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Is Aflatoxin More in the Milk of Lactating Mothers Who Have Previously Had COVID-19?

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ABSTRACT

Background: Breast milk provides the ideal nutrition for infants. It has a nearly perfect mix of vitamins, protein, and fat. Breastfeeding has many health benefits for both the mother and infant. Breast milk contains all the nutrients an infant needs in the first six months of life. The present study aimed to measure aflatoxin M1 (AFM1) levels in breast milk and identify nutritional and socio-demographic factors associated with AFM1 levels. **Methods:** This cross-sectional study was conducted to assess AFM1 levels in the breast milk of 100 mothers by enzyme-linked immunosorbent assay (ELISA), 20 of whom had COVID-19 in the past 4 months during June 2020-March 2021. The participants were given a questionnaire to answer nutritional and socio-demographic questions, such as age, place of residence, COVID-19, educational status, employment status, and body mass index (BMI). **Results:** The mean age of mothers was 29.1 ± 4.9 years. Based on the results, 39/100 samples had AFM1 more than 5 ng/l, and all 20 mothers who previously had COVID-19 had AFM1 in their milk. The mean AFM1 was 7.10 ng/l and ranged 5.37–9.01 ng/l. Consumption of milk and yogurt also significantly increased AFM1 in milk. Moreover, place of residence and BMI and COVID-19 had a statistically significant effect on the presence of AFM1. **Conclusion**: The AFM1 contamination was significantly associated with the consumption of milk and yogurt by the lactating mother. Breastfeeding mothers should be careful in their food intake and try to fit their BMI. The presence of aflatoxin in the lactating mothers who previously had COVID-19 indicates that their children are more vulnerable.

Keywords: *Aflatoxin M1; Mycotoxins; Breast milk; COVID-19*

Introduction

Mycotoxins are one of the most important chemical contaminants that can be transferred from the maternal diet to breast milk. These chemicals have carcinogenic, genotoxic, teratogenic, haemorrhages, hepatotoxic, nephrotoxic, estrogenic, immunosuppressive, and even immediate death (Safavizadeh *et al.*, 2020a, Safavizadeh *et al.*, 2020b). Aflatoxins, which are secondary metabolites, generally produced by fungi aspergillus (A. Flavus, A. Parasiticus, and A. Nomius), are one of the most widespread mycotoxins. Aflatoxins usually

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contain six compounds, including aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1, aflatoxin G2, aflatoxin M1 (AFM1), and aflatoxin M2 (AFM2) (Kumar *et al.*, 2017). diagnosed as AFM in milk 12 to 24 h later.

When an AFB-contaminated diet is used during human and animal lactation, the toxin is metabolized and is detected as AFM in the milk 12 to 24 hours later. AFM1 is ten times less carcinogenic than AFB1and is excreted in the urine and breast milk (Peles *et al.*, 2019, Prandini *et al.*, 2009). The international agency for research on cancer (IARC) organization suggested that AFM1 should be classified to be a group I carcinogen. The European commission Announced that the maximum level of AFM1 in raw milk and dairy products must not exceed 50 ng/kg, and its permissible limit for infant food is 25 ng/kg. Aflatoxin levels in an environment are under the influence of climate, geographical conditions, and humidity (Agriopoulou *et al.*, 2020). Proper infant nutrition is needed to achieve healthy growth and development. Breastfeeding is highly recommended for infant because it has many usefulness for both mother and baby. Breastfeeding is very recommended for infant nutrition it is very effective (Prell and Koletzko, 2016). The recommendation of national and international organizations is that infants should be exclusively breastfed for the first 4-6 months and should not even be given water to complete their feeding (World Health Organization, 2003). However, substances that are transferred to breast milk due to the mother's exposure to certain chemical contaminants can negatively affect the infant's health (Landrigan *et al.*, 2002). Worldwide studies to determine the prevalence of AFM1 in human milk showed that 2% of breast milk in brazil (Ishikawa *et al.*, 2016), 82% in Nigeria (Salami, 2006), 100% in eastern Iran (Sadeghi *et al.*, 2009), 35% in Serbia (Radonić *et al.*, 2017), and 89% of breast milk in Mexico (Cantú-Cornelio *et al.*, 2016) , have been contaminated with AFM1. In newborn infants, factors, such as underweight, high metabolic rates, lack of development of sufficient detoxification mechanisms, and their underdeveloped organs and tissues put them at increased risk of AFM1 exposure compared to adults (Lombard, 2014). Given that an infant has a lower capacity for carcinogen biotransformation, toxic substances can stay in their bodies for a longer time (Ghiasian and Maghsood, 2012). If there is an unauthorized amount of aflatoxin in breast milk, these people will be more vulnerable to COVID-19 (Kostoff *et al.*, 2020) . People with fungal and mycotoxin diseases have more severe symptoms of COVID-19 than others. To the best of the authors' knowledge, the present survey is the first to peruse the presence of AFM1 in breast milk in Tehran. Breastmilk has not been proven to protect infants from SARS-COV-2, the virus that causes COVID-19. Mycotoxins that contribute to a degraded/dysfunctional immune system are pervasive; they contribute to myriad (especially chronic) diseases. Decreased ability of the human immune system increases the risk of COVID-19 (Kostoff *et al.*, 2020) **.**

