

Serum aflatoxin levels of the healthy adult population living in the north and south regions of Turkey

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Submitted 14 January 2013; Final revision received 19 August 2013; Accepted 20 August 2013; First published online 18 November 2013

Abstract

Objective: To determine the serum concentrations of aflatoxin B₁ (AFB₁), aflatoxin B₂ (AFB₂), aflatoxin G₁ (AFG₁) and aflatoxin G₂ (AFG₂) in the healthy adult population living in both the Black Sea and Mediterranean regions of Turkey and to investigate the regional, seasonal and gender variability in aflatoxins (AF) exposure in these regions.

Design: Serum AFB₁, AFB₂, AFG₁ and AFG₂ concentrations were analysed by HPLC.

Settings: In total, four hundred and eighty-four serum samples were analysed.

Subjects: Four hundred and eighty-four healthy adult volunteers living in rural areas of the Black Sea and Mediterranean regions of Turkey were studied.

Results: The mean serum concentration of total AF in the Black Sea region was 1.33 ppb (min–max 0.15–3.38 ppb) and 0.90 ppb (min–max 0.18–2.48 ppb) for summer and winter, respectively. In the Mediterranean region, the mean serum concentration of total AF was determined as 0.55 ppb (range 0.04–1.72 ppb) for summer and 0.45 ppb (range 0.12–1.43 ppb) for winter. The total AF concentrations in serum samples were statistically higher in summer compared with winter for the two regions. The differences between the regions were statistically significant concerning all samples, with higher total AF concentrations in the Black Sea region.

Conclusions: The overall results suggest that the Turkish population living in these two regions is continuously exposed to AF, particularly in the summer, and that mycotoxin contamination in food should be monitored routinely for food safety and human health.

Keywords

Aflatoxins

Mycotoxin

HPLC

Healthy adult population

Turkey

Aflatoxins (AF), first discovered in the 1960s, are naturally occurring contaminants of agricultural crops formed by several fungus species of the genus *Aspergillus*. *Aspergillus flavus* and *Aspergillus parasiticus* in particular are responsible for most AF contamination of food crops worldwide⁽¹⁾. The general population is exposed to AF primarily by consuming contaminated foods such as spices, dried fruits, cereals and cereal products. The four major types of AF are aflatoxin B₁ (AFB₁), aflatoxin B₂ (AFB₂), aflatoxin G₁ (AFG₁) and aflatoxin G₂ (AFG₂). The relative proportions and amounts of the various AF on food crops depend on the *Aspergillus* species present, as well as on growing and storage conditions. Contamination is higher on crops grown in hot, humid and tropical climates generally, but also can occur in temperate climates varying from year to year. Pre-harvest AF levels increase during droughts, and post-harvest levels increase when crops are not properly dried before storage or

are not protected from insect and rodent infestations. Rapid post-harvest drying and storage in an area with a moisture content of less than 10% w/v can eliminate most contamination^(1–4).

AF are well-known human carcinogens based on sufficient evidence of carcinogenicity from studies in human subjects. AFB₁ is one of the most potent carcinogens occurring naturally and is classified as a Group I carcinogen (carcinogenic to humans) by the International Agency for Research on Cancer⁽⁵⁾. This conclusion was reaffirmed in two subsequent re-evaluations^(2,3). The International Agency for Research on Cancer concluded that there was sufficient evidence in experimental animals for the carcinogenicity of naturally occurring mixtures of AFB₁, AFG₁ and AFM₁; limited evidence for the carcinogenicity of AFB₂; and inadequate evidence for the carcinogenicity of AFG₂.

The primary target organ for AF toxicity and carcinogenicity is the liver in both man and animals. AFB₁ is

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metabolized in the liver by the cytochrome P450 system to the highly reactive AFB₁-8,9-epoxide, which is responsible for the toxicity⁽⁶⁻⁸⁾. The 8,9-epoxide metabolite can be detoxified through conjugation with glutathione, a reaction mediated by the enzyme glutathione *S*-transferase. The activity of glutathione *S*-transferase is much higher (by a factor of 3 to 5) in animal species that are resistant to AF carcinogenicity, such as the mouse, than in susceptible animal species, such as the rat. Man has a lower glutathione *S*-transferase activity than either the mouse or the rat, suggesting that people are less capable of detoxifying AFB₁-8,9-epoxide. The metabolic effects of AF include inhibition of DNA, RNA and protein synthesis; reduction in miscellaneous enzyme activities; depression of glucose metabolism; inhibition of lipid synthesis, including that of phospholipids, NEFA, TAG and cholesterol and its esters; and depression of clotting factor synthesis⁽⁹⁾. The increased risk of hepatocarcinoma is caused by deletion mutations in the *p53* tumour-suppressing gene and by activation of dominant oncogenes⁽¹⁰⁾.

