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Original Article

Aflatoxin M1 in Pasteurized Milk in Babol city, Mazandaran Province, Iran

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Abstract

Background: Aflatoxin M_1 (AFM_1) is the metabolite of aflatoxin B1 (AFB_1) and is found in milk when lactating animals are fed with contaminated feedstuff. The presence of AFM_1 in milk, pose a major risk for humans especially kids as it can have immunosuppressive, mutagenic, teratogenic and carcinogenic effects. The present study is aimed to investigate the occurrence of AFM_1 in subsidized pasteurized milk in Babol, Mazandaran Province, Iran.

Methods: Some 72 pasteurized milk packages were collected from supermarkets in various districts of city during January to March 2006. Milk samples were centrifuged and amounts of 100 µl of skimmed milk were tested for AFM_1 contamination by competitive ELISA.

Results: All the samples (100%) exhibited contamination with AFM_1. The contamination levels means in January, February, and March were 227.85, 229.64, and 233.1ng/l, respectively. The amount of AFM_1 in all the samples were above 50ng/l, the threshold set by the European community regulations.

Conclusion: Monitoring of AFM_1 level should be part of quality control procedures in dairy factories, particularly the ones providing infant's milk. Production of safer and healthier milk and other dairy products with minimum AFM_1 level can be achieved by adopting prophylactic measures including control of humidity and water content of feedstuff, which favors mould production.

Keywords: *Aflatoxin M_1, ELISA, Pasteurized milk, Iran*

Introduction

Aflatoxins are secondary toxic metabolites and found in most plant products including peanuts, copra, soya, maize, rice and wheat. Aflatoxins B_1, B_2, G_1, and G_2 are produced by special strains of *Aspergillus flavus*, and *A. parasiticus*. The species *A. flavus* merely produces AFB, while other species produce both AFB and AFG(1). Aflatoxins are produced during growth, harvesting and storage course (2). When animals consume AFB_1 contaminated feedstuff, the toxin is metabolized in the liver and execrated as AFM_1 via milk, urination, and feces (3, 4). AFM_1 is bound to milk proteins, especially casein, which leads to its presence in dairy products (2). Aflatoxins are acute toxic compounds, and have shown to be immunosuppressive, mutagen, teratogen and carcinogen (5). International Agency for Research on Cancer (IARC), WHO introduced Aflatoxins B_1 and M_1 as primary and secondary groups of carcinogenic compounds, respectively (6). The main target organ for toxicity and carcinogenicity is liver. Although mutagenic and carcinogenic level of AFM_1 is lower than AFB_1, its geotaxis activity is known to be much higher (7). AFM_1 is resistant to heat and is not degraded during pasteurization process (8). Food standards for AFM_1 level varies in different countries. According to European community and Codex Alimentary the maximum level for AFM_1 should not exceed 50 ng/kg in raw milk and processed dairy products (4). In Australia and Switzerland AFM_1 levels in children food are not allowed to be more than 10 ng/kg (9). In order to establish a limit for AFM_1

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in milk and other dairy products in Iran, preliminary studies are needed to examine its occurrence and level in different areas of Iran.

This study is aimed to investigate the amounts of AFM_1 in subsidized pasteurized milk in Babol, Mazandaran Province, Iran.

Material and Methods

During the winter 2006, some 72 pasteurized milk packages were collected from supermarkets in various districts of Babol and transferred to Department of Medical Mycology, Babol University of Medical Sciences. Milk samples were centrifuged at 3000g for 10 min at 2-8 °C and skimmed milk was directly used for quantitative analysis of AFM_1 by competitive ELISA, using AFLA M_1 Kit- code MA418 (Tecna, Trista, Italy). Simply, one hundred µl of prepared samples and standard solutions were added into each well, already coated with anti-aflatoxin M_1 antibodies by the manufacturer, and incubated at 20-25º C for 45 min. The plates were washed four times by washing buffer, 100 µl enzymes conjugate solution was added into each well and plates were incubated at 20-25° C for 15 min. The plates were washed as before and 100 µl of developing solution was added into each well and incubated at 20-25° C in a dark place for 15 min. The reaction was stopped by stop solution and absorbance was measured at 450 nm by an ELISA reader within 60 min.

