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Aflatoxin B_1, M_1 and Ochratoxin A Levels in Infant Formulae and Baby Foods Marketed in Ankara, Turkey

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ABSTRACT

Aflatoxin B_1 (AFB_1), aflatoxin M_1 (AFM_1), and ochratoxin A (OTA) that may lead to severe problems in children's health were evaluated in commonly consumed various types of baby foods. In the present study, 63 infant formulae, follow on formulae and baby foods were randomly collected from pharmacies and supermarkets in Ankara, Turkey. AFB_1, AFM_1, and OTA levels were assessed by commercially available enzyme-linked immunosorbent assay (ELISA) kits. AFB_1, AFM_1 and OTA levels were found in 87, 36.5 and 40% of the samples between 0.10-6.04 ppb, 0.06-0.32 ppb and 0.27-4.50 ppb, respectively. We suggest that mycotoxin contamination should be routinely monitored in foods for babies in order to reduce food-borne hazards in infants and young children.

Key words: aflatoxin, baby food, enzyme-linked immunosorbent assay (ELISA), food safety, mycotoxin, ochratoxin

INTRODUCTION

Mycotoxins are food contaminants produced by certain kinds of fungi and the most commonly found mycotoxins, namely aflatoxins and ochratoxins, carry potential risks for humans[1-2]. Aflatoxins are highly toxic and carcinogenic secondary metabolites produced by *Aspergillus flavus*, *A. parasiticus* and *A. nomius* in various foodstuffs[1-6]. *A. flavus* produces only aflatoxin B (AFB_1 + AFB_2), while the other two species produce both aflatoxin B and G (AFG_1 + AFG_2)[6]. Activation of aflatoxin B_1 is important in any mycotoxicological consideration of the effects of AFB_1 on organisms. AFB_1 itself is not carcinogenic, but is metabolized into an ultimate carcinogenic metabolite by oxidation of an 8,9-vinyl ether bond, and this epoxide form binds to nucleic acids and proteins[1]. AFB_1 is listed as a Group 1 agent by the International Agency for Research on Cancer (IARC), especially as a cause of hepatocellular carcinoma[2,7]. Aflatoxin M_1 (AFM_1) is the hydroxylated metabolite of AFB_1, found in the milk of humans and animals[1,4]. They may be found in milk products obtained from livestock that have ingested contaminated feed[3-6]. The toxic and carcinogenic effects of AFB_1 have been convincingly demonstrated in laboratory animals and AFM_1 is classified as a Group 2B human carcinogen[3-4] though its carcinogenic potency in sensitive species is estimated to be approximately 10 times lower than that of AFB_1[5]. In some animals, weight loss, anorexia, hemorrhage, renal damage, gastrointestinal and urogenital carcinomas have been associated with the effects of aflatoxins[1-2].

On the other hand, ochratoxin A (OTA) is found in many commodities, including cereals, cereal products, coffee, grapes, grape juice, wine, cocoa, beer, meat, milk and milk products[2,8-10]. OTA is mainly produced by *Penicillium verrusocum* in temperate climates and by a number of *Aspergillus* spp. such as *A. ochraceus*, *A. carbonarius*, *A. niger* and *A. terreus* in warmer and tropical areas[8-9]. OTA is classified as a possible human carcinogen (Group 2B) by IARC due to its nephrotoxic effects[10]. Renal function and morphology are greatly affected by at high dose of OTA, as indicated by increased kidney weight, urine volume, blood urea nitrogen, urinary glucose and proteinuria. Changes in renal enzymes and protein patterns can be used to distinguish the type of renal injury caused by OTA[2,6]. OTA is also associated with Balkan Endemic Nephropathy (BEN), which is a fatal human kidney disease[2,6], and it is a potent immunotoxin, genotoxin, mutagen and teratogen[9].

The susceptibility of infants and young children towards the adverse effects of mycotoxins is higher than that of adults so that these types of contaminations in baby foods should seriously be taken into concern. Contamination of infant formulae and baby foods is inevitable since multiple ingredients are present in infant formulae and baby foods, including milk powder, fruits or different kinds of cereals. Therefore the major aim of the present study was to assess AFB_1, AFM_1 and OTA levels in infant formulae, follow-on formulae and baby foods.

MATERIALS AND METHODS

I. *Samples*

Sixty three powered infant formulae, follow on

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formulae and baby food samples were randomly collected from pharmacies and supermarkets in the years of 2003 and 2004, in Ankara, Turkey. The samples were stored in plastic bags at -20ºC until analysis.

II. *Methods of Analysis*

The samples were screened for AFB_1, AFM_1 and OTA contents by commercial AFB_1, AFM_1 and OTA enzyme-linked immunosorbent assay (ELISA) kits (Ridascreen®, R-Biopharm AG, Darmstadt, Germany), respectively. All chemicals used in the experiments were of analytical grade.