In 2002, the International Agency for Research on Cancer reported that experimental animals infected with hepatitis B virus (woodchucks, tree shrews and transgenic mice heterozygous for the *p53* tumour-suppressor gene) were more sensitive to the carcinogenic effects of AF than uninfected animals. On the other hand, epidemiological studies have also shown that AF exposure is associated with increased risk of hepatocellular carcinoma, particularly in combination with hepatitis B virus. The potency of AF appears to be significantly enhanced in individuals with hepatitis B infection⁽¹¹⁻¹⁴⁾. The results of several cohort studies in China and Taiwan showed the association between biomarkers for AF exposure (AF metabolites in the urine and AF-albumin adducts in the blood) and primary liver-cell cancer and the association remained significant when the analyses were controlled for hepatitis B infection^(5,15). The correlation between dietary exposure to AF and the incidence of human liver cancer in some areas, especially in Africa and Asia, has been determined⁽¹⁶⁾. Due to the frequent occurrence of AF and their severe toxicity, guidelines and tolerance levels for AF have been set in several countries including Turkey^(17,18).

Turkey has encountered the AF contamination problem in different foods such as maize, nuts and hazelnuts exported and/or consumed in the country since 1967⁽¹⁹⁾. There are some studies on the levels of mycotoxins in different foods consumed and produced in Turkey, particularly on the concentrations of AF in cereals, cereal products, baby foods and infant formulas⁽²⁰⁻²⁵⁾. However, AF levels in serum and urine of healthy people living in Turkey have not been assessed and there was no study on the evaluation of serum AF levels according to region, season and/or age. Therefore, the present study was undertaken to investigate the regional, seasonal and gender variability in AF exposure of the healthy adult

population living in two different regions of Turkey by measuring serum AF concentrations by the HPLC technique.

Materials and methods

Chemicals and reagents

Methanol (MeOH) and acetonitrile (ACN) were HPLC grade and purchased from Riedel (Poole, Dorset, UK). Trifluoroacetic acid (TFA) was obtained from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals and solvents were purchased from Merck (Darmstadt, Germany).

Standards

AF standards (AFG₁, AFB₁, AFG₂ and AFB₂) were obtained from Sigma Chemical Co. Stock solutions and standards were prepared and analysed according to AOAC Method 971.22⁽²⁶⁾.

Participants

The study was conducted in the rural areas of two provinces in the Black Sea (Ordu) and Mediterranean (Antalya) regions of Turkey. These regions have suitable hot, humid and tropical climatic conditions which favour the production of AF by *Aspergillus* species that contaminate many agricultural commodities during harvesting and/or while in storage. The sampling size for each province was selected by a web-based sampling program (RAOSOFT). The volunteers were chosen by the cluster sampling method. Our sample size represents the population with the margin of error as 5% and our confidence interval was 90%. The blood samples were drawn from about twenty to twenty-five volunteers (who were living in that region for more than 5 years) from five villages of each province in health-care centres. In each village, there were about ten primary health-care centres. We randomly chose both the primary health-care centres (five centres for each village) and the healthy adult volunteers who applied to the health-care centres. Participants had no history of renal and hepatic disease. The numbers of female and male adult individuals were about the same in this sampling frame. The ages of the participants were between 17 and 64 years in the whole study group (*n* 484). In each cluster, the distributions of age and sex were normal. The weight and height of all participants were recorded, from which BMI (= [body weight (kg)]/[length (m)]²) was calculated⁽²⁷⁾. Demographic information of the participants is presented in Table 1.

Dietary information, including the frequency of food intake, was collected through a standard FFQ administered face to face. The reference period for the FFQ was about 6 months. The eight food groups were red meat, white meat, fish, vegetable, fruit, milk and milk products, legumes, and corn and cereal products. The fasting blood samples were collected in the morning in July 2007

(summer) and January 2008 (winter). The fasting blood samples were taken and the questionnaire was completed in 1 h before taking the fasting blood samples. The samples were centrifuged at 800g for 15 min and the serum was separated. All serum samples were aliquoted and stored at -20°C until analysis.

The study was approved by Hacettepe University Ethical Committee and conducted according to the Declaration of Helsinki. All individuals participated in the study voluntarily and written consent (in Turkish) was obtained before blood samples were drawn.

Extraction of aflatoxins from serum samples

After digestion of serum proteins^(28,29), the extraction method modified from Nelson *et al.*⁽³⁰⁾ was performed. First, 1 ml of serum sample was diluted with 2 ml of n-hexane and mixed for 1 min. After centrifugation at 5000g for 5 min, the upper n-hexane phase containing serum lipids was removed and discarded. This process was repeated for two more times. Next, 1 ml of chloroform was added to the remaining part of the serum; the solution was shaken vigorously for 0.5 min, vibrated for 4.5 min and centrifuged at 5000g for 10 min. The bottom chloroform layer was removed and transferred to a tube. This process was repeated for three more times. The chloroform phases collected in the tube were evaporated to complete dryness under a stream of nitrogen gas.

