Determination of Aflatoxin M1 Levels in Raw Milk Samples in Gilan, Iran

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Abstract

Aflatoxins are cancerogenic compounds produced predominantly by certain strains of the Aspergillus genus. Contamination of milk and dairy products to aflatoxin M1 is a risk for human health. Afloatoxin M1 is relatively stable during milk pasteurization and storage as well as during the preparation of various dairy products. In this study, 90 raw milk samples was obtained randomly during the Autumn and Winter of 2011, in Gilan province (Northern Iran). Samples were tested for Afloatoxin M1 (AFM1) contamination by ELISA (Enzyme Linked Immuno Sorbent Assay) technique. In 56 of the 90 raw milk samples examined (65.55%), the presence of AFM1 was detected in concentrations between 2.1–131 ng/l. AFM1 levels in 28 samples (31.11%) were higher than the maximum tolerance limit (50 ng/l) accepted by the European countries. It was concluded that widespread occurrence of AFM1 in milk samples were considered to be possible hazards for public health especially children.

Keywords: Afloatoxin M1, ELISA, raw milk

Introduction

Mycotoxins are fungal secondary metabolites that if ingested can cause a variety of adverse effects on both humans and animals [1]. Aflatoxins are a group of structurally-related toxic compounds produced by certain strains of the fungi Aspergillus flavus and A. parasiticus [2, 3]. Aflatoxins have sub-acute and chronic
effects such as liver cancer, chronic hepatitis, jaundice, hepatomegaly and cirrhosis in humans, AFM1 is classified in Group 2 as a probable human carcinogen [1, 4]. *A. parasiticus* produces four major aflatoxins: B1, B2, G1 and G2, while AFB1 is the most toxic in the group and the toxicity is in the order of B1 > G1 > B2 > G2 [5]. Aflatoxin M1 is a major metabolite of aflatoxin B1 (AFB1), which is formed when animals ingest feed contaminated with aflatoxin B1. These metabolites are not destroyed during the pasteurization and heating process. The amount of AFM1 which is found in milk depends on several factors, such as animal breed, lactation period, mammary infections etc. AFM1 could be detected in milk 12-24 h after the AFB1 ingestion, reaching a high level after a few days. When AFB1 intake is stopped, the AFM1 concentration in milk decreases to an undetectable level after 72 h [6,7,8]. Many countries have established regulations to control the levels of aflatoxin B1 in feeds and to have maximum permissible levels of aflatoxin M1 in milk to reduce this hazard [8]. The European Community and Codex Alimentarius Commission prescribed that the maximum level of aflatoxin M1 in milk and milk products should not exceed 50 ng/L [9]. Thin layer chromatography (TLC), high performance liquid chromatography (HPLC) and enzyme linked immunosorbent assay (ELISA) are the most common techniques for detecting AFM1 in milk and dairy products [10]. Some studies have been done about aflatoxin M1 contamination in raw milk in the world and their results have presented exceeded concentrations regarding the European Community and Codex Alimentarius regulatory limit [11,12, 13,14,15]. The aim of this study was to investigate the presence of AFM1 in raw milk samples produced in Gilan province (Northern Iran) by ELISA method.

### Materials and Methods

**Preparation of samples:** A total of 90 raw milk samples was obtained randomly from dairy farm during autumn and winter 2011 in Gilan province (Northern Iran). All Samples defatted through cooling Centrifuge for 10 min, 3500g at 4°C. The upper fat layer removed by spatula. 100 µl (per well) of this solution was used in the test [14, 15].

**ELISA test procedure:** ELISA kit (R-biopharm; Germany), were used for the test. Before starting the test, the reagents were brought up to room temperature. The AFM1 standards and test samples (100 µl per well) in duplicate were added to the wells of a micro-titer plate pre-coated with antibodies for AFM1 and incubated at room temperature in dark for 60 min. After the washing step, 100µl of peroxidase conjugate was added to the wells and plate was incubated again for 60 min at room temperature in dark. After the washing step, the unbound conjugate was removed during washing. Subsequently, 50 µl each substrate (urea peroxide) and chromogen (tetramethyl-benzidine) were added to the wells and incubated for 30 min in dark. Finally, 100 µl of stop solution were added to each well. The optical absorbance of each well was read at 450 nm with ELISA plate reader. Absorbance percentages were taken to the calibration curve performed with standards at different concentrations. Statistical analyses were performed.
Determination of aflatoxin M1 levels

Results

A total of 90 raw milk samples was analyzed with competitive ELISA. The occurrence of AFM1 was shown in table 1. Of the 90 samples analyzed, 56 samples (65.55%) were found to be contaminated with AFM1. 28 samples (31.11%) failed to reach the desired level of the European Communities and Codex, defined as 50ng/l. The aflatoxin M1 contamination levels were between 2.1 - 131ng/l.

