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Determining the aflatoxin M1 levels in breast milk

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Abstract. – OBJECTIVE: Mycotoxins are different toxic substances at relatively smaller molecular weight produced by some types of fungi. Aflatoxin is the most common type of mycotoxin easily reproducing in food stored for a long time in unsuitable conditions. This study determined the aflatoxin M1 (AFM1) levels in breast milk samples collected from mothers who gave birth in Kırşehir, Turkey.

MATERIALS AND METHODS: A total of 82 breast milk samples to be analyzed to determine the AFM1 levels were collected from voluntary breastfeeding mothers who gave birth in the Kırşehir Training and Research Hospital and who were randomly selected. The AFM1 levels were determined using the competitive ELISA kit.

RESULTS: The AFM1 levels in the breast milk samples of mothers who did not consume milk were lower than those of other mothers. The AFM1 levels in the breast milk samples of mothers consuming fabrication milk were lower than mothers consuming homemade milk ($p<0.01$). Additionally, the AFM1 levels in the breast milk samples of mothers consuming homemade or self-made bread were lower ($p<0.05$).

CONCLUSIONS: This study found that the nutritional habits of breastfeeding mothers affected the AFM1 levels in breast milk.

Key Words:
Breast milk, Enzyme-linked immunosorbent assay (ELISA), Aflatoxin M1, Mycotoxin.

Introduction

Breastfeeding is the most important nutrition-related practice in the neonatal and infancy periods, and national and international organizations recommend that infants are fed with breast milk only and should not be given water during the first six months. It is also recommended that breastfeeding should be continued until the 24th month in addition to supplementary infant foods after the first six months[1-3]. It is a clear fact[4] that breast milk has a lot of benefits both for the infant and the mother. Breastfeeding in newborns was determined[5] to reduce mortality and morbidity due to diseases seen in the initial periods of life. This nutrient with high nutritional value has many beneficial effects on the infant's developmental process, gastrointestinal tract and immune functions[6].

However, it has been reported[7] that breastfeeding may threaten the infant's health due to the presence of some diseases in the mother and or the exposure of the mother to some chemical agents/infections may transfer these into the breast milk. Some toxins known to pass into milk are mycotoxins[7]. The term mycotoxin derives from the Greek word "mykes" meaning fungus and the Latin word "toxicum" meaning poison[8]. Mycotoxins may be described as fungal metabolites that can cause diseases or death in humans and animals when absorbed into the body by digestion, inhalation, or skin absorption[9]. The concept of metabolite is used for products released in metabolic reactions, and secondary metabolites are described as compounds that are not necessary for the growth of the organism and are produced and exist in only a limited number of taxonomic groups. Mycotoxins are secondary metabolites with no biochemical importance regarding the growth and development of molds[10]. There are almost 400 mycotoxins described in today's world, and it is known[9] that the number of molds reproducing mycotoxins has reached 350.

Mycotoxins are toxic products of the *Aspergillus, Penicillium, Fusarium* and *Alternaria* types of fungus that can cause contamination in food[11]. Contamination of food with mycotoxins is associated with climate and agriculture methods. Thus, it shows geographical differences. Global

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climate changes have had negative impacts on food contamination with mycotoxins in recent years. The United Nations Food and Agriculture Organization (FAO)[12] warned that 25% of grains in the world contained mycotoxins at different concentrations. Almost 25% of crops in the world are exposed to contamination by molds, and this causes serious economic damages, as well as the transmission of toxic secondary metabolites. People consuming animal-based foods such as milk, meat, eggs produced from animals that are fed with plant-based food contaminated with mycotoxins may be exposed to mycotoxins[13].

