported by northeast region of Bosnia and Herzegovina were considered [12].

The coefficients 0.001 and 0.03 refer to 0.001 liver cancer cases/year/100 000 individuals per 1ng/kg bw per day of AFM1 in Hepatitis B virus surface antigen-negative ($HbsAg^{-}$) individuals 0.03 liver cancer cases/year/100 000 individuals per 1 ng/kg bw per day of AFM1 in $HBsAg^{+}$ individuals [13].

Table 1. Occurrence of aflatoxin M1 (AFM1) in milk in Bosnia and Herzegovina

Regions	Aflatoxin M1 concentration [ng/L]						Average concentration*** [ng/L]
	April-June 2014*			October-November 2014**			
	Mean±SD	Min	October-November 2014**	Mean±SD	Min	Max	
Canton Sarajevo	6.00±4.00	1.00	15.00	4.85±1.16	3.23	6.65	7.31
Herczegovina-Neretva canton	16.00±4.00	13.00	19.00	6.11±2.18	3.23	13.9	
Canton Tuzla	27.00±18.00	10.00	46.00	9.72±9.95	1.88	24.9	
Romanija region	6.00±2.00	3.00	10.00	4.23±0.42	3.65	4.96	
Central Bosnia canton	14.00±13.00	3.00	36.00	7.81±9.76	2.73	38.9	
Zenica-Doboj canton	10.00±7.00	4.00	22.00	-	-	-	
Total	11.00±10.00	1.00	46.00	6.62±6.36	1.88	38.00	

*unpublished date, ** Bilandžić et al. [10], ***Calculated by Eq. 2

RESULTS AND DISCUSSION

Estimated dietary intake of AFM1 for the general population of Bosnia and Herzegovina was in a range of 0.076-0.310 and 0.045-0.256 ng/kg bw per day in spring and autumn sampling period, respectively. Average EDI calculated using average concentration was 0.049 ng/kg bw per day. The highest EDI (0.184-0.310 and 0.065-0.168 ng/kg bw per day in spring and autumn sampling period, respectively) was determined in Tuzla canton (northeast Bosnia and Herzegovina) in both sampling periods, and the smallest one in Sarajevo canton (0.039-0.101 ng/kg bw per day) in spring and the Romanija region (0.028-0.033 ng/kg bw per day) in autumn sampling period, both regions are located in central east Bosnia and Herzegovina (Tables 2 and 3). The results of AFM1 concentration in milk in Bosnia and Herzegovina in two sampling periods are in an agreement to the previously reported fact about seasonal effect influences the concentration of aflatoxin M1. Climate conditions in Bosnia and Herzegovina region are characterized with cold winter, humid spring and relatively warm autumn. Some authors reported the higher concentration of AFM1 in cold seasons as compared to hot seasons. In cold months milking animals are mostly fed with compound feeds and thus the concentration of aflatoxin B1 increases which in turn enhances AFM1 concentration in milk. Moreover, high temperature and moisture contents also affect the presence of aflatoxin B1 in feeds. *Aspergilus flavus* and *Aspergilus parasiticus* can easily grow in feeds having moisture between 13% and 18% and environmental moisture between 50% and 60% and produce the toxin [1].

Joint FAO/WHO Expert Committee on Food Additives (JECFA) was calculated the estimated of AFM1 intake to be approximately 0.11 ng/kg bw day for the European type diet. This estimation was calculated based on the European regional consumption of milk and milk products of 340 g per person per day (the Global Environment Monitoring System (GEMS)/Food regional diets data) and the weighted mean concentration of AFM1 in the milk of 23 ng/kg [13]. Considering the deterministic approach of this estimation and considerably higher reported consumption of milk obtained from FAO report, and lower values of mean AFM1 concentration in milk, exposure to AFM1 just in one region of Bosnia and Herzegovina was slightly higher (Tuzla canton 0.184 ng/kg b.w. per day) and in the other regions even lower (average EDI 0.049 ng/kg bw per day) than this reported fo for the European type diet [13]. Other studies also confirmed a relatively low exposure to the AFM1 general adult population. Cano-Sancho et al. [14] estimated the exposure of the adult Catalonian population to be 0.039 ng/kg bw per day, Shundo et al. [15] reported intake of AFM1 by the Brazilian adult population of 0.08 ng/kg bw per day and Kos et al. [16] estimated exposure to AFM1 in Serbia through milk intake in 2013 to be 0.21 ng/kg bw per day. Taking into consideration represented exposure assessment, results from Bosnia and Herzegovina are comparable to the ones obtained from regions with climate conditions suitable for aflatoxins production.

