Kafkas Üniversitesi
Fen Bilimleri Enstitüsü Dergisi
Cilt 13, Sayı 2, 67-75, 2020

Kafkas University
Institute of Natural and Applied Science Journal
Volume 13, Issue 2, 67-75, 2020

Original Article

Investigation of the Relationship Between Blood Lipid Peroxidation and the Prevalence of Aflatoxin M1 in Milk Samples from Mothers and Cows Living in Kars and Surrounding Villages

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(İlk Gönderim / Received: 21. 06. 2020, Kabul / Accepted: 31. 12.2020, Online Yayın / Published Online: 31. 12. 2020)

Keywords:
Aflatoxin,
dairy product,
exposure,
aflatoxin metabolism.

Abstract: Aflatoxins are highly toxic secondary metabolites produced by molds. They cause various deformations in living systems. One of these is lipid peroxidation in mammals. Aflatoxins in milk are one of the most important factors that can increase lipid peroxidation in living life. Infants and children fed with milk and dairy products containing aflatoxin M1 (AMF1) have a greater effect.

In this study, aflatoxin levels were determined in 80 healthy cows and 80 breast milk samples from the same region, and the relationship between feeding patterns of mothers fed with these milk and their effects on blood lipid peroxidation were tried to be determined. Immunoassay kit (Ridascreen, Riedel-de Haen cat no. R1121 R-Biopharm GmbH, Darmstadt, Germany), a competitive enzyme, was used to determine AFM1 levels. Lipid peroxidation levels and AFM1 correlations were demonstrated in blood samples of mothers infected with AFM1. Samples were analyzed in 4 stages (collecting literature material, experimental applications, statistical analysis and report writing stage) in 24 months.

According to the results obtained in this study, it can be said that good agricultural practices should be adopted in order to maintain and maintain official control and implementation mechanisms at all stages from milk production to consumption, where aflatoxins in milk and dairy products are one of the biggest threats to public health.

Kars ve Çevre Köylerde Yaşayan Anne ve İneklerden alınan Süt örneklerinde Kan Lipit Peroksidasyonu ile Aflatoksin M1 Prevalansı Arasındaki İlişkinin Araştırılması

Anahtar Kelimeler:
Aflatoksin,
aflatoksin metabolizması,
serbest radikaller,
Antioksidan enzimler.

Özet: Aflatoksinler küfler tarafından üretilen yüksek düzeyde toksik sekonder metabolitlerdir. Canlı sistemlerde çeşitli deformasyonlara neden olurlar. Bunlardan biri, memelilerde lipid peroksidasyonudur. Sütteki aflatoksinler, canlı yaşamında lipid peroksidasyonunu artırabilen en önemli faktörlerden biridir. Aflatoksin M1(AMF1) içeren süt ve süt ürünleri ile beslenen bebek ve çocuklarda etkisi daha büyüktür.

Bu çalışmada, aynı bölgeden sağlıklı 80 inek ve 80 anne sütü numunesinde aflatoksin seviyesi belirlenerek, bu sütlerle beslenen annelerin beslenme şekilleri arasındaki ilişki ve kan lipid peroksidasyonuna etkileri belirlenmeye çalışıldı. AFM1 seviyelerini belirlemek için rekabetçi bir enzim olan immunoassay kiti (Ridascreen, Riedel-de Haen kedi no: R1121 R-Biopharm GmbH, Darmstadt, Almanya) kullanıldı. Sütleri AFM1 içeren annelerin kan örneklerinde lipit peroksidasyon düzeyleri ile AFM1 ilişkileri ortaya kondu. Örnekler 24 ayda 4 aşamada (literatür materyali toplama, deneysel uygulamalar, istatistiksel analizler ve rapor yazma aşaması) analiz edildi.

Bu çalışmada elde edilen sonuçlara göre süt ve süt ürünlerindeki aflatoksinler, halk sağlığını tehdit eden en büyük unsurlardan biri olduğu süt üretiminden tüketimine kadar her aşamada, resmi kontrol ve uygulama mekanizmalarının sağlanması ve sürdürülmesi için iyi tarım uygulamalarının benimsenmesi gerektiği söylenebilir.

