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RESEARCH ARTICLE

The Occurrence and Risk Assessment of Aflatoxin M_1 in Yoghurt Samples from Hamadan, Iran

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Abstract:

Background:

Aflatoxin M_1 (AFM_1) is a hepatocarcinogenic and hydroxylated metabolite of aflatoxin B_1, detected in milk and milk products.

Objectives:

The aim of our research was to determine the incidence and risk assessment of AFM_1 through the consumption of yoghurt in Hamadan province of Iran.

Methods:

Fifty yoghurt samples were gathered from various areas of Hamadan province, Iran and tested for AFM_1 by ELISA technique. The estimated daily intake (EDI) and the liver cancer incidence of aflatoxin M_1 were determined.

Results:

AFM_1 was detected in 43 (86%) samples, (mean: 28.56 ng/kg; range: <5-98.65 ng/kg). The level of AFM_1 in 9 (18%) samples was above the maximum tolerance limit (50 ng/kg). The AFM_1 intake through yoghurt consumption in various population groups ranged from 0.016 to 0.032 ng/kg bw/day in mean consumers and 0.019 to 0.046 ng/kg bw/day in high consumers.

Conclusion:

The AFM_1 intake through yoghurt contributed a slight part from the overall incidence of liver cancer in the Iranian population. From the findings of the current study, it can be derived that although the high percentage of yoghurt samples in Iran proved to be contaminated with AFM_1 contents, did not show a public health concern considering the European Commission (EC) and the Institute of Standards and Industrial Research of Iran (ISIRI) maximum limits.

Keywords: Aflatoxin M_1, Yoghurt, ELISA, Hamadan, Risk assessment, European commission.

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1. INTRODUCTION

Yoghurt is one of the most important and popular products among fermented dairy products, which has been used as food consumed by the world population for thousands of years. Yoghurt contains nearly all nutrients necessary to sustain life [1, 2]. Also, it is a rich source of dietary minerals including vitamins (B_6 and B_{12}), calcium (Ca), magnesium (Mg), zinc (Zn), phosphorus (P), and potassium (K), and so many others [3]. It is also a great source of essential amino acids of great biological modality, generally including premier protein levels toward milk. Nevertheless, on the useful effects of yoghurt consumption, a substantial number of studies have presented the presence of heavy metals and aflatoxin M_1 in foodstuff, which is a subject of serious concern, which has been increasing over the last few years [3].

Aflatoxin M_1 (AFM_1) is one of the groups of mycotoxin produced by *Aspergillus* species, especially *A. nomius*, *A.*

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flavus and *A. parasiticus* and found in contaminated milk (breast and animal), and dairy products. Researchers have demonstrated that the concentration of AFM_1 is a dangerous toxic and carcinogenic for humans and animals [4 - 8].

Many countries have implemented regulations to control the amount of mycotoxins groups among food and agricultural products, especially for AFM_1 in milk products. These regulations differ among several countries with respect to their economic considerations [4, 7, 9 - 12]. Hence, the European Commission (EC) and the Institute of Standards and Industrial Research of Iran (ISIRI) have set a limit of 50 ng/kg for AFM_1 in yoghurt varieties.

The immediate detection of contamination is one of the beneficial methods to control aflatoxin M_1 [13]. The current detection methods such as enzyme-linked immunosorbent assay (ELISA), high-performance liquid chromatography (HPLC), and thin-layer chromatography (TLC) are generally performed for the AFM_1 analysis [4 - 8, 11 - 14]. In Iran, the ELISA technique is the most usual and popular among researchers because it is an ordinary, rapid, and low-cost technique for the survey of AFM_1 [4 - 8, 15].

The aim of the current survey was to evaluate the occurrence and risk assessment of exposure of AFM_1 through the consumption of yoghurt in the Hamadan province of Iran.

2. MATERIALS AND METHODS

2.1. Sample Collection

The current research is a cross-sectional study. For this purpose, 50 yoghurt samples were randomly purchased from supermarkets located in popular markets in different parts of Hamadan province, Iran, from October 2017 to August 2018. Eventually, all samples were carried to the lab and kept in the refrigerator at 4 ^{o}C. All samples were analyzed for AFM_1 before the expiration date of the samples. All procedures of study were approved by the Ethics Committee of Hamadan University of Medical Sciences with No. IR.UMSHA.REC.1396.617.

2.2. Methods

The quantitative measurement of AFM_1 in samples was distinguished by competitive ELISA using AFM_1 test kit (RIDASCREEN® AFM_1 Art. No.: R1121, R-Biopharm, Darmstadt, Germany). The preparation of the yoghurt samples and AFM_1 measurement were performed according to the method described by the kit manufacturer. The mean lower limit of detection (LOD) for AFM_1 in yoghurt was 5 ng/kg.

