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ORIGINAL CONTRIBUTION

The effects of season and gender on the serum aflatoxins and ochratoxin A levels of healthy adult subjects from the Central Anatolia Region, Turkey

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Abstract
Purpose This study was undertaken to determine the effects of season and gender on serum aflatoxin (AF) levels (AFG_1, AFB_1, AFG_2 and AFB_2) and ochratoxin A (OTA) concentrations of healthy adult population living in Central Anatolia Region of Turkey.
Methods AF levels were measured by high-performance liquid chromatography (HPLC) and OTA levels were measured by enzyme-linked immunosorbent assay (ELISA) in serum samples of healthy adults ($n = 233$).
Results In summer and winter, total AF levels in females were 0.98 ± 0.10 and 0.94 ± 0.12 ng/ml and in males 1.35 ± 0.17 and 0.93 ± 0.11 ng/ml, respectively. Male subjects had significantly higher serum total AF levels in summer compared with females (~38 %). There was no marked seasonal change in AFG_1, AFB_1 and AFG_2 concentrations in the whole population, except AFB_2. Both of the genders had significantly higher OTA levels in winter compared with summer (~60 %).
Conclusions Overall results suggest that Central Anatolia residents are continuously exposed to AFs and OTA. Besides, season and gender can be effective in mycotoxin exposure.

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Introduction

Aflatoxins (AFs) and ochratoxin A (OTA) occupy peerless positions among other mycotoxins. The extensive assessment of their toxicology combined with the research of their molecular epidemiology has supplied a foundation for quantitative risk assessments based upon a comprehension of their modes of action [1, 2].

AFs are naturally occurring mycotoxins that are produced by different species of *Aspergillus*, the most notable ones being *Aspergillus flavus* and *Aspergillus parasiticus* [3]. AFs may be present in a wide range of food commodities, particularly cereals, oilseeds, spices and tree nuts. Maize, nuts (peanuts and Brazil nuts), pistachios, chilies, black pepper, dried fruit and figs are all known to be high-risk foods for aflatoxin (AF) contamination [4–6]. AF contamination is a universal problem, and AF levels in the foodstuff can vary from less than 1 μg/kg (1 ng/ml) to greater than 12,000 μg/kg (12 ppm) [7].

There are four major types of AFs occurring naturally: aflatoxin B_1 (AFB_1), aflatoxin B_2 (AFB_2), aflatoxin G_1 (AFG_1) and aflatoxin G_2 (AFG_2). *A. flavus* produces only B_1 and B_2, whereas *A. parasiticus* produces B_1, B_2, G_1 and G_2 [3, 8, 9]. AFs have been shown to be potent carcinogens, mutagens and teratogens [10–12]. AFB_1 is classified as *carcinogenic to humans* (Group I) by The International Agency for Research on Cancer (IARC) [3]. It requires metabolic activation to its ultimate carcinogenic form, aflatoxin-8,9-epoxide [13, 14].

Ochratoxins are toxic fungal mycotoxins which are produced by several food-borne species of the genera

Aspergillus and *Penicillium* as secondary metabolites. OTA is one of the most prevalent mycotoxins in the world, and it can be present in wide variety of foods, such grains, wine, dried grapes and coffee [15, 16]. It is well documented that OTA is a potent nephrotoxin which induces renal cell adenomas and carcinomas in both sexes of rats and mice [17, 18]. Several studies have demonstrated that OTA exposure is associated with Balkan endemic nephropathy (BEN) and with the etiology of human urinary tract tumors [19, 20] although a direct causal relationship is still under debate and mechanism of carcinogenicity of OTA is not clearly demonstrated. There is sufficient evidence in experimental animals for the carcinogenicity of OTA, and it is classified as *possible carcinogenic to humans (Group 2B)* by IARC [3].

Turkey has been facing the AF contamination problem for nearly half a century. Different foods produced within the country or exported are the main causes of AF exposure [33]. Several studies have been conducted on the levels of AFs and OTA in different foodstuff consumed and produced in Turkey [4, 5, 21].

Taking into account all the available information and data, this study has been undertaken to investigate the seasonal variability in AF and OTA levels in healthy adult subjects living in Central Anatolia Region of Turkey.

Materials and methods

Chemicals and reagents

HPLC grade methanol (MeOH), acetonitrile (ACN), dichloromethane, trifluoroacetic acid (TFA) and AF standard solutions (AFG_1, AFB_1, AFG_2 and AFB_2) were purchased from Sigma-Aldrich (St. Louis, MO). All other chemicals and solvents were also from Sigma-Aldrich. RIDASCREEN® OTA ELISA kits were obtained from R-Biopharm AG (Darmstadt, Germany).

