

Aflatoxin M₁ Contamination in Some Mothers Breast Milk in the City of Erbil



Avan Farouq Qadir*, Mouafaq Mohamad Ali**

*Food Science Department, Faculty of Agricultural Sciences, University of Suleimany, Sulaimaniyah, Iraq, e-mail: avanfq@gmail.com

**Food Technology Department, College of Agriculture, University of Mosul, Mosul, Iraq, e-mail: Mowafakali51@yahoo.com

Abstract:

Aflatoxin is a carcinogenic compound with high toxicity levels and is one of mutagens factors that have adverse effects on many vital body events, in addition to its effect on the body immune system. To study the occurrence of aflatoxin M₁ (AFM₁) in the mother's milk that depend on breast and mixed lactation, in the governorate of Erbil, 20 milk samples were collected from Rezgari hospital of Erbil city. The aflatoxins estimation has been done using the TLC method. The results showed the presence of AFM₁ in 16 samples (80% of total samples) with the range of 0.1-3.01 µg/l. When comparing the results with the internationally acceptable values, it shows that most of the obtained results were much higher than the value limits depending on the U.S. FDA and European Union regulations as well.

Keywords: aflatoxin M₁; breast milk; TLC method.

I. Introduction:

The mother's milk is the perfect food for her infant, providing the infant with all essential elements for growth, as well as with non-food items such as antibodies that protect from microbes and diseases [1]. Breast milk may sometimes contain tiny amounts of a wide range of contaminants due to serious environmental pollution [2], which may lead to the arrival of such contamination into the mothers' breast milk and thereafter the transfer to their infants that may cause a serious threat to their lives. Aflatoxin occurrence is the most common in such contaminants. Aflatoxin is considered as one of the most potent toxic substances known as Mycotoxins. Mycotoxins are

secondary metabolites produced by special fungal strains. Aflatoxins are produced by the fungi *Aspergillus flavus*, *Asp. nomius*, *Asp. parasiticus*, *Asp. Tamari* [3]. If a human food intake contaminated with the aflatoxins (B₁, B₂, G₁, or G₂) part of such aflatoxins will convert to aflatoxin M₁ (AFM₁) and aflatoxin M₂ (AFM₂) in the liver, where the toxin passes through the digestive system in all the stages of digestion until absorption in the duodenum, and then to be carried through the portal vein to the liver cells, there the toxin is metabolized in multiple paths, some of which end up in the secretion of an amount of toxin equivalent to about 1-

3% of the original amount of milk, mainly in the form of AFM₁ [4].

The two types AFM₁ and AFM₂ are among the hydroxylated products of the two types AFB₁ and AFB₂, respectively, when they consumed by humans or animals they appear in their milk [5]. Some describes the presence of AFM₁ in milk, particularly mothers' milk, as a precious biological indicator, a biomarker, for the purpose of determining the exposure to AFB₁ [6]. Based on a literature review, there is little information on the AFM₁ intake by breast feeding [7].

The importance of studying aflatoxins is in fact that small amounts of them can cause a reduction in growth rates and deterioration in the immune system and thus increase the influence of pathological injury [8]. Statistics indicate the death of 250,000 people annually in the world due to liver cancer caused by exposure to aflatoxins [9], and it was confirmed that AFM₁ causes cirrhosis of the bile duct, as well as localized death of liver tissue (liver necrosis) depending on the amount of doses taken [10]. Also in a survey carried out in Nigeria on 407 infants, it was found that 327 of them were patients with jaundice, which has proved its relationship with the presence of aflatoxins in the infants' serum [11].

In a study carried out in Victoria, Australia and Thailand, examined the exposure of infant to AFM₁ and of lactating mothers to AFB₁, using AFM₁ in breast milk as a biomarker for exposure to AFB₁, it was found that 11 out of 73 samples from Victoria were contaminated with AFM₁ with the range of 0.028-1.031 ng/ml. Also the study did indicate that out of 11 samples collected in Thailand, five of them were contaminated in the range of 0.039-1.736

ng/ml [12]. The percentage of Contaminated samples in France, Italy, Zimbabwe were found to be 0% (0 out of 42), 0.4% (1 out of 231; about 0.194 ng/ml) and 11% (6 out 54; ranged 0.014-0.05 ng/ml), respectively [13]. In a survey conducted in Abu Dhabi UAE, it was found that 99.5% of 445 samples contained AFM₁ in a range of 2-3000 ng/ml [14]. In another survey collecting 80 samples of breast milk in Jordan all the samples showed higher concentration of AFM₁ than the maximum tolerance limit accepted by the European Union and the United States [15].

The regulations issued by the U.S. FDA defined in detail the term that indicate the allowable amount of aflatoxins in the milk of infants as up to 0.5 µg/ml [4]. All EU member states other than Germany do not have any additional limits laid down for AFM₁. Only recently, the German national legislation put a strict maximum limit for AFM₁ at 0.01 µg/ml, for dietetic foodstuffs of infants and young children as well [16].

As a result of the seriousness of aflatoxins, the studies become an urgent need for the qualitative determination of aflatoxin types and for their quantitative estimation (quantification) as well. Therefore, this study was conducted to identify the presence of AFM₁ in some mothers' breast milk in the city of Erbil.