Determination of aflatoxin levels by HPLC method

The dry residue was derivatized with TFA according to the pre-column derivatization procedure of AOAC Method 971.22⁽²⁶⁾. Determination of AFB₁, AFB₂, AFG₁ and AFG₂ levels in the derivatized standards and samples was carried out by HPLC equipped with an auto sampler (Hewlett Packard Agilent 1100 Series, Vienna, Austria) using a fluorescence detector (excitation at 360 nm, emission at 430 nm). A Spherisorb S50DS2 column (3.8 mm i.d. and length 25 cm, 5 μm particle size; Waters, Milford, MA, USA) was used. The mobile phase was deionized water–ACN–MeOH (62:16:22, v/v/v) and the flow rate was 1 ml/min. The injection volume was 100 μl . The AF standards used ranged between 5 and 1000 pg/ml. The retention times were 6.2 min for AFG₁, 8.2 min for AFB₁, 11.8 min for AFG₂ and 17.0 min for AFB₂. Recovery studies were performed on blank samples of serum spiked with levels of 0.1 ng/ml, 0.25 ng/ml and 0.5 ng/ml for each AF standard and repeated for three times. The average recoveries were 69.7% for AFG₁, 79.6% for AFB₁, 101.3% for AFG₂ and 107.4% for AFB₂. The concentrations of AF in the samples were calculated by using the calibration curves of peak area prepared for each AF standard separately. The detection and quantification limits of the analyses were calculated according to the method of the US Environmental Protection Agency⁽³¹⁾. The detection limits were determined as 25 pg/ml for AFG₁ and AFB₁, 10 pg/ml for AFG₂ and 20 pg/ml for AFB₂.

The quantification limits were determined as 176 pg/ml for AFG₁, 106 pg/ml for AFB₁, 242 pg/ml for AFG₂ and 139.4 pg/ml for AFB₂.

Statistical analysis

The results were expressed as means and standard deviations. The distribution of the data was checked for normality using the Shapiro–Wilk test. The homogeneity of the variance was verified by the Levene test. The association between the rankings of two variables was measured by Spearman's rank correlation. The differences among the groups were evaluated with the Mann–Whitney *U* test using the statistical software package IBM SPSS version 17.0. For dependent variables, the Wilcoxon signed-rank test was also performed (data not shown). *P* values <0.05 were considered as statistically significant.

Results

Participants' characteristics

The total of 484 blood samples were collected from healthy adults living in the Black Sea and Mediterranean regions of Turkey in the period 2007–2008 and analysed for AFB₁, AFB₂, AFG₁ and AFG₂ by the HPLC technique. The number of the samples collected in summer and winter periods in both regions and the distribution according to gender are given in Table 1. In the Black Sea region, 104 matched samples were obtained for the two seasons; six samples in summer and nine samples in winter were collected from different persons. The mean age was 39.0 (SD 11.7) years (min–max 19–64 years) and the mean BMI was 26.5 (SD 4.3) kg/m² (min–max 18.6–39.1 kg/m²). In the Mediterranean region, 126 samples were from the same persons for the two seasons; nine more samples were obtained in the winter period. The mean age was 42.2 (SD 12.5) years (min–max 17–64 years) and the mean BMI was 27.5 (SD 5.2) kg/m² (min–max 15.6–53.6 kg/m²).

According to answers obtained from the FFQ, the dietary habits of the inhabitants in the two regions were not same completely. Although the populations in these regions consume vegetables, fruits and meat as well as locally produced food, the people living in the Black Sea region consumed more cereals including corn and corn products, whereas the consumption of fruit and vegetables was higher in the Mediterranean region.

Evaluation of serum aflatoxin levels

In the Black Sea region, AFB₁ levels were detectable in 90.0% of all samples collected in the summer and in 81.4% of the winter samples. The percentages of positive samples were 86.4% for AFG₁ and AFG₂ and 65.5% for AFB₂ in the summer period. In the winter, the percentages of positive samples were found to be 85.5% for AFG₁, 80.5% for AFG₂ and 43.4% for AFB₂ (Table 2).

Table 1 Demographic characteristics of the study population according to region, season and gender: healthy adults living in rural areas of the Black Sea region and the Mediterranean region of Turkey, 2007–2008