Mycotoxins that can be taken into the human body by consuming plant- and animal-based foods containing mycotoxin residues and metabolites may cause deterioration in the liver and kidney functions and have a neurotoxic impact. Some mycotoxins can be teratogenic and/or carcinogenic[14,15]. Aflatoxins are the most common type of mycotoxins easily reproducing in food stored for a long time in unsuitable conditions. These mycotoxins are detected in the serum and urine after getting metabolized following ingestion and accumulate in adipose tissue. It was determined that they pass into breast milk as lipid tissues and are also used during lactation in breastfeeding mothers due to their lipophilic properties[8].

Relevant studies[16] have identified 18 types of aflatoxins and determined that aflatoxin B1 (AFB1), B2 (AFB2), G1 (AFG1), G2 (AFG2), M1 (AFM1) and M2 (AFM2) among these, cause toxication in humans and animals. The most potent hepatocarcinogenic and hepatotoxic one among aflatoxins is AFB1. Aflatoxins taken into the body *via* contaminated feed and food are absorbed in the digestive tract, pass into the blood circulation and are conveyed by binding to albumins. Most of the aflatoxin in the blood is kept in the liver. Some of the aflatoxins bind to macromolecules such as endoplasmic steroids and various enzymes in hepatocytes, while some of them are transformed to fat- and water-soluble metabolites. If humans and animals consume food contaminated with AFB1, the AFB1 is hydroxylated by the cytochrome P450 enzyme system[17]. As a result of this hydroxylation process, AFB1 is transformed into AFM1, which is a 10-times more carcinogenic metabolite than AFB1, within 12 to 24 hours. AFM1 is excreted from the body *via* urine and breast milk[18].

In a study[19], which stated that aflatoxins are a risk factor for the development of hepatocellular carcinoma in humans, the serum AFB1, AFB2, AFG1 and AFG2 concentrations of hepatitis B patients with liver cancer were measured, and the mean AFB1 levels and total aflatoxin levels of the patients with liver cancer were significantly higher than the healthy control group. Additionally, the mean AFB1 level and total aflatoxin levels of the patients with chronic hepatitis B and liver cancer were found to be significantly higher than the hepatitis B patients with or without cirrhosis; thus, these results indicated that patients with chronic hepatitis B exposed to aflatoxins are at great risk for the development of hepatocellular carcinoma[19].

It is important to determine the mycotoxin levels in breast milk, which is the most important nutrient for the normal growth and the development of the newborn. Informing mothers about nutrition both during breastfeeding and throughout life can reduce the exposure to mycotoxins. In order to prevent the toxic effects of mycotoxins, it is important to develop preventive studies[2,20] against mycotoxins in the society and to analyze the breastfeeding practices of mothers with high mycotoxin levels in breast milk. Studies[20-27] on the aflatoxin content of breast milk may show differences in countries and regions.

Although there are similar studies[20,22-25,27] in the literature, no such study has been carried out in Kırşehir dominated by a continental climate. The cold winter season and climate conditions form a suitable environment for the development of aflatoxins in food. Furthermore, agriculture and livestock farming are the most important means of living in Kırşehir, Turkey. This allows people to make their own bread, produce their milk and dairy products and consume them. Thus, this situation may change the aflatoxin exposure of people living in Kırşehir. Therefore, determining the AFM1 levels in breast milk, which is the most important nutrient for newborns, informing mothers about nutrition during breastfeeding and throughout life and developing prevention efforts against mycotoxins are important steps in terms of preventing the toxic effects of mycotoxins.

This study was conducted to determine the correlation between AFM1 levels in breast milk samples collected from mothers who gave birth in Kırşehir, Turkey, and their nutritional habits. Thus, this study aimed to determine the concentrations of AFM1 with high toxicity in breast milk, interpret the risks and possible health impacts that newborns will be exposed to and make recommendations to take the necessary measures for eliminating these risks in the long term.

