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Assessment of aflatoxin exposure using urine biomarker in pregnant and non-pregnant women in Yazd, Center of Iran

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Abstract

Background: Aflatoxins (AFs) are one of the most prevalent toxins, which long-term exposure to them could be a risk factor for liver cancer. AFM_1 is the hydroxylated metabolite of AFB_1, therefore, the presence of AFM_1 in urine samples can give an appropriate estimation of dietary AF exposure in human.

Methods: The present study aimed to evaluate the excretion level of AFM_1 in urine samples of pregnant and non-pregnant women in Yazd, Iran. A total of 85 urine samples (42 pregnant and 43 non-pregnant) were selected randomly from women who had referred to health centers of Yazd during March to May 2017. From each participant, a 72-hour dietary recall was asked and the data were recorded and later analyzed by ELISA kits.

Results: The results showed that the mean level of AFM_1 in pregnant and non-pregnant women was 8.23 ± 2.9 and 35.5 ± 1.05 pg mL^{-1}, respectively. Excretion of AFM_1 in urine samples had a significant relationship with some demographic factors and type of consumed foods ($P < 0.05$).

Conclusion: There was a significant relationship between the education level, place of residence, and the consumption of nuts with the excretion of AFM_1. It can be concluded that some foods distributed in Yazd are contaminated with AFs, and a significant number of people are exposed to high concentrations of AFM_1.

Keywords: Diet, Demographic factors, Cancer, Aflatoxin M_1, Iran

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Introduction

Aflatoxins (AFs) are one of the most prevalent toxins, so that long-term exposure to them may lead to acute and chronic negative health effects and could be a risk factor for liver cancer. AFs are mainly produced by *Aspergillus flavus*, *Aspergillus parasiticus*, and *Aspergillus nomius* (1). These toxins are found in most of the crops, such as corn, peanuts, pistachio, soybean, coconut, rice, milk, dairy products, etc (2-4) and exist in multiple types including B_1, B_2, G_1, and G_2 (5). Aflatoxin B_1 (AFB_1) is highly toxic, mutagenic, teratogenic, and carcinogenic (6), which has been classified as Group 1 carcinogens by the International Agency for Research on Cancer (IARC) (7). For human, the extent of exposure to AFs depends on the level of consumption of polluted foods and the level of the toxin in different foods, such as dairy products (e.g. milk, cheese, and yogurt) (8), meat, meat products (9), rice (10), and eggs (11). New-born infants can be exposed to AFs whether in uterus or through breast feeding (12). Environmental exposure to AFs may cause liver cancer (13); furthermore, the existence of AFs in food may lead to both acute and chronic negative health effects (1) including immune-system suppression and liver cancer (14). Clinical symptoms of acute toxicity of AFs in human are as follows: vomiting, abdominal pain, pulmonary edema, liver necrosis (15), and sudden death (16). Besides, AFs can cause growth retardation and delayed development during infancy (17). Consumption of AF-contaminated food during pregnancy have adverse effects on infant growth, as it could be a teratogenic and jaundice-inducing toxin (18). Regulations for maximum contamination level of AFs in foods vary from country to country. According to the standards of the European Commission, the maximum allowable level of AFB_1 and total AFs in crops can be 2 and 4 μg/kg, respectively (19), while the Iranian National Standards Organization has assigned a maximum level of 5 ng/g for AFB_1 and 15 ng/g for total AFs in crops (20).

AFM_1 and AFM_2, which are hydroxylated metabolites of AFB_1 and AFB_2, can be found in milk, milk products, urine, and blood (1). After ingestion, AFB_1 is converted to its carcinogenic forms through metabolism by cytochrome P-450 enzymes in liver (1). Hence, the content of AFM_1 in urine sample can be a clue to determine the extent of human exposure to AF (21), therefore, considering the complexity of investigating the exposure to AFs by determining their concentrations in consumed foods, some researchers have suggested to estimate it by quantitative assessment of metabolites in milk, blood, and urine (22).

