RESEARCH ARTICLE
The Occurrence and Risk Assessment of Aflatoxin M<sub>1</sub> in Yoghurt Samples from Hamadan, Iran

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Abstract:
Background: Aflatoxin M<sub>1</sub> (AFM<sub>1</sub>) is a hepatocarcinogenic and hydroxylated metabolite of aflatoxin B<sub>1</sub>, detected in milk and milk products.

Objectives: The aim of our research was to determine the incidence and risk assessment of AFM<sub>1</sub> 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<sub>1</sub> by ELISA technique. The estimated daily intake (EDI) and the liver cancer incidence of aflatoxin M<sub>1</sub> were determined.

Results: AFM<sub>1</sub> was detected in 43 (86%) samples, (mean: 28.56 ng/kg; range: <5-98.65 ng/kg). The level of AFM<sub>1</sub> in 9 (18%) samples was above the maximum tolerance limit (50 ng/kg). The AFM<sub>1</sub> 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<sub>1</sub> 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<sub>1</sub> 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<sub>1</sub>, Yoghurt, ELISA, Hamadan, Risk assessment, European commission.

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<sub>1</sub> and B<sub>2</sub>), 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<sub>1</sub> in foodstuff, which is a subject of serious concern, which has been increasing over the last few years [3].

Aflatoxin M<sub>1</sub> (AFM<sub>1</sub>) is one of the groups of mycotoxin produced by Aspergillus species, especially A. nomius, A.
flavus and A. parasiticus and found in contaminated milk (breast and animal), and dairy products. Researchers have demonstrated that the concentration of AFM, 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, 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, in yoghurt varieties.

The immediate detection of contamination is one of the beneficial methods to control aflatoxin M [13]. The current detection methods such as enzyme-linked immunoabsorbent assay (ELISA), high-performance liquid chromatography (HPLC), and thin-layer chromatography (TLC) are generally performed for the AFM, 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 [4 - 8, 15].

The aim of the current survey was to evaluate the occurrence and risk assessment of exposure of AFM, 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 °C. All samples were analyzed for AFM, 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, in samples was distinguished by competitive ELISA using AFM, test kit (RIDASCREEN® AFM, Art. No.: R1121, R-Biopharm, Darmstadt, Germany). The preparation of the yoghurt samples and AFM, measurement were performed according to the method described by the kit manufacturer. The mean lower limit of detection (LOD) for AFM, in yoghurt was 5 ng/kg.

2.3. Risk Assessment for Exposure to AFM, Through Yoghurt

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

\[\text{AFM, intake (ng/kg bw/day)} = \text{the concentration of AFM, in yoghurt (ng/kg)} \times 95^{th} \text{ percentile (for high consumers) or mean (for mean consumers) of per capita yoghurt consumption (kg)/ body weight (kg) Equation 1.}\]

95\(^{th}\) percentile (for high consumers) or mean (for mean consumers) of per capita 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, 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, consumption was estimated according to Equation 2 [17]:

\[\text{Liver cancer incidence (cancers/yr/10^5 persons) = AFM, intake (ng/kg bw/day) \times potency}\]

\[\text{Potency= 0.001 \times (1-P) + 0.03 \times P}\]

In these equations, the liver cancer potency of AFM, 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, 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, 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, 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, in yoghurt samples consumed in Hamadan province are presented in Table 1. AFM, 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, 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, in milk (500 ng/l), none of the samples had levels above the maximum tolerance limit.

The AFM, intakes through yoghurt in different age-sex groups of Iran population are shown in Table 2. The mean AFM, 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, 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.

<table>
<thead>
<tr>
<th>Sample type</th>
<th>N</th>
<th>Positive (%)</th>
<th>Mean (ng/kg)</th>
<th>Standard Deviation (ng/kg)</th>
<th>No. of Positive Samples with AFM$_1$</th>
<th>Range (ng/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yoghurt</td>
<td>50</td>
<td>43 (86%)</td>
<td>28.56</td>
<td>26.39</td>
<td>7 (14%)</td>
<td>5-98.65</td>
</tr>
</tbody>
</table>

Table 2. Exposure assessment for AFM$_1$ intake in different sex-age groups of Iran population through yoghurt consumption using deterministic method.