II. Materials and Methods:

A. Preparation of samples

Milk samples were collected from mothers attending Rezgari Hospital (Erbil), for the time period from March until June 2003, using sterile glass jars, and then kept frozen until the day of detection.

The tables I, II and III include samples information on the mothers and their

Table.I: Mothers Age.

Age	Count
15-20 years old	2 mothers
20-30 years old	13 mothers
30-40 years old	5 mothers

Table.II: Infants Sex and Age.

Sex	Age	Count
Female	5 days - 6 months	11 infants
Male	1 month – 9 months	9 infants

Table.III: Mothers Lactation.

Lactation	Count	Percentage
Breast	12 mothers	55%
Mixed	8 mothers	35%

B. Extraction and Determination of Aflatoxins

The method described in [17] was followed with some modifications, 10ml of milk was mixed with 1ml of sodium chloride saturated solution and then 20ml chloroform were added in three batches in a separator funnel with slow stirring. The lower phase layer of chloroform drained slowly through the filter paper containing 1g of sodium sulfate. The filtrate (chloroform) was concentrated to dryness using a rotary evaporator Type 349/2 (Limited England Co.) at 40°C. Then, the residue was quantitatively transferred to test tubes and saved in the refrigerator until detecting the aflatoxins.

C. Aflatoxins Analysis (Qualitative)

The analysis was done using the Thin Layer Chromatography (TLC) method, using glass plate covered with Silica, with a thickness of 0.25mm and non-fluorinated

infants as well.

(Merck Co., Germany). Thin layer plate was marked with pencil before spotting an imaginary line 1cm from the bottom of the, then by using a micro syringe 25µl of AFM₁ standard were spotted along with 25µl sample extract. The TLC plate was first developed with diethyl ether to remove the fat (as a cleanup step), then the plate was developed with a solution of (diethyl ether: ethanol: H₂O, 1:4:95). Then the plate was dried and the concentration of AFM₁ in the sample was estimated by visual comparison of sample extract fluorescent spots with that of standard AFM₁ spots exposed to the UV radiation.

D. Aflatoxins Quantification

The equation (1) described by [18] was followed for aflatoxins quantification and it is:

$$\mu\text{g Aflatoxin per 5ml} = D \times M \times 10^6 / E \times 200 \times L \quad (1)$$

D = Specific light density

= (light density when wavelength is 365nm

– light density at wavelength of 420nm)

M = Molecular weight (in AFM₁ = 328)

E = Absorption coefficient (in AFM₁ = 21000)

L = Cell thickness in mm

Since we have taken 25µl from a volume of 0.5ml extract from a 10ml milk sample, therefore the aflatoxins concentration was calculated on the basis of µg/l. Both of the molecular weight and the absorption coefficient of AFM₁ were determined according to the calculations described in [19].

III. Results and discussion:

The results in Table.IV show that out of the 20 milk samples analyzed only 4 samples were negative and they did not contain any amounts of AFM₁, and the presence of AFM₁ in 16 out of 20 milk samples, that's 80% of the samples were positives and contaminated with the range of 0.1-3.01 µg/l. Among those positive samples there were only 2 samples that contained amounts ranged within 0.1-0.19 µg/l and therefore they were less than the allowable limits of 0.5 µg/l recommended by the U.S. FDA regulations. But, and according to the recent European regulations, all the positive samples were higher than the allowable limits of AFM₁ that have been set for infant foods intake. Recently the EU set the limit value to 0.025 µg/l, and the German national legislation put a limit figure of 0.01 µg/l according to the previously mentioned [16].

Discussion:

The mother's milk is a source for all nutrients needed by the baby of carbohydrates, fat, proteins, vitamins and minerals, as well as a source of immune proteins that extends the child adequate immunity to all communicable diseases. Due to environmental pollution and food pollution too, which threaten the lives of all living organisms, in addition to fungi pollution and its toxins, still there are vast

induced diseases that are neglected caused by mycotoxins, especially the type of aflatoxins that start to occur with wrong post-harvest handling and during the storage of the crops. Also the import of old food, especially that food which when dried may be delayed in transport and especially in maritime transport, which will lead to the presence of suitable conditions for the growth of fungi and secretion of toxins. In addition there are many remarks on the several types of daily food intake habits commonly found in the market, including nuts, grains, almond kernels, pistachio and some fruits and vegetables as well as the types of milk and dairy products of all kinds which are suitable for contamination with AFB₁. Such common food items could provide the chance for AFM₁ contamination in mother's milk. So the mother food intake contaminated with aflatoxin has become a natural phenomenon which led to the transfer of pollution to the infant through breast milk, where it became a threat to the life of the mother and her infant as well.

The results obtained in Erbil city indicate that the mothers as well as their infants face the seriousness of the inevitability of exposure to aflatoxins because the majority of the results values were higher than the internationally allowable limits, defined by the U.S. FDA, EU (EEC) regulations in addition to the German national legislation [4], [16].

Table.IV: Samples results for AFM₁ contamination rates according to U.S. FDA regulations.