Subjects and sampling

The study was conducted in the villages and suburbs of Ankara, the capital city of Turkey in June 2007 and January 2008. Ankara is located in Central Anatolia Region and can represent the dietary habits of the Central Anatolian people. The sampling size was selected by a web-based sampling program (RAOSOFT). The volunteers were chosen by cluster sampling method. Our sample size represents the population with the margin of error (the amount of random sampling error) as 5 %, and our confidence interval (a type of interval estimate of a population parameter) was 90 %. The blood samples were drawn from 120 (summer) and 113 (winter) volunteers (who were living in that region for more than 5 years) from 2 villages (Saraykoy and Ortakoy) in health care centers. Subjects had no history of renal and hepatic disease. The ages of the subjects were between 18 and 64 in the whole study group ($n = 233$). In each cluster, the distributions of the age and sex were normal. For summer AF detections, we collected 120 samples from adults (69 female and 51 male). For winter, we tried to reach to the same subjects; however, we could not attain to 9 male subjects; so we collected a total of 113 samples from the same subjects (71 female and 42 male), except two samples from females were only collected for winter. For the OTA detections, whole study group was not used due to the limitations in the sample volumes. As serum OTA and AF analyses are not diagnostic or descriptive tests, we were not able to find sufficient number of volunteers. On the other hand, insufficient blood volumes drawn from the some of the volunteers limited our OTA measurements throughout the study. Instead, we randomly chose mostly matched samples for the determination of OTA [for summer 34 samples (15 female and 19 male) and for winter 42 samples (25 female and 17 male)].

Fasting blood samples (3–5 ml) were collected in the morning in July 2007 (summer) and January 2008 (winter). Freshly drawn blood samples were centrifuged at $800 \times g$ for 15 min and the sera were separated. All samples were aliquoted and stored at −20 °C until analysis.

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

Dietary information, including the level and frequency of food intake, was collected through a standard food-frequency questionnaire. The weights of all subjects were also recorded. The overall impact of mycotoxins on health, however, is dependent on the concentrations and duration of exposure, the toxicity of the compound, the body weight of the individual, the synergistic effects of mycotoxin and environmental factors. Therefore, we calculated the BMIs of the subjects according to the information obtained from the questionnaires.

Determination of aflatoxin levels

After digestion of serum protein [22, 23], the extraction method modified from Nelson et al. [24] was performed. About 1 ml of serum sample was diluted with 2 ml of n-hexane and mixed for 1 min. After the centrifugation at $5{,}000 \times g$ for 5 min, upper n-hexane phase containing serum lipids was removed. This process was repeated for two more times. In each time, n-hexane phase was removed. About 1 ml of chloroform was added to the rest

part of the serum. The solution was centrifuged again at 5,000×*g* for 10 min after the shaking vigorously. The chloroform layer was transferred to another tube. This process was repeated for three more times. The chloroform phases were collected and evaporated to complete dryness under the nitrogen stream.

The dry residue was derivatizated with TFA according to the pre-column derivatization procedure of AOAC Method 971.22 [25]. Determination of AFB_1, AFB_2, AFG_1 and AFG_2 levels in the derivatized standards and samples was carried out by HPLC equipped with an auto sampler (Hewlett Packard [HP] Agilent 1,100 Series, Vienna, Austria) using a fluorescence detector (excitation at 360 nm, emission at 430 nm). A Spherisorb S5ODS2 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. AF standards used were ranged between 0.005 and 1 ng/ml. The retention times for the AFs were 6.2 min for AFG_1, 8.2 min for AFB_1, 11.8 min for AFG_2 and 17.0 min for AFB_2. Recovery studies were performed on blank samples of serum spiked with levels of 0.1, 0.25 and 0.5 ng/ml for each AF standard and repeated for three times. The average recoveries were 69.7 % for AFG_1, 79.6 % for AFB_1, 101.3 % for AFG_2 and 107.4 % for AFB_2. The concentrations of AFs in the samples were calculated using the calibration curves of peak area prepared for each AF standard separately. The detection limits (LOD) were determined as 0.025 ng/ml for AFG_1 and AFB_1, 0.01 ng/ml for AFG_2 and 0.02 ng/ml for AFB_2. The quantification limits (LOQ) were determined as 0.176 ng/ml for AFG_1, 0.106 ng/ml for AFB_1, 0.242 ng/ml for AFG_2 and 0.139 ng/ml for AFB_2. Recovery studies were performed on blank samples of serum spiked with levels of 0.1, 0.25 and 0.5 ng/ml for each AF standard and repeated for three times. The average recoveries were 69.7 % for AFG_1, 79.6 % for AFB_1, 101.3 % for AFG_2 and 107.4 % for AFB_2.