| | Sex | n | Age (years) | | | Height (cm) | | | Body weight (kg) | | | BMI (kg/m ²) | | |
|-----------------------------|---------|-----|-------------|------|---------|-------------|-----|---------|------------------|------|---------|--------------------------|-----|-----------|
| | | | Mean | SD | Min–max | Mean | SD | Min–max | Mean | SD | Min–max | Mean | SD | Min–max |
| Black Sea region | | | | | | | | | | | | | | |
| Summer | Female | 56 | 39.8 | 12.0 | 19–62 | 160.6 | 5.7 | 148–174 | 66.4 | 10.5 | 49–90 | 25.8 | 4.4 | 18.6–37.9 |
| | Male | 54 | 41.1 | 12.3 | 22–64 | 171.2 | 6.1 | 158–183 | 80.0 | 11.8 | 56–105 | 27.3 | 3.8 | 19.4–34.8 |
| | Overall | 110 | 40.5 | 12.1 | 19–64 | 165.8 | 7.9 | 148–183 | 73.1 | 13.1 | 49–105 | 26.5 | 4.1 | 18.6–37.9 |
| Winter | Female | 56 | 37.7 | 11.0 | 19–62 | 160.3 | 6.1 | 148–175 | 66.3 | 12.2 | 48–100 | 25.8 | 4.8 | 18.6–39.1 |
| | Male | 57 | 37.1 | 10.9 | 19–64 | 171.5 | 6.2 | 160–185 | 79.1 | 11.6 | 56–105 | 26.8 | 3.8 | 19.4–35.5 |
| | Overall | 113 | 37.6 | 11.1 | 19–62 | 166.1 | 8.4 | 148–185 | 73.0 | 13.8 | 48–105 | 26.4 | 4.4 | 18.6–39.1 |
| Mediterranean region | | | | | | | | | | | | | | |
| Summer | Female | 88 | 40.5 | 12.5 | 17–64 | 159.7 | 5.5 | 150–170 | 69.7 | 13.0 | 48–120 | 27.4 | 5.3 | 15.9–35.3 |
| | Male | 38 | 44.0 | 12.9 | 17–63 | 169.7 | 7.0 | 150–184 | 78.4 | 12.6 | 54–108 | 27.3 | 4.3 | 20.3–35.2 |
| | Overall | 126 | 41.5 | 12.7 | 17–64 | 162.7 | 7.5 | 150–184 | 72.3 | 13.5 | 48–120 | 27.3 | 5.0 | 15.9–35.3 |
| Winter | Female | 86 | 40.5 | 11.9 | 17–63 | 159.7 | 6.6 | 133–170 | 70.2 | 14.1 | 30–120 | 27.6 | 5.8 | 15.6–53.6 |
| | Male | 49 | 46.5 | 13.0 | 17–63 | 168.5 | 6.2 | 150–183 | 78.5 | 14.0 | 49–115 | 27.7 | 4.9 | 16.0–42.2 |
| | Overall | 135 | 42.7 | 12.6 | 17–63 | 162.9 | 7.7 | 133–183 | 73.2 | 14.6 | 30–120 | 27.6 | 5.4 | 15.6–53.6 |

Table 2 Numbers and percentages of aflatoxin-positive* samples in the study population according to region, season and gender: healthy adults living in rural areas of the Black Sea region and the Mediterranean region of Turkey, 2007–2008

| | Sex | AFG ₁ | | AFB ₁ | | AFG ₂ | | AFB ₂ | | Total AF | |
|-----------------------------|-----------------|------------------|------|------------------|------|------------------|------|------------------|------|----------|------|
| | | n | % | n | % | n | % | n | % | n | % |
| Black Sea region | | | | | | | | | | | |
| Summer | Female (n 56) | 46 | 82.1 | 49 | 87.5 | 45 | 80.4 | 32 | 57.1 | 49 | 87.5 |
| | Male (n 54) | 49 | 90.7 | 50 | 92.6 | 50 | 92.6 | 40 | 74.1 | 50 | 92.6 |
| | Overall (n 110) | 95 | 86.4 | 99 | 90.0 | 95 | 86.4 | 72 | 65.5 | 99 | 90.0 |
| Winter | Female (n 56) | 43 | 76.8 | 43 | 76.8 | 42 | 75.0 | 22 | 39.3 | 43 | 76.8 |
| | Male (n 57) | 51 | 89.5 | 49 | 85.9 | 49 | 85.9 | 27 | 47.4 | 51 | 89.5 |
| | Overall (n 113) | 94 | 85.5 | 92 | 81.4 | 91 | 80.5 | 49 | 43.4 | 94 | 85.5 |
| Mediterranean region | | | | | | | | | | | |
| Summer | Female (n 88) | 77 | 87.5 | 75 | 85.2 | 75 | 85.2 | 67 | 76.1 | 77 | 87.5 |
| | Male (n 38) | 32 | 84.2 | 35 | 92.1 | 31 | 81.6 | 29 | 76.3 | 35 | 92.1 |
| | Overall (n 126) | 109 | 86.5 | 110 | 87.3 | 106 | 84.1 | 96 | 76.2 | 109 | 86.5 |
| Winter | Female (n 86) | 70 | 81.4 | 68 | 79.1 | 68 | 79.1 | 27 | 31.4 | 70 | 81.4 |
| | Male (n 49) | 41 | 83.7 | 41 | 83.7 | 41 | 83.7 | 22 | 44.9 | 41 | 83.7 |
| | Overall (n 135) | 111 | 82.2 | 109 | 80.7 | 109 | 80.7 | 49 | 36.3 | 111 | 82.2 |

AFG₁, aflatoxin G₁; AFB₁, aflatoxin B₁; AFG₂, aflatoxin G₂; AFB₂, aflatoxin B₂; total AF, total aflatoxins.