We found that the risk of hepatocellular carcinoma (cases/year/100 000 individuals), depending on the $HBsAg^+$ prevalence (Tables 2 and 3), was relatively low (average 0.000064-0.000074 cases/year/100,000). In a population of 3,531,159 [17] with an HBV prevalence rate of 1.057% (low estimate) and 1.535% the projected increase in risk would correspond to about 0.002-0.003 additional cases per year at EDI of AFM1 of 0.049 ng/kg bw per day. Highest estimated HCC was in Canton Tuzla region (HCC 0.000240-0.000266).

Table 2. Risk characterization for of aflatoxin M1 in spring sampling periods

Regions	EDI* (ng/kg bw day)	EDI** (ng/kg bw day)	HCC*	HCC**
Canton Sarajevo	0.039	0.101	0.000051-0.000057	0.000132-0.000146
Romanija region	0.042	0.067	0.000055-0.000060	0.000088-0.00097
Zenica-Doboj canton	0.067	0.148	0.000088-0.00097	0.000193-0.000214
Central Bosnia canton	0.095	0.242	0.000124-0.000137	0.000317-0.000350
Herczegovina-Neretva canton	0.108	0.128	0.000141-0.000156	0.000167-0.000185
Canton Tuzla	0.184	0.310	0.000240-0.000266	0.000405-0.000447
Total	0.076	0.310	0.000100-0.000110	0.000405-0.000447

*"*mean exposure spring sampling period*", calculated from milk consumption according to FAO data (2012) and average AFM1 concentrations during spring sampling period, **"*the worst case exposure scenario in spring sampling period*" calculated from milk consumption according to FAO data (2012) and maximum concentrations of AFM1 in the spring sampling period.

Table 3. Risk characterization of aflatoxin M1 in sampling periods October-November 2014

Regions	EDI* (ng/kg bw day)	EDI** (ng/kg bw day)	HCC*	HCC**
Zenica-Doboj canton	-	-	-	-
Romanija region	0.028	0.033	0.000037-0.000041	0.000044-0.000048
Canton Sarajevo	0.033	0.045	0.000043-0.000047	0.000058-0.000065
Herczegovina-Neretva canton	0.041	0.094	0.000054-0.000059	0.000122-0.000135
Central Bosnia canton	0.053	0.262	0.000069-0.000076	0.000342-0.000378
Canton Tuzla	0.065	0.168	0.000085-0.000095	0.000219-0.000242
Total	0.045	0.256	0.000058-0.000064	0.000342-0.000378

*"*mean exposure in autumn sampling period*", calculated from milk consumption according to FAO data (2012) and average AFM1 concentrations during the spring sampling period, **"*the worst case exposure scenario in autumn sampling period*" calculated from milk consumption according to FAO data (2012) and maximum concentrations of AFM1 *in the autumn* sampling period.

CONCLUSION

We found a relatively low estimated daily intake of AFM1 by milk consumption in a general population of Bosnia and Herzegovina. The highest EDI was in a northeast Bosnia and Hercegovina and the lower ones in central east Bosnia and Herzegovina. Slightly higher exposure was in spring sampling period than in autumn that could be explained with climatic condition of this region and the livestock feeding practice. HCC was relatively low, but not inconsiderable.

LIMITATIONS OF STUDY

There are some limitations of this study. The data of concentrations of AFM1 used for exposure assessment and risk characterization are not representative enough. But, there are any published data on the concentration of AFM1 in milk in Bosnia and Herzegovina, except a study of Bilandžić et al. [10]. Also, we could not find any public report about the state of art of content of AFM1 in milk and milk products from the market in Bosnia and Herzegovina. Further, in this study, all calculations were made for a general adult population and taking into account just results of AFM1 concentration in raw milk samples. AFM1 is present in other dairy products and future analysis should include analysis of AFM1 content in heat-treated milk and milk for the manufacture of milk-based products. Also, more precise analysis of milk and dairy product intake must be done especially for some specific population such as toddlers, adolescents, female etc.

The ELISA test is often considered as a semi-quantitative test and inappropriate for use in risk assessment and it is suggested that all analysis of AFM1 concentration in various type of sample should be done with some of instrumental technics.

The results presented herein are the first results of the risk characterization of AFM1 associated with milk consumption in Bosnia and Herzegovina. The risk to public health seems to be negligible for the general BiH population but still, this first set of data represents a very valuable starting point for future research.