Determination of serum ochratoxin A levels

Acidified serum sample (2 ml) was extracted with dichloromethane (4 ml). After the centrifugation at 3,500×*g* for 15 min, upper aqueous phase was removed and 2 ml of the clear dichloromethane layer was transferred into another tube and extracted with 0.13 M sodium hydrogen carbonate buffer, pH 8.1 (1:1). After centrifugation at 3.500×*g* for 5 min, the upper layer was collected. This process was repeated and upper layers were combined, diluted with HCl (0.75 ml, 1 N) plus dichloromethane (2 ml) and centrifuged (3,500×*g*, 5 min). Dichloromethane layer was evaporated under the nitrogen stream. The residue was dissolved in 1 ml of 0.13 M sodium hydrogen carbonate buffer, and 50 µl sample was used per well of the ELISA plate. The OTA measurements were performed spectrophotometrically at 450 nm according to the procedure of RIDASCREEN OTA immunoassay for the quantitative analysis of OTA. Special software RIDA®SOFT Win was used for the evaluation of the absorbance values. OTA standards used were ranged between 25 and 2.025 ng/L. Recovery studies were performed on serum samples spiked with level of 300 ng/L of OTA, and the average recovery was calculated as 108.80 ± 7.71 %. The detection limit was 25 ng/L.

Estimation of dietary intake of OTA

The daily intake levels of OTA were estimated from the concentration in serum samples using equation below according to Breitholtz et al. [26].

Daily intake levels (ng/kg bw/day) = Cp × 1.34.

Cp: serum concentration of OTA (ng/ml).

Statistical analysis

The results were expressed as mean ± standard error of mean (SEM). 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 differences among the groups were evaluated with Kruskal–Wallis followed by the Mann–Whitney U test using a Statistical Package for Social Sciences (SPSS) Program version 17.0. p values <0.05 were considered as statistically significant.

Results

Demographic characteristics of the subjects

According to the answers obtained from the questionnaires, most of the subjects were from low-/middle-income families. The population was fed mostly with cereals and legumes. White bread and whole wheat bread were the choices for bread consumption, and fruit and vegetable consumption was low compared with cereal and legume consumption. The mean age of the whole population was 32.11 ± 8.45 years. The mean BMI of males in winter was 27.43 ± 0.95 kg/m^2, while in females it was calculated as 26.44 ± 0.90 kg/m^2. In summer, the mean BMI of females was 28.72 ± 0.79 kg/m^2 while in males it was found as 29.01 ± 0.82 kg/m^2. Central Anatolia Region in named as the “Cereal Silo” of Turkey and that is the reason why cereal consumption is very high among the residents.

Serum aflatoxin levels

The mean and median AF levels with seasonal differences of all subjects are summarized in Table 1. The differences between all AFG_1, AFB_1 and AFG_2 levels for summer and winter samples were statistically insignificant; however, winter AFB_2 levels were found to be significantly lower than summer AFB_2 levels ($p < 0.05$). The seasonal variations in total AF levels are given in Fig. 1a. Total AF levels showed an increase of 16 % in summer; however, the difference between summer and winter samples was not statistically significant. Females had significantly lower AFG_1 levels than males in summer ($p < 0.05$). In winter, AFG_1 levels were lower in male subjects vs. female subjects, but the difference was not significant ($p > 0.05$) (Table 2). There was not a marked difference between AFB_1 levels in summer and winter samples when we compared the genders. Besides, we did not observe a significant difference between females and males either in summer or in winter. The same was also valid for AFG_2 levels. Though we did not observe any significant change in AFB_2 levels between summer and winter in female subjects, there were marked changes between summer and winter samples of male subjects ($p < 0.05$). The seasonal variations in total AF levels in female and male subjects are given in Fig. 1b. Male subjects had significantly higher serum total AF levels in summer compared with winter ($p < 0.05$). The 25th, 50th, 75th and 100th percentile of AFs in different seasons are given in Table 2. Therefore, we can state that gender as well as season may play a role in the AF exposure.