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1. INTRODUCTION

The formation of aflatoxins is closely related to the development of molds Aflatoxins are secondary metabolites and are produced by *Aspergillus flavus* (*Asp. Flavus*) and *Aspergillus parasiticus (Asp. Parasiticus)*. (Jamali et al., 2012, Bennett, 2010; Iqbal et al., 2013).

This mycotoxin group, which has a toxic effect on the liver, is found in many cereal products due to improper storage, especially in cereal products, and in edible tissues of animals consuming contaminated cereal products. Milk and milk products containing aflatoxin residues; affects children who are more sensitive than adults. Therefore, aflatoxins in milk and dairy products consumed in large amounts constitute one of the important problems that threaten public health (Bennett, 2010; Coppock et al.2012;. Pitt, 2014).

When aflatoxin B1 and G1 are taken by the animals in the lactation period, a small portion (1-2%) of the aflatoxin M1 (AFM1) and M2 (AFM2) are excreted with milk. Aflatoxin M1 is 4-hydroxy aflatoxin B1, aflatoxin M2 is a derivative of 4-hydroxy B2. AFM1 is the toxic metabolite of AFB1, AFM2 is the hydroxylated form of AFB2 (Coppock et al.2012; Kalantari and Kalantari, 2007; Pitt, 2014).

This mycotoxin group, which is particularly toxic to liver, is found in many contaminated with products in poor storage conditions after harvesting of nutrients such as straw, grain, legumes, nuts, and peanuts. Therefore, if AFM1 residues are found in the most commonly consumed milk, this poses a potential risk to public health. Because aflatoxins cannot be completely destroyed from foods and feeds, It is emphasized by the relevant experts that measures should be taken by countries to limit the intake of aflatoxins via diet to minimize potential risks to health and to limit the consumption of aflatoxin containing foods at levels that can not be reduced. This is common in regions where the winter months are long. (Kamkar, 2008; Mulunda et al.,2013; Rahimi et al., 2009). The distribution of AFM1 in the milk of the animal fed with contaminated food is not homogeneous in milk (Ayyildiz, 2012; Galvano et al., 1996).

For babies' health, it is recommended that all infants receive breast milk during the first 6 months. Therefore, it is important to identify unwanted toxins and metabolites that can be found in human milk and pass on to the baby. Studies about the content of mycotoxins in the human milk are limited and show differences between countries.

Although aflatoxin B1 (AFB1) is a potent hepatotoxic and hepatocarcinogenic mycotoxin, the mechanism of cellular damage is not fully explained. A linear relationship between aflatoxin B1 and the amount of aflatoxin M1 in milk was reported (Kamkar et al., 2011; Tavakoli et al., 2013).

The level of malondialdehyde (MDA), which is an end product lipid peroxidation, is an indirect indicator of injury induced by reactive oxygen species (ROS). The most common radical damage in the organism is lipid peroxidation. In the cell membrane, oil (L.) radicals are formed by the emergence of a hydrogen from the fatty acids, and eventually the aldehydes, which are the cytotoxic products, form hydrocarbon gases such as pentane. Malonaldehyde, which is the most recent step of aldehydes from these toxic products, is used to determine lipid peroxidation. (Freeman and Crapo, 1981).

Mycotoxin, ochratoxin, zearalenone and deoxinivalenol levels were within different limits in previous studies. Variable findings on human milk aflatoxin levels indicate geographical differences in dietary aflatoxin exposure of nursing women, possibly depending on dietary habits. Breastfeeding is a necessary food for babies. For this reason, mothers need to stay away from aflatoxin as much as possible while feeding.