Sample Type	Samples number	Negative Samples	Positive Samples	Concentration Rate (PPb)	Concentration Rate, less than the limits (PPb)	Concentration Rate, higher than limits (PPb)
Mothers Milk	20 (100%)	4 (20%)	16 (80%)	0-3.01	0.1-0.19	0.31-3.01

IV. Conclusion:

This is the first study of aflatoxins in breast milk of some woman from Erbil. The presence of AFM₁ in the studied milk samples proved that there was a contamination in mothers' lactation, with the possibility of contamination of their food with all kinds of aflatoxins: B₁/B₂, G₁/G₂ and M₁/M₂.

Therefore there is an urgent need to control and reduce the presence of AFM₁ in

mothers' lactation. Strong attention should be paid to provide more control measures, better nutrition education, food safety/hygiene programs, improved agricultural practices (pre-harvest, post-harvest handling and storage), and firm quality control applications, so as to safeguard the mother and her infant from the exposure to any kind of aflatoxins.

References

- [1] J. Pronczuk, G. Moy, and C. Vallenas, "Breast Milk: an Optimal Food" *Environ. Health Perspec.*, (2004).
- [2] M.Mwanza, N.Lubanza, N.Mathew,"Mycotoxin and Food safety in Developing countries". Chapter 2. Page 49.2012.
- [3] G. L. Ronald and G. Santos, "Guide to Foodborne Pathogens", John Wiley & Sons, (2001).
- [4] H. P. VanEgmond, U. K. Svensso, and T. M. Fremy, "Mycotoxins in Milk and Milk Products", Monograph on residues and contamination in milk products International Dairy Federation, Brussels (Belgium), (1997).
- [5] G. Wei, U. R. Mashall, and D. P. H. Hsieh, "Characterization of Water Soluble Glucuronide and Sulphate Conjugates of Aflatoxin B₁, Urinary excretion in Monkey, Rat and Mouse" *Food Chem. Toxicol.* 23, pp. 809-813, (1985).
- [6] S. A. Ghiasain, and A. H. Mghsood, "Infants' Exposure to Aflatoxin M₁ from Mother's Breast Milk in Iran" *Iranian J. Public Health* 41(3), pp.119-126, (2012).
- [7] N. Polychronaki, P. Turner, Y. Gong, H. Amar, M. Abdel-Wahhab, H. El-Nezami, "Determination of AFM₁ in breast milk in a selected group of Egyptian mothers" *Food Additives Contamination.*23(7), pp. 700-8, (2006).

- [8] L. L. Charmley, H. L. Trenholm, D. B. Prelusky, and A. Rosenberg, "Economic Looses and Decontamination" *Nat. Toxins* 3, pp. 199-203, (1995).
- [9] J. D. Groopman, A. Zarba, F. Sheabar, G. N. Wagon, R. Montesano, and C. P. Wild, "Molecular Dosimetry of Aflatoxin B₁ Virus Infection", *Proc. Am. Cancer Res.* 31, pp. 230-237, (1990).
- [10] I. F. H. Purchase, "Acute Toxicity of Aflatoxin M₁ and M₂ in One-Day Old Ducklings" *Food Cosmet. Toxicol.* 5, pp.339, (1971).
- [11] O. Sodeinde, "Neonatal Jaundice, Aflatoxin and Naphthols Report of a Study in Ibadan, Nigeria" *Annals of Tropical Paediatrics* 15, pp. 107-113, (1995).
- [12] H. S. El-Nezami, G. Nicoletti, G. E. Neal, D. C. Donohue, and J. T. Ahokas, "Aflatoxin M₁ In Human Breast Milk Samples from Victoria, Australia and Thailand" *Food Chem. Toxicol.* 33(3), pp. 173-179, (1995).
- [13] S. A. Navas, M. Sabino, and D. B. Rodriguez-Amaya, "Aflatoxin M₁ and Ochratoxin A in a Human Milk Bank in the City of Sao Paulo, Brazil" *Food. Addit. Contam.* 22(5), pp. 457-462, (2005).
- [14] A. M. Saad, A. M. Abdelgagirt, and M. O. Moss, "Exposure of Infant to Aflatoxin M₁ from Mothers' Breast Milk in Abu Dhabi, UAE" *Food Addit. Contam.* 12(2), pp.255-261, (1994).
- [15] S. S. Omar, "Incidence of Aflatoxin M₁ in Human and Animal Milk in Jordan" *J. Toxicol. Environ. Health A*, 57(22-23), pp. 1404-9, (2012).
- [16] Mariko Kubo, "Mycotoxin Legislation Worldwide", European Mycotoxin Awareness Network, (last update February 2012), (2012).
- [17] A.O.A.C. Association of official Analytical Chemists, "Official Methods of Analysis", 4th ed. Virginia USA, (2000).
- [18] J. Nabney, and B. F. Nesbitt, "A Spectrophotometric Method of Determining the Aflatoxin" *Analyst.* 90, pp. 155-160, (1965).
- [19] J. G. Heathcote, and Hibbert, "Aflatoxin: Chemical and Biological Aspect", Elsevier Science, pp. 212, (1978).