Serum ochratoxin A levels

The mean and median OTA levels of different seasons are summarized in Table 3. Winter samples had significantly higher OTA levels (59 %) compared with summer samples. The seasonal differences in OTA levels in male and female subjects are also summarized in Table 4. The female winter samples had markedly higher OTA levels (60 %) compared with summer female samples. Besides, winter male samples showed significantly higher OTA levels (61 %) compared with summer male samples.

Table 1 Age and body mass index (BMI) of the subjects from Central Anatolia Region, Turkey

	Age (years)	BMI (kg/m^2)
Female summer ($n = 69$)	32.01 ± 9.12	28.72 ± 0.79
Female winter ($n = 71$)	31.12 ± 8.24	26.44 ± 0.90
Male summer ($n = 51$)	30.87 ± 8.47	29.01 ± 0.82
Male winter ($n = 42$)	29.25 ± 8.14	27.43 ± 0.95

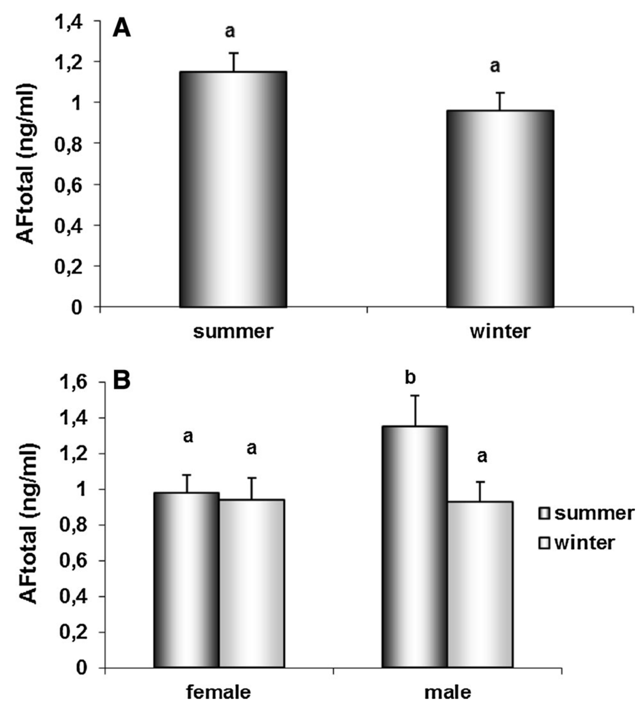

Fig. 1 Total aflatoxin levels of subjects from Central Anatolia Region, Turkey. **a** The seasonal variations in total AF levels. **b** The seasonal variations in total AF levels in female and male subjects. Results for mean values are given as ±SEM. $p < 0.05$ is considered as statistically significant. [a,b]Means with each bar that do not share the same letters are significantly different from each other

The daily intake levels for OTA are given in Table 5. Winter daily intake levels of OTA were significantly higher (59 %) compared with summer daily intake levels. The daily intake levels for OTA in male and female subjects are also given in Table 5. The daily intake levels of OTA in winter female subjects were markedly higher (60 %) compared with summer female OTA intake levels. Besides, OTA daily intake levels of winter male samples were significantly higher (61 %) compared with summer male OTA intake levels.

Discussion

Early work showed that experimental animals and humans have the metabolic capacity to convert AF into the carcinogenic metabolite, AF epoxide [1, 6, 27, 28]. Subsequent dose–response studies as well as case studies performed on small groups of human subjects in India, China, Malaysia, The Gambia and Kenya evaluated both dietary AF intake and levels of urinary AF biomarkers [29–32]. However, there are not many studies in the literature on the serum AF levels of different populations and present studies were performed on limited number of subjects [33, 34].

Table 2 Seasonal differences in aflatoxin levels of female/male and total healthy subjects from Central Anatolia Region, Turkey