The data of this first investigation indicating that there is a need for more precise research on the consumption frequency of milk, especially among the specific population (e.g. toddlers, child, adolescents), to evaluate a reliable risk assessment per population.

REFERENCES

[1] Yitbarek, M.B., Tamir, B. (2014). Mycotoxines and/or aflatoxines in milk and milk products: Review. *International Journal of Agricultural Sciences,* 4 (10), 294-311.

[2] Khaniki, J. (2007). Chemical contaminants in milk and public health concerns: A review. *International Journal of Dairy Science*, 2(2), 104-115.

[3] Pardakhti, A.R., Maleki, S. (2019). Risk assessment of aflatoxin M1 contamination of milk in Iran. *International Journal of Environmental Research,* 13, 265-271.

[4] Ali, N., Hashim, N.H., Yoshizawa, T. (1999). Evaluation and application of a simple and rapid method for the analysis of aflatoxins in commercial foods from Malaysia and the Philipines. *Food Additives and Contaminants*, 16, 273-280.

[5] Govaris, A., Roussi, V., Koidis, A., Botsoglou, N.A. (2001). Distribution and stability of aflatoxin M1 during processing, ripening and storage of Telemes cheese. *Food Additives and Contaminants*, 18, 437-443.

[6] Peng, K.Y., Chen, C.Y. (2009). Prevalence of aflatoxin M1 in milk and it's potential liver cancer risk in Taiwan. *Journal of Food Protection,* 72, 1025-1029.

[7] The Commission of the European Communities. (2016). Commission Regulation (EC) No. 1881/2006 of 19. December 2006 setting maximum levels for certain contaminants in foodstuffs*. Official Journal*, *L 364/5*, 1-39.

[8] Ured za Zakonodavstvo. (2018). Pravilnik o izmjenama pravilnika o najvećim dozvoljenim količinama određenih kontaminanata u hrani. *Službeni glasnik BiH,* 84, 15-18 (In Bosnien).

[9] Regional Office for Europe and Central Asia Food and Agriculture Organization of the United Nations. (2012). The Meat and Dairy Sector in Bosnia and Herzegovina, Preparation of IPARD Sector Analyses in Bosnia and Herzegovina, GCP/BIH/007/EC, FAO, 2012.

[10] Bilandžić, N., Tanković, S., Jelušić, V., Varenina, I., Solomun Kolanović, B., Luburić, E.B.,Cvetnić E. (2016). Aflatoxin M1 in raw and UHT cow milk collected in Bosnia and Herzegovina and Croatia, *Food Control*, 68, 352-357.

[11] Udovicki, B., Djekic, I., Kalogianni, E., P., Rajkovic, A. (2019). Exposure assessment and risk characterization of aflatoxin M1 intake through consumption of milk and yoghurt by student population in Serbia and Greece Bozidar *Toxins,* 11, 205.

[12] Petrovic, J., Salkic, N.N., Ahmetagic, S., Stojic, V., Mott-Divkovic, S. (2011). Prevalence of chronic Hepatitis B and Hepatitis C among first time blood donors in Northeast Bosnia and Herzegovina: An estimate of prevalence in general population. *Hepatitis Monthly,* 11(8), 629-633.

[13] FAO. (2001). JECFA Safety evaluation of certain mycotoxins in food. Fifty-sixth meeting of the Joint FAO/WHO Expert Committee on food additives. In FAO Food Nutric Paper, FAO, Rome, Italy.

[14] Cano-Sancho, G., Sanchis, V., Marín, S., Ramos, A.J. (2013). Occurrence and exposure assessment of aflatoxins in Catalonia (Spain). *Food and Chemical Toxicology,* 51, 188-193.

[15] Shundo, L., Navas, S.A., Lamardo, L.C.A., Ruvieri, V., Sabino, M. (2009). Estimate of aflatoxin M 1 exposure in milk and occurrence in Brazil. *Food Control,* 20, 655-657.

[16] Kos, J., Levic, J., Duragic, O., Kokic, B., Miladinovic, I. (2014). Occurrence and estimation of aflatoxin M1 exposure in milk in Serbia. *Food Control,* 38, 41-46.

[17] Agency for Statistics of Bosnia and Herzegovina. (2016). *Census Of Population, Households And Dwellings In Bosnia And Herzegovina, Final Results*. Agency for Statistics of Bosnia and Herzegovina, Sarajevo.