According to the instructions of the manufacturer, for the determination of AFB_1, 2 g powered sample was mixed with 3 mL phosphate-saline buffer (PBS) and 0.2 mL amylase solution, and extracted with 7 mL methanol (100%, v/v). The entire sample solution was filtered through folded paper filter. Two milliliters of the filtrate was mixed with two milliliters distilled water and extracted with 3 mL dichloromethane. The organic layer was evaporated with weak nitrogen stream. The dry residue was dissolved in 1 mL methanol, and then 1 mL distilled water and 1.5 mL n-heptane were added. After the extraction, 100 µL methanol layer was diluted with 400 µL of test sample dilution buffer and 50 µL of this was applied on the AFB_1 test plate.

In order to determine the AFM_1 contamination of the samples, 2 g of each powered sample was extracted with 20 mL methanol: water (70:30) using a shaker at room temperature. After centrifugation, upper cream layer was completely removed by aspirating using a pasteur pipette. One hundred microliter skimmed portion was directly applied on the AFM_1 test plate.

Determination of OTA by ELISA, according to OTA test procedure, was performed by adding 2.5 mL of 1 N HCl on 2 g of each powered sample, shaking at room temperature and later extracting with 4 mL dichloromethane. The dichloromethane phase was collected and mixed with 2 mL of 0.13 M sodium bicarbonate, pH: 8.1. Aqueous phase was collected and diluted with 1 mL of the buffer, and 50 µL of this was used for the OTA test.

The optical densities were measured at 450 nm by using an ELISA 96-well microplate reader (Sunrise Gmblt, Tecan, Austria). The linear standard curves for AFB_1, AFM_1 and OTA are shown in Figure 1.

Mycotoxin contamination in each sample was expressed as parts per billion (ppb). The lower detection limits for AFB_1, AFM_1 and OTA by ELISA were 0.025, 0.050 and 0.025 ppb, respectively. Sample, which contained the mycotoxins under the limit mentioned above, was accepted as a negative contamination. The recovery ratios for AFB_1, AFM_1, and OTA kit assays were 68% with a mean coefficient of variation (CV) 18%, 95% with a mean CV 15%, and 85% with a mean CV 14%, respectively.

RESULTS AND DISCUSSION

ELISA as a screening method is used quickly to detect mycotoxin levels in foods[4,6-7], and the result gives crude ideas concerning the mycotoxin contamination.

The commercial baby foods and processed milk- and/or cereal-based foods for infants and young children in the study were collected from supermarkets and pharmacies in Ankara, but they are also distributed to all areas in Turkey. Therefore, the results might give ideas about the contamination profile of aflatoxin B_1, aflatoxin M_1 and ochratoxin A for these infant formula and baby food in Turkey. Data on the occurrence and presence of mycotoxin in foods and feeds are needed to estimate a possible exposure degree and also to establish the regulatory limits.

As shown in Table 1, the incidence of AFB_1 contamination in these infant formulae and baby foods was very high, since 55 (87%) samples contained AFB_1 above the detection limit, 0.025 ppb and were considered positive contamination. However, eight samples with AFB_1 over the permissible level of 1 ppb were accepted for infant formulae and baby foods in Turkey. The average AFB_1 level was found as 0.89 ± 1.10 ppb in 55 samples. The detected levels were at 0.10 ppb in minimum and 6.04 ppb in maximum. Using a similar approach by Beretta *et al.*[8], the risk of AFB_1 intake from baby foods can be estimated. If a 5-month-old child with a body weight of 6.5 kg can consume 26 g of formula per portion, according to the manufacturer's suggestion, with the highest quantity

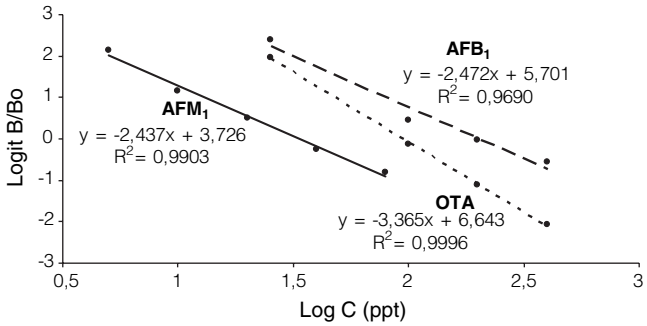

Figure 1. Samples of the ELISA linear standard curves of the AFB_1 (---), AFM_1 (—) and OTA (...).