	AFG1 (ng/ml)	AFB1 (ng/ml)	AFG2 (ng/ml)	AFB2 (ng/ml)
Female summer ($n = 69$)	0.48 ± 0.04^{a}	0.26 ± 0.03^{a}	0.22 ± 0.03^{a}	0.21 ± 0.02^{ab}
	Median: 0.42	*Median: 0.20*	*Median: 0.17*	*Median :0.20*
	Min: 0.04	*Min: 0.03*	*Min: 0.01*	*Min: 0.04*
	Max: 1.04	*Max: 1.48*	*Max: 0.82*	*Max: 0.60*
	Detectable: 61	*Detectable: 64*	*Detectable: 58*	*Detectable: 45*
	Non-detectable: 8	*Non-detectable: 5*	*Non-detectable: 11*	*Non-detectable: 24*
Female winter ($n = 71$)	0.65 ± 0.09^{ab}	0.22 ± 0.02^{a}	0.22 ± 0.02^{a}	0.16 ± 0.02^{ab}
	Median: 0.54	*Median: 0.18*	*Median: 0.18*	*Median: 0.12*
	Min: 0.04	*Min: 0.03*	*Min: 0.03*	*Min: 0.02*
	Max: 5.22	*Max: 0.68*	*Max: 0.73*	*Max: 0.52*
	Detectable: 49	*Detectable: 55*	*Detectable: 49*	*Detectable: 44*
	Non-detectable: 22	*Non-detectable: 16*	*Non-detectable: 22*	*Non-detectable: 27*
Male summer ($n = 51$)	0.73 ± 0.13^{b}	0.28 ± 0.03^{a}	0.26 ± 0.03^{a}	0.22 ± 0.02^{a}
	Median: 0.45	*Median: 0.24*	*Median: 0.21*	*Median: 0.18*
	Min: 0.05	*Min: 20.03*	*Min: 0.03*	*Min: 0.02*
	Max: 4.14	*Max: 0.92*	*Max: 0.90*	*Max: 0.67*
	Detectable: 48	*Detectable: 47*	*Detectable: 44*	*Detectable: 42*
	Non-detectable: 3	*Non-detectable: 4*	*Non-detectable: 7*	*Non-detectable: 9*
Male winter ($n = 42$)	0.51 ± 0.04^{ab}	0.22 ± 0.03^{a}	0.21 ± 0.03^{a}	0.14 ± 0.02^{b}
	Median: 0.47	*Median: 0.17*	*Median: 0.16*	*Median: 0.11*
	Min: 0.04	*Min: 0.03*	*Min: 0.02*	*Min: 0.02*
	Max: 1.19	*Max: 0.78*	*Max: 0.73*	*Max: 0.47*
	Detectable: 35	*Detectable: 487*	*Detectable: 31*	*Detectable: 32*
	Non-detectable: 7	*Non-detectable: 4*	*Non-detectable: 9*	*Non-detectable: 10*
Total summer ($n = 120$)	0.59 ± 0.06^{a}	0.27 ± 0.02^{a}	0.24 ± 0.02^{a}	0.20 ± 0.01^{a}
	Median: 0.31	*Median: 0.21*	*Median: 0.18*	*Median: 0.18*
	Min: 0.02	*Min: 0.03*	*Min: 0.02*	*Min: 0.04*
	Max: 4.14	*Max: 1.48*	*Max: 0.90*	*Max: 0.67*
	Detectable: 109	*Detectable: 111*	*Detectable: 102*	*Detectable: 87*
	Non-detectable: 11	*Non-detectable: 9*	*Non-detectable: 18*	*Non-detectable: 33*
Total Winter ($n = 113$)	0.59 ± 0.06^{a}	0.22 ± 0.02^{a}	0.21 ± 0.02^{a}	0.15 ± 0.01^{b}
	Median: 0.50	*Median: 0.17*	*Median: 0.16*	*Median: 0.11*
	Min: 0.04	*Min: 0.03*	*Min: 0.02*	*Min: 0.02*
	Max: 5.22 Detectable: 84	*Max: 0.78*	*Max: 0.73*	*Max: 0.52*
	Non-detectable: 29	*Detectable: 93*	*Detectable: 82*	*Detectable: 76*
		Non-detectable: 20	*Non-detectable: 31*	*Non-detectable: 37*

Results for mean values are given as ±SEM

$p < 0.05$ is considered as statistically significant

[a,b] Means with each column that do not share the same letters are significantly different from each other

Studies on human serum samples mostly have limited sample sizes compared with the current study. Hassan et al. [35] measured the AFB_1 levels in the serum and milk of mothers ($n = 50$) and serum of infants ($n = 50$) in Egypt. Twenty-four out of 50 mothers and their infants had been contaminated with AF with the following mean contamination levels of 8.9 ± 4.2 ng/ml (mothers' serum), 1.9 ± 0.6 ng/ml (mothers' milk) and 1.8 ± 0.9 ng/ml (infants' serum). Tchana et al. [36] showed that AFB_1 was present in the 63.9 % of the serum samples obtained from liver patients ($n = 36$) in Cameroon using HPLC. On the other hand, the urinary levels of AF and OTA were determined in children (134 boys and 110 girls) living in Sierra Leone in two seasons, and the levels of AFB_1 and AFB_2 in the dry season were found to be higher compared with the rainy season [37]. In 2007, serum AF and OTA levels were also measured in children living in the same city and the rate of detection of AF and OTA in the sera of school children in July was found to be 57 and 19 %, respectively [38].