*Positive samples were those with detectable levels (>25 pg/ml for AFG₁ and AFB₁; >10 pg/ml and 20 pg/ml for AFG₂ and AFB₂, respectively).

In the Mediterranean region, the distribution of positive samples collected in both seasons was similar to that in the Black Sea region. The percentages of positive samples collected in the summer were 86.5% for AFG₁, 87.3% for AFB₁, 84.1% for AFG₂ and 76.2% for AFB₂. In the winter 82.2%, 80.7%, 80.7% and 36.3% of the samples contained detectable levels of AFG₁, AFB₁, AFG₂ and AFB₂, respectively (Table 2).

The mean serum concentration of total AF determined in all samples in the present study was 1.12 ppb (min–max 0.15–3.38 ppb) and 0.50 ppb (min–max 0.04–1.72 ppb) for the Black Sea and Mediterranean regions of Turkey, respectively.

The mean serum AF concentrations detected in all samples according to gender, season and region are summarized in Tables 3 and 4. In the Black Sea region, we did not observe any significant differences in AF levels between the sexes, except for AFB₂. Females had

significantly higher AFB₂ levels than males only in winter ($P < 0.05$; Table 3). However, AFB₁, AFB₂ and total AF levels were found to be significantly higher in summer samples of females than males in the Mediterranean region. In winter, there was not any significant difference in all AF levels measured between males and females (Table 4).

AFG₁, AFB₁, AFG₂ and total AF levels in the Black Sea population (n 110) were significantly higher than those in the Mediterranean population in the summer period (Fig. 1). The mean serum concentration of total AF in the Black Sea region was 1.33 ppb (min–max 0.15–3.38 ppb) and 0.90 ppb (min–max 0.18–2.48 ppb) for summer and winter, respectively. In the Mediterranean region, the mean serum concentration of total AF was 0.55 ppb (min–max 0.04–1.72 ppb) for summer and 0.45 ppb (min–max 0.12–1.43 ppb) for winter. In winter, AFG₁, AFB₁, AFG₂, AFB₂ and total AF levels were found to be

Table 3 Serum aflatoxin levels of the study population according to season and gender: healthy adults living in rural areas of the Black Sea region of Turkey, 2007–2008

| Season | Sex | n | AFG ₁ (ppb) | | | AFB ₁ (ppb) | | | AFG ₂ (ppb) | | | AFB ₂ (ppb) | | | Total AF (ppb) | | |
|--------|--------|----|------------------------|-------|-------------|------------------------|-------|-------------|------------------------|-------|-------------|------------------------|-------|-------------|--------------------|-------|-------------|
| | | | Mean | SD | Min–max | Mean | SD | Min–max | Mean | SD | Min–max | Mean | SD | Min–max | Mean | SD | Min–max |
| Summer | Female | 56 | 0.225 ^a | 0.120 | 0.038–0.619 | 0.885 ^a | 0.418 | 0.232–2.066 | 0.247 ^a | 0.178 | 0.010–0.858 | 0.136 ^a | 0.096 | 0.020–0.474 | 1.371 ^a | 0.665 | 0.149–2.979 |
| | Male | 54 | 0.223 ^a | 0.179 | 0.063–1.241 | 0.800 ^a | 0.466 | 0.125–1.913 | 0.207 ^a | 0.166 | 0.020–1.036 | 0.106 ^a | 0.087 | 0.022–0.541 | 1.288 ^a | 0.685 | 0.209–3.384 |
| Winter | Female | 56 | 0.145 ^b | 0.111 | 0.033–0.588 | 0.513 ^b | 0.393 | 0.112–2.185 | 0.209 ^a | 0.071 | 0.065–0.363 | 0.111 ^a | 0.136 | 0.018–0.676 | 0.899 ^b | 0.513 | 0.177–2.347 |
| | Male | 57 | 0.126 ^b | 0.066 | 0.030–0.286 | 0.521 ^b | 0.363 | 0.116–2.297 | 0.234 ^a | 0.083 | 0.059–0.478 | 0.069 ^b | 0.045 | 0.020–0.191 | 0.904 ^b | 0.474 | 0.186–2.482 |

AFG₁, aflatoxin G₁; AFB₁, aflatoxin B₁; AFG₂, aflatoxin G₂; AFB₂, aflatoxin B₂; total AF, total aflatoxins.
^{a,b}Mean values within a column with unlike superscript letters were significantly different ($P < 0.05$).