Table 1. Occurrence of aflatoxin B_1 in baby foods and infants formulae

AFB_1	Contamination level, ppb		
	Frequency distribution, n (%)		Average
Sample	≤0.025	>0.025	Mean ± SD
Milk-based, *n* = 29	2 (7)	27 (93)	0.73 ± 1.11
Cereal-based, *n* = 25	3 (12)	22 (88)	0.80 ± 0.44
Milk + cereal-based, *n* = 9	3 (33)	6 (67)	1.93 ± 2.08
Total (*n* = 63)	8 (13)	55 (87)	0.89 ± 1.10

of AFB_1 detected (6.04 ppb or μg/kg), 24.16 ng AFB_1/kg b.w. would be taken at a time. AFB_1 is the most potent carcinogen known in mammals, the risk assessment of which is very well established.

The levels of aflatoxin M_1 in 24 samples were higher than 0.05 ppb, accepted as permission limit in Turkey, as shown in Table 2. This value is equal to the detection limit for AFM_1 in the study. Aflatoxin M_1 was detected at low levels (≤ 0.05 ppb) in 63.5% of the infant formulae and baby foods, and between levels 0.06 and 0.32 ppb in 36.5% of the samples. With a similar calculation to AFB_1 mentioned above, a child with body weight of 6.5 kg would uptake the maximum 1.23 ng AFM_1/kg b.w. at a time. Since AFM_1 is a genotoxic carcinogen, the risks against infants and young children from AFM_1 exposure need careful consideration[5].

According to the European Commission[11], the maximum level of ochratoxin A in baby foods and processed cereal-based foods for infants and young children was 0.5 ppb. Since OTA limit was not established in the same foods in our country, the Commission Regulation[11] was considered as the guide in the study. Twenty five samples were found contaminated with OTA, and the OTA levels in 19 among these samples exceeded the permission level (Table 3). The detected minimum level of OTA in the tested samples was 0.27 ppb while 4.50 ppb was the maximum. According to the instructions on the label, a baby consumes 25 g of formula per portion. If a 5-month-old child with body weight of 6.5 kg and infant formula with the highest quantity of ochratoxin A found (4.50 ppb or μg/kg) were considered, the maximum portion intake would be 17.30 ng OTA per body weight (kg). The provisional tolerable weekly intake (PTWI) is 0.1 μg/kg b.w. for ochratoxin A[12] so that there is a mild toxicological risk for a child that consumes a formula with OTA contamination slightly above the permitted level. On the other hand, considering these results, it could be concluded that mycotoxin incidence in samples selected from commonly consumed in Turkey, is not a serious public health problem at the moment. However, the total daily mycotoxin intake with the other sources of mycotoxin could be an important risk for infants and young children.

Our results showed that some samples studied were contaminated with AFB_1, AFM_1 and OTA. This situation might cause serious health problems in infants and babies such as poor growth, suppressed immune system, and cancer. An accurate prediction of the possible health impact of individual mycotoxins in foods for the vulnerable group is difficult; possible additive and synergistic effects of multiple mycotoxins make the task even more complex and the long-term effects are beyond foresight. Therefore infant foods must be routinely tested for the mycotoxins presence at every step of manufacturing and marketing.

Climatic and environmental conditions during growth, harvest and storage have great influence on mycotoxin levels, which are probably also reflected in the levels in feedstuffs[2,6,10]. Since it is difficult to remove the mycotoxin once formed, the best way of control is prevention. However, many measures have been to minimize the occurrence of moulds such as alternative methods of soil cultivation, development of mould resistant species and drying and storage techniques[6].

We suggest that manufacturers of foods for infants and young children should give an extreme importance to mycotoxin content. We believe that manufacturers, pediatricians, health-care personnel and parents should be provided with enough information and training to minimize health hazards and to form the public policies. In order to protect public health, it is essential to keep contaminants at levels toxicologically acceptable. Ultimately, surveillance should be continuous, widespread and must be conducted by the government and related ministries as the quality of the end product depend on the precise controlling at every step of the production.

Table 2. Occurrence of aflatoxin M_1 in baby foods and infants formulae

AFM_1	Contamination level, ppb		
	Frequency distribution, n (%)		Average
Sample	≤0.050	>0.050	Mean ± SD
Milk-based, *n* = 29	16 (55)	13 (45)	0.06 ± 0.03
Cereal-based, *n* = 25	19 (76)	6 (24)	0.06 ± 0.03
Milk+cereal-based, *n* = 9	5 (56)	4 (44)	0.18 ± 0.09
Total (*n* = 63)	40 (63.5)	23 (36.5)	0.07 ± 0.05

Table 3. Occurrence of ochratoxin A in baby foods and infants formulae

OTA	Contamination level, ppb		
	Frequency distribution, n (%)		Average
Sample	≤0.025	>0.025	Mean ± SD
Milk-based, *n* = 29	22 (76)	7 (24)	0.50 ± 0.33
Cereal-based, *n* = 25	11 (44)	14 (56)	1.82 ± 1.54
Milk+cereal-based, *n* = 9	5 (56)	4 (44)	2.38 ± 1.22
Total (*n* = 63)	38 (60)	25 (40)	1.54 ± 1.40

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