Urinary biomarker has been used to estimate exposure to AFB_1 in many studies. In a study by Lei et al, AFM_1 was detected in 84% of the pregnant women (23). Ezekiel et al reported that the mean urinary AFM_1 levels were significantly higher in the semi-urban population compared to the rural population (24). Ali et al also reported that the mean level of urinary AFM_1 was higher in winter than in summer, and level of AFM_1 in urine did not show significant associations with the participants' food consumption pattern (25). Although our previous study revealed the occurrence of AFM_1 in urine of volunteers (26), there is not enough information about urinary concentration of AFM_1 in Iranian women. Since AFs introduce mutagenic, carcinogenic, and teratogenic properties which cause anemia in women, particularly in pregnant women (27), and are risk factors for jaundice in infants (28), the main aim of this study was to evaluate the excretion level of AFM_1 in urine samples of pregnant and non-pregnant women living in Yazd, Iran, as a biomarker of AFB_1 exposure. Furthermore, the relationship between demographic factors and dietary intake with AFM_1 excretion was investigated.

Materials and Methods

Participant recruitment

This study was conducted on resident population of Yazd, the capital of Yazd province located in the central part of Iran (29). A total of 85 urine samples (42 pregnant and 43 non-pregnant) were taken randomly from women who had referred to Yazd health centers (Azadshahr, Panbekaran, Maskan, and Safaieh) during March to May 2017. Before beginning the study, informed consent was obtained from participants to follow ethical regulations. Participants were selected among those who were eligible, such as pregnant women (20-year-old or older, in the last trimester of pregnancy) and non-pregnant women (20 to 50 years of age, not in lactation or menstrual period). Prior to urine sampling, each participant was asked about demographic factors (Table 1). Also, food frequency questionnaire (FFQ) was asked for intake of typical food items, such as rice, milk, dairy products, meat, nuts, traditional confection of Yazd, traditional *Halva* and *Tahini* (made from sesame and sugar) in 72 hours. The sterile plastic falcons were used to collect urine samples. The samples were transferred immediately to the laboratory and stored at -20°C.

Determination of the AFM_1 metabolite in urine

ELISA kits were applied to measure the level of AFM_1 in urine samples, and ELISA kits (6827 BN Arnhem, Euro Proxima Company, Arnhem, Netherlands) were used for detection of AFM_1. The features of ELISA kits are as following: LOD: 6 pg/mL, LOQ: 9.42 pg/mL, recovery: 95%. The samples were prepared according to the manufacturer's instruction. All of the samples were centrifuged (Kubota Centrifuge Model 2810, Tokyo, Japan) at 2000×g for 10 minutes at 4°C. Then, they were diluted with sample dilution buffer with volume ratio of 1:1. Subsequently, 100 μL of the AFM_1 standard solution and test samples (diluted urine) and 10 μL of standard 80 ng/L were added in duplicate to the wells of the plate, followed by 1 hour incubation at room temperature (25°C) at dark. The solutions were removed from the *microtiter* plate and

Table 1. Socio-demographic characteristics of participants

Socio-demographic Factors	Pregnant No. (%)	Non-pregnant No. (%)
Age (year)		
20-30	40 (95.2)	14 (32.6)
30-40	2 (4.8)	13 (30.2)
40-50	0 (0)	16 (37.2)
Education level		
Primary	8 (19)	26 (60.5)
Secondary	22 (52.4)	10 (23.3)
University	12 (28.6)	7 (16.3)
Occupational status		
Employed	10 (23.8)	11 (25.6)
Housewife	32 (76.2)	32 (74.4)
Monthly income		
< 195$	8 (19)	12 (27.9)
195 – 387 $	22 (52.4)	17 (39.5)
>387 $	12 (28.6)	14 (32.6)
Geographic region		
Azadshahr	10 (23.8)	10 (23.3)
Panbekaran	10 (23.8)	10 (23.3)
Maskan	11 (26.2)	11 (25.6)
Safaieh	11 (26.2)	12 (27.9)

washed three times with rinsing buffer. In the next step, 100 µL of conjugate (Aflatoxin M-HRPO) was added to each well of the plate, except zero standard maximal wells, and incubated for 30 min at 25°C at dark. The solutions were again removed from the microtiter plate and washed three times with rinsing buffer. Thereafter, 100 µl of enzyme substrate was added to each well and incubated for 30 minutes at room temperature (20-25°C). The reaction was stopped by adding 100 µL stop solution to each well, and absorbance of each well was read at 450 nm via a microplate reader (ELX 800 UV, Bio-Tek Instruments, Inc. optical density of solutions in 6-, 12-, 24-, 48 or 96-well microplates in the wavelength range from 400 nm to 750 nm). A standard curve was drawn by plotting absorbance values against AFM_1 concentrations. The absorption intensity was inversely proportional to AFM_1 concentration in urine samples.