Table 4 Serum aflatoxin levels of the study population according to season and gender: healthy adults living in rural areas of the Mediterranean region of Turkey, 2007–2008

| Season | Sex | n | AFG ₁ (ppb) | | | AFB ₁ (ppb) | | | AFG ₂ (ppb) | | | AFB ₂ (ppb) | | | Total AF (ppb) | | |
|--------|--------|----|------------------------|-------|-------------|------------------------|-------|-------------|------------------------|-------|-------------|------------------------|-------|-------------|--------------------|-------|-------------|
| | | | Mean | SD | Min–max | Mean | SD | Min–max | Mean | SD | Min–max | Mean | SD | Min–max | Mean | SD | Min–max |
| Summer | Female | 88 | 0.131 ^a | 0.064 | 0.038–0.289 | 0.235 ^a | 0.153 | 0.040–1.130 | 0.161 ^a | 0.058 | 0.054–0.446 | 0.099 ^a | 0.055 | 0.023–0.233 | 0.581 ^a | 0.295 | 0.038–1.719 |
| | Male | 38 | 0.101 ^a | 0.061 | 0.033–0.341 | 0.185 ^b | 0.087 | 0.060–0.556 | 0.156 ^a | 0.044 | 0.017–0.222 | 0.074 ^b | 0.047 | 0.026–0.238 | 0.469 ^b | 0.227 | 0.040–1.322 |
| Winter | Female | 86 | 0.075 ^a | 0.103 | 0.032–0.627 | 0.189 ^b | 0.123 | 0.055–0.862 | 0.177 ^a | 0.066 | 0.068–0.410 | 0.034 ^c | 0.009 | 0.020–0.055 | 0.448 ^b | 0.203 | 0.121–1.430 |
| | Male | 49 | 0.062 ^a | 0.053 | 0.032–0.332 | 0.193 ^b | 0.105 | 0.041–0.531 | 0.166 ^a | 0.051 | 0.043–0.323 | 0.053 ^c | 0.068 | 0.021–0.306 | 0.449 ^b | 0.156 | 0.194–0.921 |

AFG₁, aflatoxin G₁; AFB₁, aflatoxin B₁; AFG₂, aflatoxin G₂; AFB₂, aflatoxin B₂; total AF, total aflatoxins.
^{a,b,c}Mean values within a column with unlike superscript letters were significantly different ($P < 0.05$).

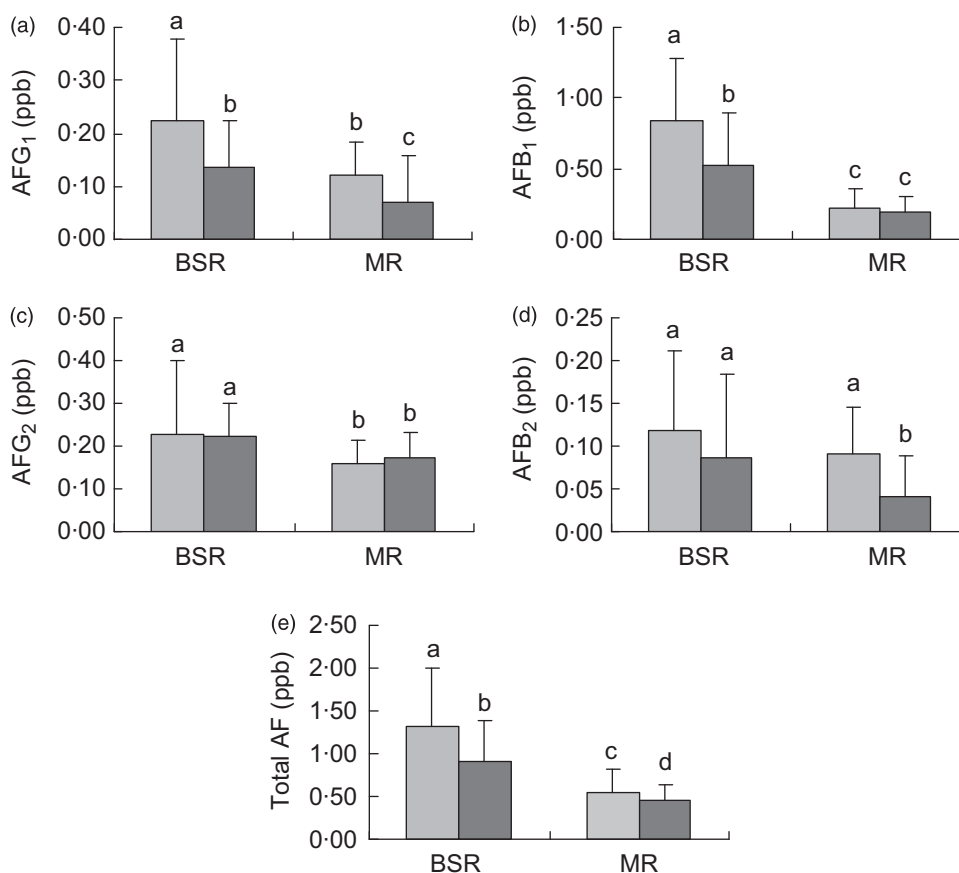


Fig. 1 Serum aflatoxin levels in the healthy adult population living in rural areas of the Black Sea region (BSR) and the Mediterranean region (MR) of Turkey according to season (□, summer (July 2007); ■, winter (January 2008)): (a) aflatoxin G₁ (AFG₁); (b) aflatoxin B₁ (AFB₁); (c) aflatoxin G₂ (AFG₂); (d) aflatoxin B₂ (AFB₂); (e) total aflatoxins (total AF). Values are means with their standard deviations represented by vertical bars; BSR: *n* 110 for summer, *n* 113 for winter; MR: *n* 126 for summer, *n* 135 for winter. ^{a,b,c,d}Mean values with unlike superscript letters were significantly different ($P < 0.05$)