Aflatoxins are a group of mycotoxins having mutagenic, carcinogenic and immunosuppressive properties. Aflatoxin M1 (AFM1) is the main hepatic carcinogenic metabolite of Aflatoxin B1 (AFB1). Aflatoksin M_1 (AFM_1), Aflatoksin B_1 (AFB_1)' in başlıca hepatik karsinojenik metabolitidir. Feeding of milk-giving animals and mothers with food contaminated with AFB1, this toxin passes into milk as 1-6% AFM1. The presence of AFM1 in dairy products has proven to be a potential risk for human health, particularly for infants and children. Aflatoxins undergo metabolic changes in the liver and become toxic after being converted to various epoxide derivatives. These derivatives, which are responsible for toxic and carcinogenic effects, affect liver cells at the molecular level. It disrupts protein synthesis by inhibiting DNA and RNA synthesis, causing carcinogenic, genotoxigenic, teratogenic, nephrotoxigenic, hepatotoxigenic, reproductive diseases, immunosuppressive effects, mental retardation and development disorders. The short life of the pasture during the vegetation period of Kars and the cold and rainy period of the rest of the year brings the necessity of keeping the animal foods closed. Negative environmental conditions (humidity, heat, ventilation etc.) accelerates the formation of aflatoxins and passes directly to humans and animals in contact with these nutrients. It is inevitable that the aflakoxins that reach the mother and children fed the milk fed with these nutrients bring with them many negative health problems. In our country, very few studies have been found on AFM1 in breast milk and cow's milk. Therefore, a new and comprehensive study is needed. In the study, AFM1 ratio was determined in milk samples in the region. The relationship between lipid peroxidation and blood samples was determined in determining the transmission and protection methods of AFM1. By demonstrating the relationship between AFM1 and peroxidation, a new model for direct and carcinogenic effects of toxins will be contributed (Kamkar et al., 2011; Tavakoli et al., 2013; Freeman and Crapo, 1981).

Thus, problems that may occur due to toxins in milk and other foods have been identified, preventive measures have been taken, health and economic problems will be reduced.

Different AFM1 contamination levels were determined in many foods consumed in our country. Turkey also AFM 1 levels in food are important for biological metabolites and it is important that the specified standards. Prevention of aflatoxin contamination can be achieved by strict control over agricultural, production and storage conditions. It would also be helpful to disseminate the study to a large scale by taking into account the dimensions of rural and urban regions.

The aim of this study is to determine the presence of aflatoxins in milk and dairy products at all stages from milk production to consumption, draw attention to risk limits. In addition, blood samples were taken and lipid peroxidation levels were determined and their relationship with AFM1 was tried to be revealed.

2. MATERIAL AND METHODS

All experimental applications in the study were performed in the research laboratories of the Faculty of Veterinary Medicine, University of Kafkas and Faculty of Science.

Sampling

Fifteen milliliters of milk samples were taken using sterile milking machines from 80 breastfeeding mothers whose volunteering certificates were obtained and consuming cow's milk daily, and from 80 cows in the regions where the mothers lived. The collected milk was brought to the laboratory with a -4 oC cooler until the experiment day. The samples were frozen within one day and kept at -20 ° C until

analysis for aflatoxins and heavy metals. Blood samples taken into heparinized vacuuming tubes were divided into plasma and red blood cells by sniffering. The plasma was frozen (−20 ˚C) and stored until further analysis. RBC samples were washed three times with 0.9% NaCl and held for 20 hours at 20 ºC until analysis time.

Quantitative Analysis of AFM1 Concentration

Immunoanalytic german kit was used to measure the AFM1 levels in the milk taken in the designated regions (Ridascreen, Riedel-de Haen cat no: R1121 R-Biopharm GmbH, Darmstadt, Germany). Samples were centrifuged at 3500 g for 10 minutes and their oil-free sub-phases were taken and incubated in the dark for 30 minutes at room temperature (RT). After removing the supernatant, it was washed twice with 250 µl wash buffer. In the next step, 100 µl of peroxidase conjugate AFM1 was added to each sample and allowed to darken for 15 minutes. Then 1000 µl of substrate / cromogen was added. After waiting for fifteen minutes, the reagent was measured at 450 nm in ELISA after addition and shaking.

Measurement was performed by assuming the 0.5, 10, 20, 40 v3 80 ng / l (ppt) AFM1 standard provided by the kit. The AFM1 concentrations of samples were evaluated by using RIDASOFTVIN program, provided by R-Biopharm.

Analytical procedures

Lipid peroxidation was determined by measuring the amount of thiobarbituric acid-reacting substance (TBARS) in plasma according to the method of Placer (1966) et al.

The values of MDA reactive material were expressed in terms of TBARS (nmol/ml plasma).

Statistical Analysis:

In the analyzes made using the SPSS software program, P values below 0.05 were considered statistically significant.

3. RESULTS

AFM1 values and antioxidant relationships between lipid peroxidation showed differences in the study conducted with 80 cows in the same region and milk samples taken from 80 mothers fed daily cow milk in the same region.