Over 100 nations worldwide have set maximum tolerable levels (MTLs) of AF in food in order to limit AF exposure [39]. In industrialized nations, these standards provide public health protection, but seem to have little

Table 3 Seasonal differences in 25 percentile, 50 percentile, 75 percentile and 100 percentile levels of aflatoxin levels in healthy subjects from Central Anatolia Region, Turkey

	AFG1 (ng/ml)	AFB1 (ng/ml)	AFG2 (ng/ml)	AFB2 (ng/ml)
25th percentile				
Summer	0.15	0.06	0.03	0.04
Winter	0.14	0.04	0.03	0.03
50th percentile				
Summer	0.32	0.15	0.11	0.12
Winter	0.40	0.12	0.11	0.06
75th percentile				
Summer	0.59	0.29	0.27	0.24
Winter	0.63	0.25	0.27	0.15
100th percentile				
Summer	1.29	0.54	0.49	0.40
Winter	1.19	0.47	0.46	0.32

Table 4 Seasonal differences in ochratoxin A levels of female/male and total healthy subjects from Central Anatolia Region, Turkey

	Number of samples	Serum OTA concentrations (ng/ml)			
		Min	Max	Median	Mean
Female Summer	15 *Detected: 15* *Non-detected: 0*	0.03	1.55	0.17	0.31 ± 0.10^{a}
Female Winter	25 *Detected: 24* *Non-detected: 1*	0.05	1.12	0.43	0.49 ± 0.05^{b}
Male Summer	19 *Detected: 19* *Non-detected: 0*	0.07	1.01	0.21	0.32 ± 0.06^{a}
Male Winter	17 *Detected: 11* *Non-detected: 6*	0.05	0.90	0.47	0.51 ± 0.05^{b}
Total Summer	*34* *Detected: 34* *Non-detected: 0*	0.03	0.02	0.21	0.31 ± 0.05^{a}
Total Winter	*42* *Detected: 35* *Non-detected: 7*	0.05	0.11	0.44	0.50 ± 0.04^{b}

Results for mean values are given as ±SEM

$p < 0.05$ is considered as statistically significant

[a,b] Means in columns that do not share the same letters are significantly different from each other

effect in less developed countries, for several reasons [7]. First, the food consumed from subsistence farms, which are widespread in less developed countries, does not usually enter any regulatory inspection for AFs [40, 41]. Second, many people consume such high levels of contaminated products that their daily AF exposure would still render them vulnerable to disease in these countries [42]. Third, if less developed countries attempt to export food, they may find their export markets severely jeopardized by strict AF standards. This results in potential risk of exporting the best foods and keeping the worst domestically [43].

OTA existence has been demonstrated in different biological fluids. Studies on seasonal variability of OTA levels in human biological fluids are limited [12, 44]. The mean OTA levels do not exceed 1 ng/ml in healthy populations of most European countries [45]. Scott et al. [46] found 0.88 ng/ml as the mean level of OTA in 144 Canadian subjects. In Japan, the researchers detected plasma OTA levels in 184 healthy volunteers living around Tokyo, with 85 % of them found to be positive. However, the mean value of OTA was 0.068 ng/ml, which is lower than that the mean levels reported in Europe and Turkey, possibly due to completely different dietary habits, such as consumption of fish and other marine species [47].

There are studies in the literature concerning the serum OTA levels of different healthy populations, including Turkey [48, 49]. In the present study, we found that the mean OTA concentration in summer was 0.31 ± 0.05 ng/ml, while in winter OTA levels were 0.50 ± 0.04 ng/ml, still not exceeding 1 ng/ml. Only two samples in summer and two samples in winter exceeded 1 ng/ml. In both of the genders, winter serum OTA concentrations were markedly higher vs. summer samples. Rosner et al. [50] measured the serum levels of OTA in German population ($n = 927$). The percentage of the positive specimens was 98.1, and 1.9 % of specimens exhibited levels <0.06 ng/ml. The majority of the specimens were concentrated in the 0.11–0.50 ng/ml range. Cholmakov-Bodechtel et al. [51] conducted a survey on 2005 adults and 574 children from the various regions of Germany in order to calculate the mean OTA intake from food consumption. These subjects completed a 3-day food consumption protocol and a food-frequency questionnaire covering the preceding 4 weeks. The daily total OTA intakes by children and adults were calculated to be 27.9 and 39.9 ng. The relative intake by children is 0.97 and by adults 0.58 ng/kg b.w. The highest intake, 1.3 ng/kg b.w., was found for children of age 4–6 years. Because of their high consumption frequency, cereal products are the main contributors for all groups of exposed subjects. Furthermore, coffee (14.5 %) and beer (9.8 %) play a role in adults, whereas in children fruit juices, primarily red grape juice (15.4 %) and the food group of sweets (chocolate/cereal-based bars/biscuits) (9.9 %), were found to be important OTA sources.