Materials and Methods

Breast milk samples were collected from women who presented to the Department of Pediatrics at the Kırşehir Research and Training Hospital between May and October 2019. The participants included 82 mothers who gave birth at the hospital or brought their infants to the hospital for routine follow-ups. Breast milk samples of the mothers on different lactation days were collected in this study. The samples of 82 women who voluntarily participated in the study were collected by themselves or with the help of nurses. The contents of a voluntary consent form were read to the mothers included in the study, and those who agreed to participate signed the form. Mothers who did not agree to participate in the study were excluded. Additionally, a questionnaire form to determine sociodemographic data, living conditions and nutrition habits was filled out by the participants face to face. The approval of the Kırşehir Ahi Evran University Faculty of Medicine Clinical Studies Ethics Committee was obtained before the study was conducted (Decision No.: 2019-07/80, Date: 09.04.2019).

Preparation of Samples

Breast milk samples of 5-10 ml in sterile containers were collected from the participants. The collected samples were frozen immediately and kept at -80°C until the analysis. The samples were gradually defrosted at 4°C while they were protected from light exposure before the analysis, and they were then centrifuged at 3.500 g at 10°C for 10 minutes. The top milk layer that accumulated on the top after the centrifugation step was aspirated with a straw, and the remaining nonfat supernatant was used for the analysis.

Quantitative Analysis of AFM1 in the Samples with ELISA

An AFM1 ELISA (CK-bio-19614; Shanghai Coon Koon Biotech Co., Shanghai, China) test kit based on the antigen-antibody reaction was used for the quantitative analysis of AFM1 in the breast milk samples. All procedures were carried out as instructed by the manufacturer.

50 µl of the standard solution and 50 µl of the samples were added to separate wells of the microtiter plate. The standards and samples were examined in pairs. Then, 50 µl of the biotinylated antigen was added to the wells and incubated in dark at 37°C for 60 minutes. The fluid in the wells was discharged after the incubation process, and the remnant of the fluid was eliminated by turning the plate upside down and lightly tapping it on absorbent paper. The wells were washed with 350 µl washing solution five times. Then, 50 µl of Avidin-HRP was added to the wells and incubated in dark at 37°C for 60 minutes. After the fluid in the wells was drained after the incubation process, the wells were washed five times with 350 µl of washing solution. Then, 50 µl of Chromogen A and 50 µl of Chromogen 2 were added to the wells and incubated in dark at 37°C for 15 minutes. Lastly, 50 µl of the stopping solution was added to the wells. Absorbance was measured at 450 nm in the ELISA plate reader within 15 minutes after the addition of the stopping solution.

AFM1 levels in the breast milk samples were determined in units of µg/L. Quantification in the breast milk samples was performed using the standard curve ranging from 0.1 to 2 µg/L. The lower detection limit was 0.1 µg /L according to the test protocol.

Statistical Analysis

The statistical analysis was conducted using the Statistical Package for the Social Sciences version 21.0 software for Windows (IBM Corp., Armonk, NY, USA). The normality assumptions of the continuous variables were tested with Kolmogorov-Smirnov and Shapiro-Wilk tests. The homogeneity assumption of the variances was tested using Levene's test. The descriptive statistics of the variables are presented as mean∓standard deviation, while their frequencies are presented as n (%). Independent-samples *t*-test and One-Way Analysis of Variance (ANOVA) were used for the univariate analyses of the variables based on the type of the variable and the analysis results of the aforementioned assumptions. Duncan's multiple comparison test was used for the comparisons of groups with statistically differences between them as a result of the ANOVA. Results with a *p*-value lower than 0.05 were considered statistically significant in all statistical analyses.

Results

Basic Information on Participants

The study included 82 mothers. The mean age of the mothers was 27.75∓5.71, almost half of them (43.9%) were secondary school graduates, and the majority (85.4%) were housewives. Most of the mothers did not smoke (79.3%). More than half of the mothers lived in the city (64.6%), 67.1% lived in apartments, 64.6% had central heating, and almost half of them (45.1%) had a place with a south front. Most mothers stated that food did not become moldy in their home environment (91.5%).