higher in Black Sea samples compared with Mediterranean samples (Fig. 1). AFG₁, AFB₁ and total AF levels in the Black Sea region and AFG₁, AFB₂ and total AF levels in the Mediterranean region were higher in summer compared with winter (Fig. 1). The highest AFB₂ and AFG₂ levels were also seen in the summer samples collected from the Black Sea region, but no significant differences were observed due to the high standard deviation. The lowest AF levels were measured in the winter samples from the Mediterranean region (Fig. 1). Total AF levels were 32% and 18% lower in winter samples compared with summer samples in the Black Sea and Mediterranean regions, respectively.

There was no correlation and relationship between the dependent variables (the levels of AF) and independent variables (age and BMI).

Discussion

The contamination of crops with mycotoxins is a major determinant of trade for all economies. Major risks are

inevitable climatic conditions such as unseasonable rains or high humidity⁽³⁾. The possible role of AF in influencing human health has been researched mainly in relation to their role as a carcinogen. It is well documented that contamination of foods with AF may cause liver cancer in man^(14,16). However, AF also have a major effect on the immune system and these toxins may also affect the epidemiology of many diseases and health risks in those countries where the toxin is uncontrolled⁽⁸⁾. A non-harmful level of AF has not yet been identified.

There are a few studies in the literature demonstrating AF levels in serum^(30,32,33). Corcuera *et al.*⁽³⁴⁾ reported that the level of AFB₁ in plasma from rats given a single dose of 0.25 mg AFB₁/kg body weight by oral gavage was 24.8 ng/ml and 9.5 ng/ml at 10 min and 30 min, respectively. Studies on human serum samples mostly have limited sample sizes compared with the current study. In a study performed by Hassan *et al.*⁽³⁵⁾, the researchers measured the AFB₁ levels in the serum and milk of mothers (*n* 50) and serum of infants (*n* 50) in Egypt. Twenty-four out of fifty mothers and their infants had been contaminated with AF with the following mean

contamination levels: 8.9 (SD 4.2) ng/ml (mothers' serum), 1.9 (SD 0.6) ng/ml (mothers' milk) and 1.8 (SD 0.9) ng/ml (infants' serum). Tchana *et al.*⁽¹⁴⁾ showed that AFB₁ was present in 63.9% of the serum samples obtained from patients with liver diseases (*n* 36) in Cameroon using HPLC. In another study conducted in the UK using ELISA, AF levels of blood donors (*n* 27) were found not to be higher than 20 pg/ml and it was reported that present UK guideline tolerances for AF in imported food were effective in limiting human exposure to toxic AF in the UK diet⁽³⁶⁾. To our knowledge, there is not any study in the literature evaluating the AF levels in the healthy adult population in Turkey. Our study is the first to assess the blood AF levels in healthy adults along with seasonal and regional variations and the correlations with gender in these particular regions. The highest total AF concentration was found in the summer samples collected from the population living in the Black Sea region. The differences between the regions and seasons were statistically significant. In the Black Sea region, AFB₂ levels were found to be higher in females compared with males only in winter. In the Mediterranean region, AFB₁, AFB₂ and total AF levels were higher in females *v.* males only in summer. According to the FFQ, Black Sea residents consumed more cereals including corn and corn products, whereas the Mediterranean population consumed more vegetables, fruits and meat as well as locally produced food. We can suggest that the differences in serum AF levels between the regions are due to the consumption of more cereals in the Black Sea region compared with the Mediterranean region whose residents tend to consume more fresh products. In Turkey, it was reported that the concentration of total AF in wheat samples ranged from 10.4 to 643.5 ng/kg. Sixty per cent of the samples collected in Black Sea and Eastern Anatolia regions of Turkey were found to be positive for total AF⁽²²⁾. In the study of Giray *et al.*⁽³⁷⁾, the levels of AF and ochratoxin A (OTA) in corn samples grown and consumed in the Black Sea region were reported to be higher than in other regions. The humidity of the Black Sea region can cause favourable conditions for the growth of moulds and production of toxins.