2.3. Risk Assessment for Exposure to AFM_1 Through Yoghurt

The risk of AFM_1 intake through yoghurt was carried out by the deterministic approach and calculated according to the following equation [16, 17]:

AFM_1 intake (ng/kg bw/day) = the concentration of AFM_1 in yoghurt (ng/kg) × 95th percentile (for high consumers) or mean (for mean consumers) of per capita yoghurt consumption (kg)/ body weight (kg) Equation 1.

95th percentile (for high consumers) or mean (for mean consumers) of per capital yoghurt consumption was obtained by food frequency questionnaires (FFQ), Before the study, FFQ was prepared and given to people with different age-sex groups to complete it during a month. The people were randomly selected. For all participants in FFQ study, written informed consent was obtained.

For risk assessment, AFM_1 value in yoghurt samples in which the concentration of this mycotoxin was lower than LOD was considered as LOD [17].

The liver cancer incidence due to AFM_1 consumption was estimated according to Equation 2 [17]:

Liver cancer incidence (cancers/yr/10^8 persons) = AFM_1 intake (ng/kg bw/day) × potency

Potency= 0.001 × (1-P) + 0.03 × P

In these equations, the liver cancer potency of AFM_1 in individuals positive for negative hepatitis B surface antigen (HBsAgz +) and individuals negative for negative hepatitis B surface antigen (HBsAgz -) was considered as 0.03 and 0.001 cancers/year/ ng of AFM_1/ kg body weight/ day in a population of 100,000 [17]. According to previous studies, HBsAgz + prevalence rate in Iran was 1.7% [18].

2.4. Statistical Analysis

The concentrations of AFM_1 in milk samples were analyzed by SPSS Statistics 16.0 for Windows. One-side t-test was applied to compare the mean concentration of AFM_1 samples with the maximum acceptable amount of the ISIRI and European Union (50 ng/kg) regulation. Differences between values were considered significant at $P \leq 0.05$.

3. RESULTS

The occurrence and levels of AFM_1 in yoghurt samples consumed in Hamadan province are presented in Table **1**. AFM_1 was detected above an acceptable level of 86% (43/50) in the analyzed samples, ranging from <5 to 98.65 ng/kg. Levels of the AFM_1 in 9 (18%) yoghurt samples exceeded the ISIRI and European union *i.e.* 50 ng/kg. On the other hand, considering the US FDA [19] limits for AFM_1 in milk (500 ng/l), none of the samples had levels above the maximum tolerance limit.

The AFM_1 intakes through yoghurt in different age-sex groups of Iran population are shown in Table **2**. The mean AFM_1 intake in various population groups ranged from 0.016 to 0.032 ng/kg bw/day in mean consumers and 0.019 to 0.046 ng/kg bw/day in high consumers. Data regarding the potential liver cancer risk of AFM_1 in yoghurt in the Iranian population were estimated and presented in Table **2**.

Table 1. The occurrence of AFM_1 in yoghurt samples from Hamadan (Iran) market.

Sample type	N	Positive	Mean (ng/kg)	Standard Deviation (ng/kg)	No. of Positive Samples with AFM_1			Range (ng/kg)
					<5 ng/kg	5-50 ng/kg	>50 ng/kg	
Yoghurt	50	43 (86%)	28.56	26.39	7 (14%)	34 (68%)	9 (18%)	<5-98.65

Table 2. Exposure assessment for AFM_1 intake in different sex-age groups of Iran population through yoghurt consumption using deterministic method.

Group and Sex	No. of Consumers	Mean Weight (kg)	Mean Consumers			High Consumers (percentile 95)		
			Yoghurt intake (kg/day)	AFM_1 intake (ng/kg bw/day)	Estimation of cancer risk (cancers/yr/10^8 persons)	Yoghurt intake (kg/day)	AFM_1 intake (ng/kg bw/day)	Estimation of cancer risk (cancers/yr/10^8 persons)
Infant (4-9 years)								
Male	56	27.56	0.025	0.026	0.039	0.034	0.036	0.054
Female	68	25.12	0.027	0.032	0.048	0.040	0.046	0.069
Teenagers (10-19 years)								
Male	85	52.16	0.034	0.019	0.028	0.042	0.024	0.035
Female	68	48.15	0.037	0.023	0.034	0.045	0.028	0.041
Adults (20-65 years)								
Male	95	78.65	0.042	0.016	0.023	0.051	0.019	0.028
Female	89	64.25	0.045	0.020	0.030	0.054	0.024	0.036
Elderly (>65 years)								
Male	45	71.25	0.045	0.018	0.027	0.055	0.023	0.034
Female	53	59.36	0.046	0.023	0.034	0.058	0.029	0.043

Table 3. The occurrence and levels of aflatoxin M_1 (ng/kg) in various yoghurt samples published in previous studies.