The youngest infant of the mothers included in the study was 1 week old, while the oldest was 34 weeks old. The mean age of the infants was 2 weeks. Based on the average infant age, two age groups were formed as <2 weeks and 2+ weeks. The effect of lactation on aflatoxin level was investigated. It was determined that the aflatoxin mean of the <2 week group (2.10∓0.42) was lower than the aflatoxin mean (2.23∓0.59) of the 2+ week group. However, the difference between aflatoxin levels was not statistically significant (p=0.266). When the mothers were asked how many times they breastfed in a day, it was determined that they breastfed 12 times as the mean number of their breastfeeding occasions. The mother with the lowest breastfeeding rate breastfed her infant 2 times a day, while the mother with the highest breastfeeding rate breastfed her infant 20 times a day.

The Presence and Levels of AFM1 in the Breast Milk Samples and Variables Examined for Correlations

In the study, the mean AFM1 concentration in the breast milk samples of the mothers was found as 2.17∓0.52 µg/L. No statistically significant difference was found in the statuses of AFM1 in the breast milk of the mothers based on their ages, places of residence or educational levels. The frequencies of the responses given by the participants to the questionnaire and the comparisons regarding the effects of the information obtained from these responses on the AFM1 values are summarized in Table I. As shown in Table I, milk consumption had a statistically significant effect on the AFM1 levels in breast milk ($p<0.01$). The AFM1 levels in the breast milk samples of the mothers who did not consume milk were lower than those of the other mothers. The AFM1 levels in the breast milk samples of the mothers consuming fabrication milk were lower than the mothers who boiled their milk or purchased homemade milk.

Consuming chili flakes had a significant effect on the AFM1 levels in breast milk ($p<0.01$). Moreover, the AFM1 levels in the breast milk samples of the mothers who consumed chili flakes every day were quite lower than those of the other mothers. The AFM1 levels in the breast milk samples of the mothers consuming chili flakes once or twice a month were higher than the other mothers. The effect of the way the mothers consumed bread on the AFM1 values in their breast milk was statistically significant ($p<0.05$). Accordingly, the aflatoxin levels in the breast milk samples of the mothers consuming homemade or self-made bread were lower.

Discussion

Breast milk is a balanced nutritional source containing nutrients in appropriate amounts according to the needs of each infant and with high biological availability[28]. The American Academy of Pediatrics (AAP) and the World Health Organization (WHO)[3] recommend that infants should be fed with only breast milk for the first six months, supplementary infant foods should be started after the seventh month, and breastfeeding should be continued at least until two years of age for ideal nutrition[3]. This situation has resulted in an increase in the frequency and duration of breastfeeding in many countries, as well as enabling the increasing number of studies conducted on breast milk.

Relevant studies[7,11,18,29] have emphasized the chemical contaminants in breast milk. These chemicals do not naturally exist in breast milk and are introduced to it by the metabolic processes of substances taken into the body in different ways. The transfer of chemical contaminants into breast milk can occur with inhalation or skin contact, while the most common way of transfer is by consuming contaminated food. The concentrations of chemical contaminants that pass into breast milk vary according to the amount and metabolic process of contaminants to which the mother is exposed. The molecular weight, capacity to bind to plasma proteins and lipophilic properties of the chemical are the most important factors changing the amount of transfer[29]. An important group of most common chemical contaminants is mycotoxins. These are mycotoxins that are produced by Aflatoxin, *Aspergillus* and *Penicillium* types of fungi and have potentially detrimental effects on health[11].