Results

A total of 72 pasteurized milk samples were analyzed with the competitive ELISA. All the samples (100%) were found contaminated with AFM_1. The contamination means in January, February and March were 227.85, 229.64, and 233.1 ng/l, respectively. The highest contamination was observed in March and the lowest in January. AFM_1 contamination ranged from 178.8 to 253.5 ng/l (mean value 230.2), which shows that the contamination level in all samples (100%) exceeded the European community regulations (50 ng/l). There was no significant relationship between AFM_1 contamination level and different months of winter ($P \leq 0.05$) (Table 1, 2).

Table 1: The AFM_1 contamination levels in milk samples during winter 2006 in Babol, Mazandaran Province, Iran

AFM_1 concentration ng/l	>250	201-250	151-200	51-150	<50
Number of assayed milk samples	4	62	6	0	0
%	5.5	86	8.5	0	0

Table 2: Number and mean values of AFM_1 positive milk samples during winter 2006 in Babol, Mazandaran Province, Iran

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Months	Number of Samples tested	Contaminated samples > 50 ng/l, (%)	Mean±SE
Jan.	24	24 (100)	227.85±4.98
Feb.	24	24(100)	229.64±4.19
Mar.	24	24(100)	233.1±4.07
Total	72	72(100)	230.2±1.89

Discussion

The occurrence of Aflatoxin in food is a serious global health problem, particularly in developing countries. Aflatoxins are well documented as cancer potency factors as 4.6-28.2% of annual hepatocarcinoma cases worldwide are caused by theses toxins (10). The presence of AFM_1 in milk and other dairy products is a concern throughout the world and many countries have set threshold limits for milk used by adults and infants. Despite a considerable progress in food industry in Iran during the past two decades, a few reliable data

is available on contamination levels of milk and other dairy products with AFM_1. The earliest study in Iran dates back to 1982, in which 52 liquid milk samples from Tehran showed to be contaminated with AFM_1, ranging from 23 to 3000 µl/l (11). Also, in another study on pasteurized milk samples from Tehran, AFM_1 contamination showed to be above the threshold (50ng/kg) set by European Union (11). However, in two separate studies in Sarab and Shiraz on 111 ad 624 milk samples, AFM_1 contamination level was below the maximum tolerance limit of European Union (12). A recent study in Tehran showed that the amount of AFM_1 in 78% of liquid milk samples was higher than the maximum tolerance limit accepted by European Union. However, a validated High Performance Liquid Chromatography (HPLC) analysis of milk samples from five different regions in Iran (Gorgan, Hamadanm, Rasht, Shiraz and Tehran) showed that the AFM_1 contamination levels were much lower than those obtained by quantitative ELISA (13). Our results in Babol showed that AFM_1 contamination levels in 100% of milk samples were about 4 folds more than European Union standard. The difference in AFM_1 contamination levels may be attributed to different methods of evaluation and seasonal variations. Thus, adopting a reliable and uniform standard method for measurement of AFM_1 contamination in milk and other dairy products is a crucial step. Monitoring AFM_1 levels should be part of quality control procedures in dairy factories, particularly the ones providing infant's milk, as young animals were found to be more susceptible to aflatoxins than adults (14). An accurate knowledge on the seasonal variation of AFM_1 contamination in milk from different geographical areas in Iran can provide valuable information on factors affecting AFB_1 production, the primary alfatoxin that is metabolized to AFM_1. The amount of AFB_1 in animal feed can be minimized by taking care of cultural phases, including harvest and storage practices, that present critical points for fungal growth and mycotoxin production (2). If reduction of AFM1 concentration in feedstuff is not possible in some areas, addition of aflatoxin adsorbents to the contaminated feedstuffs can be considered as an alternative method (2).

Ethical Considerations

Ethical issues including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc. have been completely observed by the authors.

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