Table 1. Occurrence of AFM1 in raw milk samples from Northern Iran

<table>
<thead>
<tr>
<th>AFM1 levels ng/l</th>
<th>Sample No.</th>
<th>(%)</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not detected</td>
<td>31</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>&lt; 10</td>
<td>8</td>
<td>8.88</td>
<td>2.1- 9.7</td>
</tr>
<tr>
<td>11-25</td>
<td>13</td>
<td>14.44</td>
<td>11.2- 21.5</td>
</tr>
<tr>
<td>26-50</td>
<td>10</td>
<td>11.11</td>
<td>27.5-48</td>
</tr>
<tr>
<td>≥ 50</td>
<td>28</td>
<td>31.11</td>
<td>51.2- 131</td>
</tr>
<tr>
<td>Total Samples</td>
<td>90</td>
<td>65.55</td>
<td>2.1- 131</td>
</tr>
</tbody>
</table>

Discussion

Under favorable conditions of temperature and humidity, these fungi grow on certain foods and feeds, resulting in the production of aflatoxins [16]. The contamination of milk and milk products with AFM1 display variations according to geography, country and season. The pollution level of AFM1 is differentiated further by hot and cold seasons, due to the fact that grass, pasture, weed and rough feeds are found more commonly in spring and summer than in winter [17]. Maximum limits for aflatoxin M1 in milk and milk products in various countries shown in table 2 [4, 18].
Several surveys were performed in order to determine the AFM1 levels in raw milk samples (Table 3) [14]. In Kuwait, 54 samples of dairy products were analyzed for aflatoxin M1, 28% were contaminated with AFM1 [11]. KIM et al, determined the incidence of AFM1 in pasteurized milk as 76% in Korea, with a mean concentration of 18 pg/g [19].

Table 3. The occurrence of aflatoxin M1 in raw milk samples in other studies

<table>
<thead>
<tr>
<th>Location</th>
<th>Number of milk samples</th>
<th>Percent of contaminated milk samples above 50ng/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Argentina</td>
<td>56</td>
<td>0</td>
</tr>
<tr>
<td>Greece</td>
<td>166</td>
<td>1.8</td>
</tr>
<tr>
<td>Indonesia</td>
<td>342</td>
<td>21</td>
</tr>
<tr>
<td>Italy</td>
<td>296</td>
<td>1.7</td>
</tr>
<tr>
<td>UAE</td>
<td>59</td>
<td>56</td>
</tr>
<tr>
<td>Thailand</td>
<td>310</td>
<td>84</td>
</tr>
<tr>
<td>Iran</td>
<td>100</td>
<td>78</td>
</tr>
</tbody>
</table>

In study Celik et al, Seventy-five samples (88.23%) were found to be contaminated with AFM1, and 48 samples (64%) exceeded the legal level of AFM1 in milk according to the Turkish Food Codex and Codex Alimentarius limit (50 ng/kg) [4]. Rastogi et al, reported that 75% of liquid milk samples exceeded European Communities and Codex Regulations [20]. In our study, Of
the 90 samples analyzed, 56 samples (65.55%) were found to be contaminated with AFM1 and contamination levels were between 2.1 – 131 ng/l. Milk and milk products, are a major nutrient for humans, especially children. For this reason, AFM1 in milk and dairy products should be controlled systematically [21]. According to observations, the levels of contamination of milk by AFM1 seem to vary in many studies. These variations may be related to different reasons such as milk manufacturing procedures, type of milk, conditions of milk ripening, geographical region, the country, the season and the analytical methods employed [22]. According to results obtained, incidence and contamination levels of AFM1, seem to be a serious problem for public health. For this reason, milk and dairy products have to be inspected and controlled continuously for AFM1 contamination and animal feeds should be checked regularly for AFB1 and storage conditions of feeds must be taken under strict control.

References


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