Aflatoxin presence in food is significantly determined by the conditions of air humidity and temperatures. Moisture, drying time of a product, ambient relative humidity, fungi, and spore intensity, as well as elevated temperatures, may be listed as some of the factors affecting the

Table I. Descriptive statistics of the variables and comparisons of groups.

	n (%)	AFM1 level µg/L	p
Education Level			
Illiterate	6 (7.3)	1.8∓0.65	0.139
Primary School	17 (20.7)	2.30∓0.65	
Secondary School	36 (43.9)	2.27∓0.46	
University	21 (25.6)	2.02∓0.41	
Postgraduate	2 (2.4)	1.99∓0.37	
Employment Status			
Housewife	70 (85.4)	2.19∓0.52	0.344
Employed	12 (14.6)	2.03∓0.53	
Smoking			
Yes	17 (20.7)	2.23∓0.46	0.591
No	65 (79.3)	2.15∓0.53	
Heating type			
Heating stove	27 (32.9)	2.27∓0.64	0.497
Central heating	53 (64.6)	2.13∓0.45	
Electrical stove, air conditioner, etc.	2 (2.4)	2.06∓0.61	
Place of residence			
Village	9 (11.0)	2.32∓0.66	0.709
Town	2 (2.4)	1.92∓0.13	
District	18 (22.0)	2.06∓0.47	
City	53 (64.6)	2.19∓0.52	
Monthly Income, Turkish Liras			
0-800	3 (3.7)	2.24∓0.46	0.382
801-1,600	11 (13.4)	2.16∓0.81	
1,601-2,400	25 (30.5)	2.33∓0.44	
2,401-3,000	19 (23.2)	2.17∓0.49	
3,000+	22 (26.8)	2.02∓0.46	
Type of housing			
Single house	26 (31.7)	2.16∓0.58	0.852
Apartment	55 (67.1)	2.18∓0.49	
House front			
South	37 (45.1)	2.12∓0.55	0.766
West	6 (7.3)	2.13∓0.27	
East	3 (3.7)	1.79∓0.68	
North	18 (22.0)	2.11∓0.47	
Do you use any herbal mixture/tea, etc. purchased from an herbalist?			
Yes	22 (26.8)	2.20∓0.45	0.764
No	60 (73.2)	2.16∓0.54	
Does your food become moldy in your home environment?			
Yes	6 (7.3)	2.24∓0.47	0.711
No	75 (91.5)	2.16∓0.52	
What do you do with moldy food?			
I throw away all of it	70 (85.4)	2.19∓0.53	0.832
I scrape the moldy part and use the rest	6 (7.3)	2.06∓0.56	
I take the moldy part completely and use the rest	4 (4.9)	2.12∓0.46	
Supplementary infant food			
Yes	13 (15.9)	2.37∓0.60	0.145
No	67 (81.7)	2.13∓0.50	
Are you using herbal products to boost lactation?			
Yes	25 (30.5)	2.34∓0.49	0.059
No	56 (68.3)	2.10∓0.52	
Tomato/Pepper paste storage status			
I do not store these	0 (0.0)	-	0.483
Room temperature	7 (8.5)	2.04∓0.67	
Refrigerator	75 (91.5)	2.18∓0.51	
Spices			
I do not store these	6 (7.3)	2.38∓0.63	0.584
Room temperature	70 (85.4)	2.16∓0.52	
Refrigerator	6 (7.3)	2.12∓0.49	