Accordingly, the distribution of aflatoxin M1 in cow milk samples (Table.1), the distribution of aflatoxin M1 in breast milk samples (Table 2), and descriptive statistics for breast milk and cow milk samples are given in the results (Table 3). In addition, lipid peroxidation levels were determined in the examination of the blood samples taken (Table 4).

Among the investigated breast milk from volunteers, 40 out of 80 samples (58.75 %) were found to be contaminated with AFM1 with the range of 0.00 and 17.86 ng/l. In breast milk, the AFM1 milk sample was 55% while the average value was 3.87 ng / kg. MDA levels in the mother blood were found to be 5.20 1.41 nmol/ml plasma.

Table 1. Aflatoxin M1 distribution in cow milk samples

Samples	**AFM_1 (ng/kg)**	**Rate (%)**	**Total (%)**	**Min-Max (ng/kg)**
33	0.00	41,25	41,25	0.00-17.86
7	1.00-3.90	8,75	58,75	
17	4.00-6.90	21,25		
5	7.00-9.90	6,25		
8	10.00-12.90	10,0		
4	13.00-15.90	5,0		
6	16.00-18.90	7,5		
80(Total)		100.0		

Table 2. Aflatoxin M1 distribution in breast milk samples

Saples	**AFM1 (ng/kg)**	**(%)**	**(%)**	**Min-Max (ng/kg)**
36	0.00	45	45	0.00-6.68
28	1.00-3.90	35	55	
16	4.00-6.90	20		
80 (Total)		100.0	100.0	

Table 3. Descriptive statistics and test results for breast milk and cow milk samples

Samples	**n**	$\bar{x}$ **AFM_1(ng/kg)**	**SH**	**95%CI**	**t**	**p**
Breast milk	80	3.87	0.24	0.35-1.35	0.81	0.422
Cow milk	80	9.28	0.32	0.63-1.93		
$\bar{x}$ ***: mean,*** ***SH:*** *Standard error; 95%CI: 95% confidence interval*						

Table 4. MDA analysis results in mother blood samples

MDA (nmol/ml plasma **)** $\bar{X}$				
		S	**F/t**	**p**
Breast milk	5,20	1,41	0,63	0,54

4. DISCUSSION

Milk and dairy products, which make up a large proportion of the food source of vulnerable children, pose a significant risk to public health if they carry AFM1 residues.

Aflatoxin B1 is taken into the body with moldy grains, sacrificial products that are eaten by pregnant women and moldy feeds of animals. In some studies, some animals exposed to aflatoxins prenatally showed growth retardation as fetal. It was found that AFM1 was found in breastmilk and the mothers were exposed to

AFB1 by feeding them and therefore, babies fed with breast milk received AFM1 and they were found to have forward cancer risk. Due to the reasons mentioned above, many foods, especially breast milk and baby food have been investigated in terms of aflatoxin M1 content. Although there are very few studies about infant formula in our country, no study on breast milk was found. Today, people living in rural areas have very limited time to follow the developments in the field of health. With such projects, science will be transformed into products.

Aflatoxins are a group of mycotoxins having mutagenic, carcinogenic and immunosuppressive properties. These are the secondary metabolites of Aspergillus flavus, Aspergillus parasiticus and Aspergillus nomius. Aspergillus are major contaminants in foodstuffs. Major toxins AFB1 and AFB2 produced by Aspergillus flavus are AFG1 and AFG2 produced by Aspergillus paraciticus. Aflatoxin B1 is the most hepatotoxic and carcinogenic of the natural toxins. AFB1 has been under the control of the FDA in recent years because of being extremely toxic and extremely common in basic food and nutrients. Aflatoxin M1 is the main hepatic carcinogenic metabolite of Aflatoxin B1, fed to AFB1-contaminated foods of milk-giving animals and mothers.

In this first study in Kars, AFM1 analysis results of milk samples were found to be positive by 55%. The mean AFM1 concentration of our samples was 3.87 ± 0.24 ng / l. It is not possible to make local comparisons. Turkey and some other countries are working on this issue is available.

The presence of AFM1 in dairy products has proven to be a potential risk for human health, especially for infants and children. Considering that infants and children have to consume more milk than adults and their bodies are more sensitive than adults, it is emphasized that AFM1, which may occur in milk and dairy products, is an important source of health. Aflatoxins undergo metabolic changes in the liver and become toxic after being converted to various epoxide derivatives. These derivatives, which are responsible for toxic and carcinogenic effects, disrupt the protein synthesis by inhibiting DNA and RNA synthesis by affecting the liver cells at the molecular level (Tirmenstein & Mangipudy, 2014).