The incidence of AF in foods and feeds is relatively high in tropical and subtropical regions, where climatic conditions provide optimal conditions for the growth of moulds^(38,39). Environmental conditions such as temperature, humidity and sunlight can affect the survival of pathogens able to live external to the host, as is the case for mycotoxigenic fungi. Thermo-tolerant species are adapted to warmer climate and, for example, *A. flavus* may become more problematic than *Penicillium verrucosum* (i.e. OTA) in temperate Europe⁽⁴⁰⁾. In the present study, total AF levels were elevated in the summer period compared with winter for two regions. Overall results suggest that the Turkish population living in these regions is continuously exposed to AF, especially in summer. For the

Black Sea region, the consumption of fruits, legumes, corn and cereal products was found to be higher in summer than in winter. The consumption of fruits was found to be higher in summer than in winter for the Mediterranean region. The climate in both the Black Sea and Mediterranean regions is suitable for the occurrence of mycotoxins due to the hot and humid conditions especially in the summer period. To our knowledge, studies in the literature concerning the changes in AF levels in man according to season are limited. The urinary levels of AF and OTA were determined in children living in Sierra Leone in two seasons and the levels of AFB₁ and AFB₂ in the dry season were found to be higher compared with the rainy season⁽⁴¹⁾. In 2007, serum AF levels were also measured in children living in the same city and the rate of detection of AF in the sera of schoolchildren in July was found to be 57%⁽⁴²⁾. Wild *et al.*⁽⁴³⁾ also reported that in Gambia AF-albumin adduct levels were significantly higher in the dry season than in the wet season among 350 subjects. These results are in agreement with the current study as we also determined higher serum AF levels in summer season for both of the regions.

A number of complicated and expensive approaches have been used to determine AF exposure in human populations, including analysis of AF metabolites and AF-DNA or AF-protein adducts⁽⁴⁴⁻⁴⁷⁾. In the present study, we determined AF levels in serum samples after the digestion of serum proteins and this provided instantaneous monitoring of AF exposure in the Black Sea and Mediterranean populations.

In our previous study, we determined the OTA levels in the Black Sea and Mediterranean regions using ELISA. The differences between mean values of OTA in the samples collected in summer and winter for each region were statistically significant⁽⁴⁸⁾, in agreement with the results obtained in the present study. This shows that in summer mycotoxin production increases because of environmental factors. Besides, in such circumstances, conditions of storage might elevate the mycotoxin contamination in stored food products. Synergistic and additive effects between mycotoxins may also enhance their toxicity. Additional studies on the synergism among mycotoxins must be performed to predict possible *in vivo* effects of such multi-contaminations.

In Turkey, the incidence of cancer has increased over the years. The most five frequent types of cancer observed in 2002 in Turkey were lung cancer, breast cancer, stomach cancer, skin cancer and bladder cancer, consecutively⁽⁴⁹⁾. Liver cancer is the eleventh most common type of cancer in males, affecting 2.1 per 100 000 Turkish males, while in females it is the fifteenth most common type of cancer, affecting 1.3 per 100 000 Turkish females⁽⁵⁰⁾. The rough rate of liver carcinoma in Antalya for males and females was 2.5/100 000 and 2.1/100 000, respectively. For the Black Sea region, the rough rate of liver carcinoma in Samsun (a province close to Ordu) for

males and females was reported to be 2.0/100 000 and 0.5/100 000, respectively⁽⁵⁰⁾. The exposure to AF in the population living in these regions may be one of the factors affecting the induction of liver cancer.

Although the present study contributes to the literature by presenting the AF levels of the healthy adult Turkish population, it has some limitations. The number of the subjects used in our study could have been increased. The study could have been conducted on more provinces for it to reflect the whole Turkish population. The analyses of AF contamination of foods consumed in the two provinces could have given more strength to the current study, as this would reflect the relationship between the consumption of AF-contaminated food and blood AF levels.

The food consumed is usually food that families have produced, stored and prepared without any consideration for the risks of AF contamination. Where trade does occur, the least contaminated foods and feeds are exported; this may lead to enhanced exposure of the producers, because the more highly contaminated products are retained at home for consumption by a population that is already at the greatest risk of AF exposure⁽⁵¹⁾.

Conclusion

We suggest that novel training programmes on AF contamination should be developed, especially for farmers. New scientific knowledge and improved techniques for harvesting, handling and storage can reduce or eliminate the contamination problem with mycotoxins. Besides, in scientific practice, the importance of overall daily dietary intake of all fungal toxins should not be underestimated. Moreover, a wide variety of foods, particularly cereals, should be monitored routinely for mycotoxins to protect human health and the risk of economic loss.

Acknowledgements

Sources of funding: This study was funded in part by a grant from Hacettepe University Research Fund (contract grant number: 0401301002). The funder had no role in the design, analysis or writing of this article. *Conflict of interest:* The authors declare that there are no conflicts of interest. *Authors' contributions:* S.A., S.S. and P.E. contributed to study concept and design, analysis and interpretation of the data, statistical analysis of the data, and drafting of the manuscript. B.K.G. contributed to study supervision, study concept and design, analysis and interpretation of the data, and drafting of the manuscript. G.S. contributed to study concept and design, and drafting of the manuscript. All authors have seen and approved the final version of the manuscript.

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