Coronel et al. (2011) investigated the plasma OTA concentrations of the Spanish population ($n = 325$) living in a plain region ($n = 243$) and a mountain region

Table 5 Seasonal differences in estimated daily ochratoxin A intake of female/male and total subjects from Central Anatolia Region, Turkey

	Estimated daily intake levels (ng/kg bw/day)			
	Min	Max	Median	Mean
Female summer ($n = 15$)	0.04	2.08	0.23	0.41 ± 0.13^{a}
Female winter ($n = 25$)	0.07	1.51	0.58	0.66 ± 0.07^{b}
Male summer ($n = 19$)	0.10	1.35	0.29	0.43 ± 0.07^{a}
Male winter ($n = 17$)	0.23	1.20	0.63	0.69 ± 0.07^{b}
Summer total ($n = 34$)	0.04	2.08	0.28	0.42 ± 0.07^{a}
Winter total ($n = 42$)	0.07	1.51	0.59	0.67 ± 0.05^{b}

Results for mean values are given as ±SEM

$p < 0.05$ is considered as statistically significant

[a,b] Means in columns that do not share the same letters are significantly different from each other

($n = 82$) of the province of Lleida, during winter and summer seasons. Half of the samples presented OTA levels up to 0.5 ng/mL, the 76.9 % of the population presented levels up to 1 ng/mL, 21.2 % ranged between >1 and 4 ng/mL, and only a 1.8 % was contaminated in levels from >4 to 11 ng/mL, with mean a mean of 0.86 ng/ml and median of 0.54 ng/mL. The distribution was similar when the population was sorted by gender and age. When the variation of the seasons in each region was evaluated separately, significant differences were found between summer and autumn in the mountain region ($p = 0.0194$), and the highest median was found in summer [52].

Palli et al. [53] reported that 85 % of the serum samples collected from healthy Italian adults contained 0.2–1.0 ng/ml OTA (mean: 0.56 ng/ml) and males had higher OTA levels compared with females. Peraica et al. [54] performed a study on plasma OTA levels of healthy people living in five Croatian cities. Both of the research groups observed higher OTA levels in summer period in contrast to our results obtained from Central Anatolia Region and in agreement with our recent study conducted on the serum samples obtained from Black Sea and Mediterranean regions. Erkekoglu et al. [44] suggested that the higher values of OTA in serum samples collected during the summer could have been related to particular climate conditions and seasonal variations in dietary and drinking habits of Italian, Croatian and Turkish people. However, regional differences influence nutritional habits of residents and in Central Anatolia Region, and the dietary habits differ from the seaside parts of Turkey as well as from Europe. Inhabitants of Central Anatolia mainly consume cereals, legumes and bread rather than vegetables or fruits, particularly in winter. Therefore, storage of cereals and legumes in improper conditions after harvesting may contribute to higher serum OTA levels observed in winter samples.

Assaf et al. [55] reported that mean plasma OTA concentration of blood donors in Lebanon was 33 % OTA positive, with no gender and age difference. Wafa et al. [56] reported that mean urine OTA concentrations ($n = 25$) was 0.01 ng/ml in Egyptian population. In a study by Khalef et al. [57], 67 % of the serum samples obtained from Algerian population were OTA positive and the mean OTA level was determined as 2.8 ng/ml, which is a higher value compared with the results of the current study. Mean serum OTA levels of the healthy population in Tunisia varied with location, dietary habits, the way of food storage and/or climate, as also observed in the study of Erkekoglu et al. [44] conducted in Black Sea and Mediterranean regions of Turkey and herein. Maaroufi et al. [58] determined an overall incidence of OTA contaminated sera of 52 % in the control population (range 0.7–7.8 ng/ml).