Table continued

Table I *(continued)*

	n (%)	AFM1 level µg/L	p
Grains			
I do not store these	7 (8.5)	2.17∓0.32	0.829
Room temperature	71 (86.6)	2.16∓0.55	
Refrigerator	4 (4.9)	2.33∓0.15	
Dairy Products			
I do not store these	0 (0.0)	-	-
Room temperature	1 (1.2)	1.76-	
Refrigerator	81 (98.8)	2.18∓0.52	
Dried nuts			
I do not store these	12 (14.6)	2.28∓0.48	0.337
Room temperature	68 (82.9)	2.15∓0.53	
Refrigerator	2 (2.4)	2.09∓0.10	
How often and how do you consume the following food groups?			
Cheese			
How			
Self-made	12 (14.6)	2.18∓0.64	0.197
Homemade	13 (15.9)	2.41∓0.57	
Fabrication	55 (67.1)	2.13∓0.46	
I do not consume it	0 (0.0)	-	
How often			
Every day	53 (64.6)	2.09∓0.54	0.279
Once or twice a week	18 (22.0)	2.29∓0.44	
Three or five times a week	6 (7.3)	2.17∓0.41	
Once or twice a month	4 (4.9)	2.62∓0.57	
Milk			
How			
Self-made	20 (24.4)	2.27∓0.58	**0.022***
Homemade	15 (18.3)	2.46∓0.49	
Fabrication	40 (48.8)	2.05∓0.46	
I do not consume it	6 (7.3)	1.86∓0.50	
How often			
Every day	20 (24.4)	2.04∓0.50	0.273
Once or twice a week	32 (39.0)	2.20∓0.52	
Three or five times a week	11 (13.4)	2.42∓0.54	
Once or twice a month	13 (15.9)	2.21∓0.50	
Yogurt			
How			
Self-made	33 (40.2)	2.22∓0.53	0.575
Homemade	15 (18.3)	2.30∓0.52	
Fabrication	30 (36.6)	2.08∓0.50	
I do not consume it	1 (1.2)	2.28∓-	
How often			
Every day	42 (51.2)	2.06∓0.51	0.284
Once or twice a week	22 (26.8)	2.24∓0.54	
Three or five times a week	12 (14.6)	2.35∓0.50	
Once or twice a month	3 (3.7)	2.35∓0.65	
Chili flakes			
How			
Self-made	8 (9.8)	2.32∓0.39	0.767
Homemade	3 (3.7)	2.31∓0.17	
Fabrication	64 (78.0)	2.15∓0.54	
I do not consume them	5 (6.1)	2.28∓0.46	
How often			
Every day	17 (20.7)	1.87∓0.46	**0.004***
Once or twice a week	25 (30.5)	2.28∓0.53	
Three or five times a week	14 (17.1)	1.96∓0.30	
Once or twice a month	19 (23.2)	2.41∓0.57	

Table continued

Table I *(continued)*

	n (%)	AFM1 level µg/L	p
Isot (A type of indigenous pepper of Şanlıurfa, Turkey)			
How			
Self-made	2 (2.4)	2.36∓0.59	0.907
Homemade	2 (2.4)	2.22∓0.06	
Fabrication	45 (54.9)	2.18∓0.54	
I do not consume it	30 (36.6)	2.12∓0.52	
How often			
Every day	3 (3.7)	1.68∓0.42	**0.036***
Once or twice a week	14 (17.1)	2.42∓0.51	
Three or five times a week	6 (7.3)	2.41∓0.34	
Once or twice a month	28 (34.1)	2.06∓0.51	
Bread			
How			
Self-made	8 (9.8)	1.78∓0.64	**0.046***
Homemade	2 (2.4)	1.69∓0.68	
Fabrication	69 (84.1)	2.24∓0.47	
I do not consume it	1 (1.2)	2.05∓-	
How often			
Every day	78 (95.1)	2.17∓0.53	0.660
Once or twice a week	0 (0.0)	-	
Three or five times a week	2 (2.4)	2.01∓0.38	
Once or twice a month	0 (0.0)	-	
Tomato/Pepper Pastes			
How			
Self-made	15 (18.3)	2.29∓0.58	0.739
Homemade	23 (28.0)	2.21∓0.42	
Fabrication	41 (50.0)	2.12∓0.54	
I do not consume them	1 (1.2)	2.28∓-	
How often			
Every day	59 (72.0)	2.10∓0.53	0.149
Once or twice a week	8 (9.8)	2.26∓0.40	
Three or five times a week	8 (9.8)	2.32∓0.55	
Once or twice a month	5 (6.1)	2.60∓0.49	
Meat and meat products			
How			
Self-made	9 (11.0)	2.33∓0.65	0.568
Homemade	7 (8.5)	2.12∓0.56	
Fabrication	61 (74.4)	2.16∓0.50	
I do not consume them	2 (2.4)	2.56∓0.24	
How often			
Every day	11 (13.4)	2.13∓0.44	0.172
Once or twice a week	41 (50.0)	2.14∓0.47	
Three or five times a week	18 (22.0)	2.07∓0.59	
Once or twice a month	9 (11.0)	2.53∓0.67	
Dried fruits/vegetables			
How			
Self-made	28 (34.1)	2.23∓0.54	0.545
Homemade	15 (18.3)	2.14∓0.54	
Fabrication	30 (36.6)	2.19∓0.52	
I do not consume them	8 (9.8)	1.93∓0.40	
How often			
Every day	9 (11.0)	2.25∓0.48	0.462
Once or twice a week	30 (36.6)	2.13∓0.54	
Three or five times a week	17 (20.7)	2.21∓0.41	
Once or twice a month	13 (15.9)	2.41∓0.64	
Grains			
How			
Self-made	5 (6.1)	1.76∓0.48	0.100
Homemade	9 (11.0)	1.92∓0.46	