This study aimed to measure AFM1 levels in breast milk and identify nutritional and socio-demographic factors associated with these levels.

Materials and Methods

Study design and participants: This cross-sectional study was conducted to determinate AFM1 levels in milk samples of 100 breastfeeding women. The inclusion criteria consisted of women who delivered their infants in hospitals under the auspices of the Islamic Azad University of Tehran and women who were infected by COVID-19 during the past 4 months. The exclusion criterion was having an underlying disease. Milk samples were collected during June 2020-March 2021 by simple random sampling. The subjects were questioned by a physician at the time of the sample donation. They were asked to complete a questionnaire about medical history, living conditions, and dietary habits. This questionnaire was based on the World Health Organization (WHO) guidelines and were translated to Persian

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language by certified translators (Elaridi *et al.*, 2017).

Sample preparation: Hand expression or manual breast pump was used to collect 15-20 ml of breast milk samples into sterile plastic milk containers during regular feeding of infants. Breast milk samples were transported on ice in an icebox to the laboratory, were stored at -20°C, and protected from light until analysis.

Determination of AFM1: The technique used in this study was based on an international standard developed for cow's milk which was validated for mother's breast milk samples (Martin *et al.*, 2016). Milk samples were warmed to 37°C. Vertex was given to homogenize the breast milk samples. Five ml breast milk was transferred into centrifuge tubes and centrifuged for 10 min at 3,500 g. After the centrifugation process, the top layer was extracted with a Pasteur pipette, and the remaining supernatant (separated milk) was skimmed to be used for testing. In this test, AFM1 ELISA kit (Germany, Biopharm-R) was used for measurement. Wells were coated with antibodies directed against anti-AFM1 antibodies, and then anti-AFM1 antibodies, standards or sample solutions, enzyme conjugate, and substrate chromogen were added. A standard curve for AFM1 was constructed with six points (0, 5, 10, 20, 40, and 80 ng/l). The detection limit of the test kit was 5 ng/l. Accordingly, samples less than 5 ng/l were considered to be a negative finding for the presence of AFM1.

Ethic considerations: All studies were conducted under the supervision of the ethics committee of the Islamic Azad University.

Data analysis: Comparison between AFM1 content in milk and each of the factors was evaluated by SPSS Version 25.0 for Windows. The P-value < 0.05 was considered to be statistically significant.

Results

The mean age of the volunteer mothers was 29.1 ± 4.9 years (ranged 19-44 years). The socio-demographic characteristics of the participants based on AFM1 levels in breast milk samples are summarized in **Table 1**. No sample was above the EU limit (25 ng/l).