Location	No. of Samples	No. Positive Samples (%)	Detection Method	Mean (ng/kg)	Range (ng/kg)	Exceeded Regulation, n (%)	Reference
Iran	61	30 (49.2)	TLC	26	15 – 102	10 (16.4)	Fallah *et al*. [34]
Turkey	80	70 (87.5)	ELISA	66.1	10 – 475	16 (20)	Atasever *et al*. [28]
Iran	60	59 (98.33)	ELISA	51.66	6.2 – 87	38 (63.33)	Issazadeh *et al*. [35]
Turkey	26	26 (100)	ELISA	238	125 – 269	26 (100)	Tosun *et al*. [27]
Pakistan	96	59 (61)	HPLC	90.4	4 – 615.8	28 (47)	Iqbal *et al*. [34]
Pakistan	96	32 (33.33)	HPLC	90.4	LOD – 880	21 (21.87)	Iqbal *et al*. [37]
Turkey	50	50 (100)	ELISA	55.28	40.62 – 72.04	5 (10)	Temamogullari & Kanici [38]
Iran	60	48 (80)	ELISA	130.5	19.7 – 319.4	3 (5)	Rahimi [39]
Iran (Traditional)	40	40 (100)	ELISA	33.6	6 – 91	1 (2.5)	Mason *et al*. [25]
Iran	42	10 (23.8)	ELISA	15.1	6.3 – 21.3	0	Bahrami *et al*. [40]
South Korea	55	15 (27.27)	HPLC	51*	20 – 150	NR	Kim-Soo *et al*. [41]
Malaysia	5	2 (40)	ELISA	16.45*	7.5 – 31	0	Nadira *et al*. [42]
Pakistan	66	26 (39.39)	HPLC	56	LOD – 196.3	8 (12.12)	Iqbal *et al*. [43]
Qatar	21	16 (76.1)	ELISA	31.32	4.16-38.21	0	Hassan *et al*. [44]
Kenya	38	NR	ELISA	117	17 – 1100	25 (65.78)	Lindahl *et al*. [45]
China	27	15 (55.5)	ELISA	17.2	4 – 47	0	Guo *et al*. [46]

*Mean of positive samples; NR: Not reporte

4. DISCUSSION

Yoghurt is a favorite fermented dairy product, which is used as part of the popular diet in Iran because yoghurt is useful to affect human's health and has nutritional value. Various types of fermented dairy products have been made and consumed in individual households in Iran, Turkey, Qatar, Lebanon, and other Middle Eastern countries for centuries.

Considering the present findings, we detected a high level of AFM_1 contamination in yoghurt samples from Iran. In a prior survey, Cano-Sancho *et al*. [16] reported the lower level of AFM_1 at a detectable level in yoghurt samples; but in a recent study, Altun *et al*. [20] detected AFM_1 in 100% of yoghurt samples. Various studies by other researchers from different countries have previously been conducted on high or low contamination levels of AFM_1 in yoghurt. Table **3** shows the compilation of data level of AFM_1 contamination in yoghurt samples from previous studies from several countries measured by different techniques including HPLC and ELISA.

Hassan and Kassaift [21] from Lebanon using ELISA method reported that 49 (72%) of 68 samples of yoghurt were detected with AFM_1 and in 9 (14%) samples, the amount of AFM_1 was higher than the EU regulations (50 ng/kg), this result is approximately similar to our research results. The other survey from Iran by Tavakoli *et al* [7] that were done with ELISA technique on 50 samples of yoghurt, 35 (70%) of samples were contaminated with AFM_1. Also, 6 (17.4%) samples of yoghurt had greater AFM_1 content than the limit allowed in European Union (EU) 50 (ng/kg).

The other obtained results reported by Tabari *et al*. [22] determined AFM_1 levels in 120 yoghurt samples from Guilan province in Iran using the ELISA method. They have reported that 100% of the samples found aflatoxin M_1 by a mean concentration of 28.2 ng/kg. Also, 16 (13.3%) samples were above the permissible quantity according to the EC (50 ng/kg). However, this result is in contrast to our findings, that showed 86% (43/50) were contaminated with AFM_1.The other conducted results were revealed in Serbia by Tomašević *et al*. [23], which were observed with ELISA, from 56 samples of yoghurt, all samples (100%) were contaminated with AFM_1. Also, 22 (39.2%) samples were above the permissible level according to the EC (50 ng/kg). But, our results reported that approximately some of the yoghurt samples were contaminated with AFM_1. This result from Serbia is similar to another study from Iran by Nikbakht *et al*. [24]. They detected that all yoghurt samples (100%) were contaminated with AFM_1; and also, 20 (22.22%) of the samples were above the permissible level according to EU (50 ng/kg).