In a study performed on 62 healthy people, the average OTA concentration was found to be 0.53 ± 1.00 ng/ml [59]. Abid et al. [60], a follow-up study between 1991 and 2000, concerning OTA levels of kidney disease patients and healthy population in Tunisia, reported that mean blood OTA levels were 3.35 ng/ml in 1991, 2.25 ng/ml in 1994, 2.6 ng/ml in 1997 and 1.22 ng/ml in 2000 in the healthy groups. The percentage of OTA positive individuals in the healthy control group significantly decreased throughout the years; however, all values are higher than the mean OTA levels in Turkish healthy population possibly due to climate and geographical conditions or dietary habits. Filali et al. [61] reported the mean plasma OTA concentration of Moroccan population as 0.29 ng/ml, with no gender difference. Morocco residents seemed to be less contaminated compared with their neighbors.

There are also some studies on blood OTA levels of nephropathy and cancer patients in Middle East and North African countries. Ozcelik et al. [49] analyzed the serum OTA levels in the patients with different kinds of urinary disorders (hemodialysis patients $n = 35$; peritoneal dialysis patients $n = 28$; bladder cancer patients $n = 15$; renal stone patients $n = 15$; totally $n = 93$) and in control group ($n = 40$) in Isparta, Turkey, and OTA levels were 0.40 ± 0.28 ng/ml in healthy adults in the study, which is a close value to the results of the current study.

Blood OTA concentration has been reported to be a good index for predicting OTA intake. Therefore, the daily intake of OTA was estimated from the mean concentration of serum samples according to Breitholtz et al. [24]. In a study performed by Thuvander et al. [62], the mean plasma level of OTA was determined as 0.2 ng/ml in Scandinavian healthy people ($n = 406$), without any gender difference. The daily intake of OTA was calculated as 0.26 ng/kg bw/day. Due to the high consumption of locally produced pork

with high OTA levels, Assaf et al. [55] reported that in healthy blood donors ($n = 250$) living in Lebanon the mean daily intake level of OTA was 0.23 ng/bw/day.

Several organizations, including World Health Organization (WHO), tried to bring a clear assumption to the "tolerable daily intake (TDI)" levels of OTA in humans. The TDI of 5 ng OTA/kg b.w./day based on their calculations on the carcinogenicity studies [63]. WHO suggested a provisional TDI of 16 ng OTA/bw/day. Joint Expert Committee on Food Additives (JECFA) established a Provisional Tolerable Weekly Intake (PTWI) of 112 ng/kg bw/week (equivalent to 16 ng/kg bw/day) and reconfirmed this value, but rounded it off to 100 ng/kg b.w. per week corresponding to approximately 14 ng/kg bw/day [64]. All daily intake values estimated in the present study are below the TDI values given by all the regulatory authorities, and our results suggest that Central Anatolia population seems to be continuously exposed to OTA.

In the recent years, several biomarkers were suggested to indicate the exposure to AFs. AF exposure biomarkers include urinary AF metabolites and AF-albumin adducts in peripheral blood. These biomarkers have been applied in studies of many populations worldwide and are well validated [65]. On the other hand, microRNAs are suggested to be potential biomarkers for predicting the onset of AF exposure in human beings [66]. Besides, the results of a number of studies confirm that accumulation of a mutated form of protein p53 after exposure to high levels of AF may be a factor that triggers the production of anti-p53 antibodies, which are suggested to be important biomarkers of AF exposure [67].

Although the present study contributes to the literatures by presenting the AF and OTA levels of healthy Turkish population living in Central Anatolia Region (the second largest region of Turkey), it has some limitations: The number of the subjects used in this study could have been increased. The study could have been conducted on more provinces. The analyses of AF and OTA contamination of foods consumed in Ankara could have given more strength to the current study. On the other hand, adding the measurement of one of AF biomarkers would have amplified our findings.

In conclusion, detecting OTA and AF levels in serum provides very important information about the degree of exposure of population in Central Anatolia Region. It is also used in estimation of the overall risk characterization. Mycotoxin risk management strategies need to be taken into account as an option, and necessary measures must be implemented to ensure that foods not complying with the maximum levels of AF and OTA are not marketed. Surveillance on food contaminants including AFs and OTA should be conducted by government and related ministry continuously. Using scientific knowledge and improved techniques for harvesting, handling and storage will reduce or eliminate contamination problem with mycotoxins and prevent the threat to human health and the risk of economic loss.

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Conflict of interest The authors declare no conflict of interest.

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