<table>
<thead>
<tr>
<th>Group and Sex</th>
<th>No. of Consumers</th>
<th>Mean Weight (kg)</th>
<th>Yoghurt intake (kg/day)</th>
<th>AFM$_1$ intake (ng/kg bw/day)</th>
<th>Estimation of cancer risk (cancers/yr/10$^5$ persons)</th>
<th>Yoghurt intake (kg/day)</th>
<th>AFM$_1$ intake (ng/kg bw/day)</th>
<th>Estimation of cancer risk (cancers/yr/10$^5$ persons)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infant (4-9 years)</td>
<td>Male</td>
<td>56</td>
<td>27.56</td>
<td>0.025</td>
<td>0.026</td>
<td>0.039</td>
<td>0.034</td>
<td>0.036</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>68</td>
<td>25.12</td>
<td>0.027</td>
<td>0.032</td>
<td>0.048</td>
<td>0.040</td>
<td>0.046</td>
</tr>
<tr>
<td>Teenagers (10-19 years)</td>
<td>Male</td>
<td>85</td>
<td>52.16</td>
<td>0.034</td>
<td>0.019</td>
<td>0.028</td>
<td>0.042</td>
<td>0.024</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>68</td>
<td>48.15</td>
<td>0.037</td>
<td>0.023</td>
<td>0.034</td>
<td>0.045</td>
<td>0.028</td>
</tr>
<tr>
<td>Adults (20-65 years)</td>
<td>Male</td>
<td>89</td>
<td>78.65</td>
<td>0.045</td>
<td>0.016</td>
<td>0.023</td>
<td>0.051</td>
<td>0.019</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>89</td>
<td>64.25</td>
<td>0.045</td>
<td>0.020</td>
<td>0.030</td>
<td>0.054</td>
<td>0.024</td>
</tr>
<tr>
<td>Elderly (&gt;65 years)</td>
<td>Male</td>
<td>45</td>
<td>71.25</td>
<td>0.045</td>
<td>0.018</td>
<td>0.027</td>
<td>0.055</td>
<td>0.023</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>53</td>
<td>59.36</td>
<td>0.046</td>
<td>0.023</td>
<td>0.034</td>
<td>0.058</td>
<td>0.029</td>
</tr>
</tbody>
</table>

Table 3. The occurrence and levels of aflatoxin M$_1$ (ng/kg) in various yoghurt samples published in previous studies.

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

*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, Ahun 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, contamination in yoghurt samples from previous studies from several countries measured by different techniques including HPLC and ELISA.

Hassan and Kassafit [21] from Lebanon using ELISA method reported that 49 (72%) of 68 samples of yoghurt were detected with AFM, and in 9 (14%) samples, the amount of AFM, 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. Also, 6 (17.4) samples of yoghurt had greater AFM, content than the limit allowed in European Union (EU) 50 (ng/kg).

The other obtained results reported by Tabari et al. [22] determined AFM, 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, 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. 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. 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. 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, and also, 20 (22.2%) 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, 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, 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, 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, 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, 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, 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, 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,. Also, the other surveys revealed that the quality of raw materials in yoghurt is effective on the presence and levels of AFM,; it is also noted that little or no reduction in aflatoxin M, 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/10^5 persons [32] and AFM, intake through yoghurt contributed 0.023-0.048 cancers/yr/10^5 person for mean consumers and 0.028-0.069 cancers/yr/10^5 person for high consumers. Therefore, our findings indicated AFM, in yoghurt contributed a slight part from the overall incidence of liver cancer in the Iranian population. The intake of AFM, 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, intake through milk and yoghurt was 0.025–0.033 case or cancers/yr/10^5 person in China that it was similar to our study while in Serbia and Greece was 3.6–0.47 and 0.7–0.9 case or cancers/yr/10^5 person, respectively that it was higher than the current study [17, 33]. The dispenses were related to the AFM, 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, 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, in milk and dairy products. Hence, it is important to use fast methods in the detection of AFM, in milk and dairy products; and also, the Iranian public health authorities have to monitor ceaselessly to detect AFM, 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.
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