Aflatoxins are highly soluble in fat. They are rapidly absorbed from the area of exposure, particularly in the respiratory and gastrointestinal tract (Agag, 2004). Aflatoxins are more absorbed when ingested by the mouth. Aflatoxin M1 can be detected in milk within 6-24 hours after AFB1 is taken with feed and reaches maximum level in 12-48 hours (Kamkar et al. 2014; Tsakiris et al., 2013). Aflatoxins are primarily metabolized by the liver to the hydroxylate or the reactive epoxide for aflatoxin M1 which is less harmful (Bbosa et al., 2013).

The association of high amounts of aflatoxin hepatocellular carcinoma with food has been widely discussed in epidemiological studies. In the first investigations, samples from different geographical areas were studied and a connection was made with primary liver carcinoma. The important coincidence of the possible role of aflatoxin in hepatocellular carcinoma has led to studies on the association of unknown diseases such as Kwashiorkor and Reyeors syndrome. Although there was no general opinion on the definition of Kwashiorkor, the relationship between edema and hypoalbumin and kwashiorkor was accepted in international scientific circles. Many international studies have shown the relationship between aflatoxin and kwashiorkor. Therefore, accurate exposure of AFM1 exposure and necessary precautions should be taken.

According to Turkish Food Codex Contaminants Regulation, the maximum limit of aflatoxin M1 in milk used in the production of raw milk, heat-treated milk and milk-based products is 0.050 μg / kg. This level is 0.025 μg / kg in infant formulas and continuation

formulas (Ulven et al., 2011). It is known that AFM1, which has a toxic effect on liver, has many negative effects on health (Bbosa et al., 2013; Abbès et al., 2010; Williams et aı. 1998).

In our study, AFM1 levels in breast milk were determined as 3.87 ng / l. and many other studies (Turkey, Cyprus, Iran, Colombia and Cyprus) showed a significant parallelism (Diaz and Sanchez, 2015; Atasever et al., 2014; Mahdavi et al., 2010; Jurewicz et al., 2013; Kunter et al, 2016). Researchers found AFM1 levels in breast milk between 60.90 and 299.99 ng/l in Turkey (Gürbay et al., 2010). Some other studies reported that the mean of AFM1 levels was found to be 401 ng/l in Sudan (Elzupir et al., 2012), 664 ng/l in Thailand (El-Nezami et al., 1995), ng/l in Australia, and 25 ng/l in Nigeria (Adejumo et al., 2013).

In this study, AFM1 level in cow milk was found to be 9.28 ng/kg. In our study, AFM1 values in both breast milk and cow milk were low. This may be due to the climate and geography of the region. That is to say, the incidence of aflatoxin in foods in the cold regions is less than the hot regions.

There is little research showing the relationship between blood lipid peroxidation and blood milk contaminated with AFM1. In this study MDA and AFM1 contamination levels found in breast milk were low. In the literature, different studies are compatible with this study (Shen et al.,1994; Souza et al., 1999; Grintzalis et al., 2014)..

In all studies, there was a parallel relationship between milk AFM1 levels and blood MDA levels. In our study, MDA levels in the mother blood were found to be 5.20 1.41 nmol/ml plasma.

Milk and dairy products containing aflatoxin; children who are more sensitive than adults are also consumed in large amounts. Therefore, aflatoxins in milk and dairy products are one of the major problems that threaten public health. It has been determined that AFM1 levels in commercially consumed milk are within legal limits. However, it is still stated that it may pose a risk for children fed with milk for a long time (Madali and Ayaz, 2017).

In our country, studies on nutrients in various regions showed that the rates of alfatoxin were high. Therefore, we needed to make this study because of possible contamination through food chain. If aflatoxin is present in food in a region, all living things in this region, especially infants, are at risk.

This study is the first to use breast milk and cow's milk in the region. For this reason, we think that it will be an important guide to studies on breast milk and other dairy products. In addition, taking into account the seasonal changes in the regions in the future, making new measures and practices regarding the storage, transportation and preservation methods of winter animal food will be important in terms of developing health and agricultural policies in preventing import and export losses.

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