AFM1 level was upper the detection limit in 39% of the samples. Among those samples, the mean level of AFM1 was 7.10 ng/l (ranged 5.37–9.01 ng/l). There was no statistically significant difference between the AFM1 groups in mothers' ages, educational status or employment status ($P > 0.05$). However, there was no significant difference between the two groups regarding place of residence. A larger percentage of mothers who were living in Tehran had detectable levels of AFM1 in their breast milk (31%) than mothers who were living in suburbs.There was also a significant difference in body mass index (BMI) between the groups. There is no statistically significant relationship observed between the number of previous months and aflatoxin. AFM1 was present in the breast milk of all mothers who previously had COVID-19.

Table 2 summarizes nutritional risk factors with AFM1. No statistically significant difference was observed in the presence of AFM1 in breast milk compared to intake of white cheeses, bread, meat and meat products, eggs, nuts, and seeds. A significantly higher percentage of mothers who consumed milk and yogurt had detectable levels of AFM1 in their breast milk compared to mothers who did not consume milk and yogurt.

Table 1. Socio-demographic characteristics of the participants based on aflatoxin M1 levels in breast milk .

Socio-demographic characteristics	Aflatoxin M1 level		P-value[a]
	≥ 5 ng/l, (n=39)	< 5 ng/l, (n=61)	
Age (year)			
19-30	18 (33.9)[b]	35 (66.1)	0.32
31-44	21 (44.6)	26 (55.4)	
Place of residence			
Tehran City	31(43.6)	41 (56.4)	0.048
the suburbs	8 (28.5)	20 (71.5)	
Having COVID-19 during the past 4 months			
Yes	20 (100)	0 (0.0)	0.026
No	19 (23.7)	61 (76.3)	
Educational status			
No academic degree	2 (33.3)	4 (66.7)	0.06
Academic degree	37 (40.2)	55 (59.8)	
Employment status			
Housewife	19 (43.1)	25 (56.9)	0.09
Employed	20 (35.7)	36 (64.3)	
Body mass index (kg/m^2)			
≤18.5	0 (0.0)	0 0.0)	
18.5 – 24.9	3 (5.7)	49 (94.3)	0.03
25.0 – 29.9	11 (47.8)	12 (52.2)	
≥30.0	25 (100)	0 (0.0	

[a]: Chi-square test; [b]: N (%)

Table 2. Nutritional risk factors of the participants based on aflatoxin M1 levels in breast milk.

Nutritional risk factor	Aflatoxin M1 level		P-value[a]
	≥ 5 ng/l, (n=39)	<5 ng/l, (n=61)	
Milk			
1–2 times/week	9 (15.2)	50 (84.8)	0.02
>2 times/week	30 (73.1)	11 (26.9)	
White cheese			
1–2 times/week	2 (28.5)	5 (71.5)	0.08
>2 times/week	37	55	
Bread, pasta			
1–2 times/week	0 (0.0)	1 (100)	0.1
>2 times/week	39 (39.3)	60 (60.7)	
Cereal, rice			
1–2 times/week	1 (33.3)	2 (66.7)	0.19
>2 times/week	37 (39.7)	59 (60.3)	
Meat and meat products			
1–2 times/week	19 (31.6)	41 (68.4)	0.31
>2 times/week	20 (50.0	20 (50.0)	
Eggs			
1–2 times/week	14 (32.5)	29 (67.5)	0.73
>2 times/week	25 (43.8)	32 (56.2)	
Yogurt			
1–2 times/week	29 (42.0)	40 (58.0)	0.01
>2 times/week	10 (32.2)	21 (67.8)	
Nuts and seeds			
1–2 times/week	35 (45.5)	42 (54.6)	0.2
>2 times/week	4 (17.3)	19 (82.7)	

[a]: Chi-square test; [b]: N (%)