Table continued

Table I ***(continued)***

	n (%)	AFM1 level µg/L	p
Fabrication	65 (79.3)	2.23∓0.52	
I do not consume them	2 (2.4)	2.35∓0.21	
How often			
Every day	8 (9.8)	2.16∓0.47	0.670
Once or twice a week	45 (54.9)	2.17∓0.55	
Three or five times a week	18 (9.8)	2.13∓0.43	
Once or twice a month	8 (9.8)	2.39∓0.56	
Dried nuts			
How			
Self-made	0 (0.0)	-	0.245
Homemade	0 (0.0)	-	
Fabrication	78 (95.1)	2.17∓0.51	
I do not consume them	2 (2.4)	2.60∓0.14	
How often			
Every day	9 (11.0)	2.10∓0.43	0.828
Once or twice a week	31 (37.8)	2.13∓0.56	
Three or five times a week	13 (15.9)	2.09∓0.35	
Once or twice a month	26 (31.7)	2.24∓0.59	
Spices			
How			
Self-made	4 (4.9)	2.23∓0.80	0.724
Homemade	4 (4.9)	2.24∓0.34	
Fabrication	68 (82.9)	2.14∓0.53	
I do not consume them	5 (6.1)	2.41∓0.35	
How often			
Every day	29 (35.4)	2.08∓0.49	0.405
Once or twice a week	22 (26.8)	2.27∓0.45	
Three or five times a week	14 (17.1)	2.06∓0.55	
Once or twice a month	12 (14.6)	2.33∓0.61	

N=82. The lower detection limit of the AFM1 test kit was 0.1 µg /L. *Significant difference between groups at $p \leq .05$.

reproduction of aflatoxins[30]. Climate conditions are the most critical characteristic that influences the availability of AFM1 in the mother's milk according to several researchers[21,31]. The summer season is hot and dry, while the winter season is cold and rainy in Kırşehir, Turkey. This study found that place of residence, heating state and the front of the house did not cause a significant difference in the aflatoxin values of the samples collected for this study.