In a previous study performed in Iran, Mason *et al*. [25] revealed that in 37 (92.5%)out of 40 industrial yoghurt samples, aflatoxin M_1 was detected in concentration between <5 and 71 ng/kg; and in 3 (7.5%) samples, the contamination level exceeded the maximum permissible limit (50 ng/kg). In agreement with our research, these reports proved a widespread incidence of aflatoxin M_1 in yoghurt samples ready and consumed in Iran. Compared to some reports from several countries, our results showed higher contamination. In Turkey, for example, aflatoxin M_1 was detected in 2 (3.3%) out of 60 yoghurt samples with a range of 24 to 28 ng/kg. Also, none of the samples had aflatoxin M_1 above the maximum tolerance limit (50 ng/kg) set by the EU [26]. In another survey from China using the ELISA method [17], it was observed that in 8 (4.49%), out of 178 samples, aflatoxin M_1 was present. The contamination level was detected in 8 (4.49%) of the samples which were above 50 ng/kg according to the EU.

According to several studies carried out in different neighboring countries of Iran, Turkey Altun *et al*. [20]; Tosun & Ayyildiz [27]; Atasever *et al.* [28] detected a high incidence of aflatoxin M_1 in yoghurt samples (100%, 100% and 87.5%, respectively). These results were detected by the ELISA method.

As seen in Table **3**, the contamination levels of aflatoxin M_1 in yoghurt samples vary from one study to another. This variability can be explained by different factors such as geographical region, yoghurt-making procedures, analytical method employed and seasons variability [4, 29]. On the other hand, the previous study by Iha *et al*. [30] showed that the process of fermentation of yoghurt has no effect on aflatoxin M_1. Also, the other surveys revealed that the quality of raw materials in yoghurt is effective on the presence and levels of AFM_1. It is also noted that little or no reduction in aflatoxin M_1 levels occurs as a result of pasteurization [31].

The incidence of liver cancer in Iran was 3.53 cancers per year per 10^5 persons or 3530 cancers/yr/10^8 persons [32] and AFM_1 intake through yoghurt contributed 0.023-0.048 cancers/yr/10^8 person for mean consumers and 0.028-0.069 cancers/yr/10^8 person for high consumers. Therefore, our findings indicated AFM_1 in yoghurt contributed a slight part from the overall incidence of liver cancer in the Iranian population. The intake of AFM_1 and liver cancer incidence due to the consumption of this mycotoxin through yoghurt and milk was reported in other countries including China, Spain, Greece and Serbia [16, 17, 33]. The range of liver cancer incidence or hepatocellular carcinoma (HCC) due to AFM_1 intake through milk and yoghurt was 0.025–0.033 case or cancers/yr/10^8 person in China that it was similar to our study while in Serbia and Greece was 3.6–0.4.7 and 0.7–0.9 case or cancers/yr/10^8 person, respectively that it was higher than the current study [17, 33]. The dispenses were related to the AFM_1 level and consumption value of yoghurt.

CONCLUSION

From our findings of the current study, it can be derived that although a high percentage of yoghurt samples in Iran proved to have AFM_1 contents, but it does not show a public health concern considering the European Commission (EC) and the Institute of Standards and Industrial Research of Iran (ISIRI) maximum limits. However, regarding the important role of milk, especially dairy products in the human diet, there is a huge concern about the presence of AFM_1 in milk and dairy products. Hence, it is important to use fast methods in the detection of AFM_1 in milk and dairy products; and also, the Iranian public health authorities have to monitor ceaselessly to detect AFM_1 contamination.

AUTHORS' CONTRIBUTIONS

Ali Heshmati, and Amir Sasan Mozaffari Nejad conceived, designed, analyzed, and interpreted the data; Tayebeh Ghyasvand and Amir Sasan Mozaffari Nejad performed data collection. Ali Heshmati and Amir Sasan Mozaffari Nejad wrote the first draft and finalized it. All authors read and approved the final manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

All procedures of the study were approved by the Ethics Committee of Hamadan University of Medical Sciences, Iran with No. IR.UMSHA.REC.1396.617.

HUMAN AND ANIMAL RIGHTS

Not applicable.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The data that support the findings of this study are with the corresponding author, [ASMN], and can be made available on reasonable request.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest, financial or otherwise.

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