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Discussion

One potential source of AFM1 in breast milk is the use of contaminated dairy products by lactating mother. Higher AFB1 concentrations are expected in animal feed, then, more AFM1 is disposed into the animal milk and carried into derived dairy products. In previous studies, the attendance of AFM1 in breast milk was significantly related to the educational level of the lactating mother. Mothers with lower educational levels may have a lower socioeconomic status and then are more likely to consume food contaminated with AFM1, since higher quality products tend to be more expensive (Elaridi *et al.*, 2017), which is not in line with the present study. Analysis of AFM1 concentrations in breast milk and consumption of various food groups indicated that the consumption of milk and yogurt was positively correlated with AFM1 contamination. A study conducted in Ilam, Iran, indicated no significant associations between consumingmilk and dairy products, meat, fish, legumes, grain products, fruits, and nuts with the concentration of AFM1 in women's breast milk (Maleki *et al.*, 2015). In a study conducted in Turkey, housewife mothers who lived in wet and dank houses, or ate spices or dried fruits and vegetables had a significantly greater prevalence of AFM1 in their breast milk compared to those who had jobs, did not report moisture or mildew in the home, or did not eat spices or dried fruits and vegetables (Karayağiz Muslu and Özdemir, 2020). Research in Mexico showed that AFM1 level was significantly associated with consuming egg, cola, drink, and sunflower oil (Cantú-Cornelio *et al.*, 2016) . A study conducted in Hamedan, Iran, reported that there was no significant association between food and aflatoxin levels in milk (Samiee *et al.*, 2020). The results of a study in Shahrekord, Iran, showed that consuming bread, rice, and non-alcoholic beer beverage significantly increased the risk of AFM1 incidence in breast milk (Jafari *et al.*, 2017).

Recent studies have examined the harmful impacts of toxic lifestyle, iatrogenic, biotoxic, environmental/ occupational, and psychosocial/socioeconomic factors on the health of the immune system directly or indirectly. Depending the method of accumulating the results, there were +1000–2000 factors that can degrade the immune system, and the number was viewed as a gross underestimate. Some of the factors in the present study included lifestyle, iatrogenic, mycotoxins, environmental, psychosocial/ socioeconomic factors. These factors reduce the level of the immune system, and ultimately, increase the risk of developing COVID-19 (Kostoff *et al.*, 2020) .

In this study, it was found that higher levels of aflatoxin were reported in the breast milk of mothers who gave birth in Tehran than in the suburbs. Moreover, the higher the BMI of lactating mothers, the higher the aflatoxin level. The level of aflatoxin in the breast milk of mothers with more BMI was higher. These results are consistent with a study in Egypt (Polychronaki *et al.*, 2006). Aflatoxin was present in all lactating mothers who previously had COVID-19.

Consumption of milk and yogurt is one of the sources of aflatoxin according to previous studies and causes this substance to appear in the breast milk of mothers who have consumed contaminated milk and yogurt. In this study, it was found in 39% of lactating mothers, which is in line with other studies (Iqbal and Asi, 2013, Issazadeh *et al.*, 2012, Safavizadeh *et al.*, 2020a).

Conclusion

This study showed that place of residence and BMI affect the level of aflatoxin in breast milk, which is due to the pollution in Tehran compared to its suburbs and also the adipose tissue of women with high BMI. The relationship between COVID-19 and aflatoxin has not been studied in any article and for the first time it was found that aflatoxin was higher in breast milk of mothers who previously had COVID-19. Mothers who consumed more milk and yogurt in the week had more aflatoxins in their milk, which can be very worrying. The results of this study were consistent with other studies and showed that consumption of foods, especially contaminated dairy products, increases breast milk aflatoxins. The present report on AFM1 concentrations in breast milk is the first of its kind in Tehran. A high prevalence of AFM1

(39%) was found with a range of 5.37–9.01 ng/l in 100 collected breastmilk samples. The AFM1 contamination was significantly associated with the consumption of milk and yogurt by the lactating mother. Given that none of the samples exceeded the 25 ng/l of EU limit, more studies are warranted to survey AFB1 contamination in various food products in Iran. Further studies with larger samples will help examine the mycotoxins in breast milk and help identify many of the factors that contribute to their growth. Increasing knowledge about mycotoxins and enforcing suitable governmental control and ratification mechanisms would reduce mycotoxin contamination.

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Conflict of interest

The author declares that there is no conflict of interest.

Author' contributions

The experiments and writing the manuscript were done by Sepideh Seifi.

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