Diet is an important factor for aflatoxin exposure because of mycotoxins' biochemical reactions and the potential of these toxins to be transferred into breast milk by accumulating in adipose tissue. Some researchers[27,32,33] have claimed that no correlation exists between the mother's diet and the concentration of AFM1 in her milk. Some studies[18,22,31] showed that peanut, red pepper, dried fig and various dehydrated goods, as well as grains and dairy goods, have a high risk of containing aflatoxin. Dried fruits and spices pose the risk of containing aflatoxins that might originate from poor dehydration and/or improper preservation quality. Mycotoxins might reproduce inside fruits at any stage before, during and after harvest or during drying, packaging and storage processes. A high sugar content, the harvesting technique and drying conditions in dried fruits enable mold growth and growth of aflatoxins[34,35]. This study found that milk consumption had a statistically significant effect on the aflatoxin levels in breast milk. The aflatoxin levels in the breast milk samples of the mothers who did not consume milk were lower than those of the other mothers. The aflatoxin levels in the breast milk samples of the mothers consuming fabrication milk were lower than the mothers who produced their milk or consumed homemade milk. The way the mothers consumed bread had a significant effect on the aflatoxin values of their milk samples. Accordingly, the aflatoxin values in the breast milk samples of the mothers consuming homemade or self-made bread were lower. This study found that consuming chili flakes had a significant effect on the aflatoxin values in breast milk. The aflatoxin values in the breast milk samples of the mothers who consumed chili flakes every day were quite lower than those of the other mothers. The aflatoxin values in the breast milk samples of the mothers consuming chili flakes once or twice a month were higher than those in the other mothers. It may be considered that not storing chili flakes under suitable conditions had a role in this. Since individuals who regularly consume chili flakes do not store these foods at home for a long time, their aflatoxin values might have been low.

There are studies[23,32,36] reporting no link between AFM1-contaminated mother's milk and sociodemographic characteristics like maternal education level, workplace or the location of the mother's residence. This study found that sociodemographic factors such as education level and workplace had no significant effect on the aflatoxin levels. However, it was found that living in the village or city affected aflatoxin values, although the difference was statistically insignificant. Other studies[37,38] have suggested a correlation between maternal education level and profession and AFM1 contamination in the mother's milk. Some studies[21,37] have shown a negative correlation between maternal educational levels and AFM1 contamination. The authors suggested that sociodemographic characteristics affect nutritional habits, thus affecting the possibility of AFM1 contamination in the mother's milk.

The present study was limited by the number of samples and inability to compare the AFM1 levels in the breast milk of mothers living in different geographical regions.

Conclusions

The results obtained in this study indicated that it is necessary for healthcare providers to educate pregnant women and breastfeeding mothers about how to choose lower-risk foods to protect infants from the harmful effects of AFM1. Additionally, such education curricula must draw attention to consuming fresh and ripe food, paying attention to product labels and best before dates and proper food storage, as well as informing readers about the process of foods getting stale and the risks of feeding rotten food to pets. Public announcements explaining the dangers of consuming rotten food and the importance of storing food under suitable conditions will also be beneficial. The suitability of storage rooms, temperature, humidity, light and other conditions to the food in question is also important. Long-term studies conducted with more participants will make more detailed contributions in terms of the scientific literature on the existence of mycotoxins in human milk and assist in identifying several factors playing a part in mycotoxin production.

Conflict of Interest

The authors have no conflicts of interest to declare.

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Authors' Contribution

Conceptualization, E. Ünlü; methodology, E. Ünlü, G. Turna Saltoğlu, G. Ünlü and H. Tozak Yıldız; validation, G. Turna Saltoğlu, G. Ünlü and H. Tozak Yıldız;; formal analysis, E. Ünlü , G. Turna Saltoğlu, G. Ünlü and H. Tozak Yıldız; investigation, E. Ünlü , G. Turna Saltoğlu, G. Ünlü and H. Tozak Yıldız; data curation, E. Ünlü , G. Turna Saltoğlu, G. Ünlü and H. Tozak Yıldız; writing-original draft preparation: G. Turna Saltoğlu, G. Ünlü and H. Tozak Yıldız; writing-review and editing: G. Ünlü, H. Tozak Yıldız; visualization, E. Ünlü, G. Turna Saltoğlu; supervision, E. Ünlü, G. Turna Saltoğlu. All authors have read and agreed to the published version of the manuscript.

Ethics Approval

The study was approved by the Kırşehir Ahi Evran University Faculty of Medicine Clinical Studies Ethics Committee before the study was conducted (Decision No: 2019-07/80, Date: 09.04.2019).

Informed Consent

The contents of a voluntary consent form were read to the mothers included in the study, and those who agreed to participate signed the form.

Data Availability Statement

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