Statistical analysis

The data were analyzed using SPSS version 20 (IBM SPSS Inc., Chicago, IL). Mann-Whitney U test was used to compare the two groups and determine the relationship between food intake and the concentration of AFM_1 in urine samples. Kruskal-Wallis one-way ANOVA test was used to determine the relationship between types of consumed rice (Native, Imported, or Both), place of residence (Azadshahr, Panbekaran, Maskan, Safaieh), and concentration of AFM_1. Statistically significant level was considered at $P < 0.05$.

Results

Forty-two pregnant and forty-three non-pregnant respondents, with a mean age of 31.4 ± 9.5 years (25.2 ± 3.4 years for pregnant women and 37.4 ± 9.6 years for non-pregnant women), participated in this study. The results showed that from a total of 85 participants, 80 people (94.1%) had excreted AFM_1 in the range of 0.4 - 67.8 pg mL^{-1}. Excretion of AFM_1 in urine samples had a significant relationship ($P < 0.05$) with some demographic factors, such as age, education level, income level, and place of residence, as well as consumption of some types of foods including rice, nuts, traditional confection of Yazd, Tahini and traditional Halva. However, there was no significant relationship between the excretion of AFM_1 and occupational status as well as consumption of milk, dairy products, and meat (Tables 2 and 3). In the non-pregnant group, all patients (100%) had excreted AFM_1, and a significant relationship was observed between some demographic factors (i.e., age, body mass index [BMI], education level, income level, and place of residence) and food consumption (i.e., rice, nuts, traditional confection Yazd, Tahini and traditional Halva) with the excretion of AFM_1. AFM_1 was found in 90.47% of urine samples of pregnant women. There was a significant relationship between the education level, place of residence, and the consumption of nuts with the excretion of AFM_1 (Tables 4 and 5).

Discussion

The presence of AFM_1 in urine samples can give an appropriate estimation of dietary AF exposure in human (21). The present results showed that AFM_1 level in Yazd population was higher than that in some countries, such as Brazil, Egypt, and Guinea (22,30,31). Urinary tests have shown that high percentage of the population of several countries in Asia and Africa are exposed to AFs. For instance, Sabran et al (21) found that the mean concentration of AFM_1 in urine samples of Malaysian population (n = 22) was 42.1 pg mL^{-1}. The range of urinary AFM_1 detected by Ali et al was 31-348 ng mL^{-1} (32). De Cássia Romero et al evaluated urinary excretion of AFM_1 in Brazilians, and reported that 65% of them showed contamination with a mean concentration of 1.8 pg mL^{-1} (22). Polychronaki et al detected AFM_1 in urine samples of 38% of Egyptians and 86% of Guineans (30). Ali et al. reported that AFM_1 was detected in more than 40% of all urine samples of Bangladeshi population at a range of 1.7-104 pg/mL in summer and at a range of 1.8-190 pg/mL in winter season (25). Schwartzbord et al reported a significant correlation between the dietary intake of AFB_1 and the AFM_1 concentration in urine samples (33). In the present study, the excretion of AFM_1 was observed in about 94% of women in Yazd, and the range of excreted AFM_1 was 0.4-67.8 pg mL^{-1} with a mean concentration of 22.03 ± 1.9 pg mL^{-1}. There was a significant relationship between the excretion of AFM_1 and age, as the excretion of AFM_1 increased by increasing age. Hence, the highest levels of AFM_1 excretion were observed in age group of older than 30 years. On the other hand, there was no significant association between age and excretion of AFM_1 in pregnant women; nonetheless, excretion of AFM_1 was observed in some of older participants (31-40 years) of this group. Lei et al reported a significant relationship between the excretion level of AFM_1 and age, as they noticed that the concentration of AFM_1 was conspicuously high in the age group of older than 28 years (23). As well, the results of the study done by Ali et al revealed that the highest mean AFM_1 level (101 ± 71 pg mL^{-1}) was observed in the age group of 50-60 years (25). Epidemiological studies have shown that there is a direct relationship between intake of AFB_1 and liver cancer, so that over 90% of liver cancer cases are diagnosed after the age of 45 years, when the excretion level of AFM_1 is high (34).

To avoid the effect of false weight gained during pregnancy, this parameter was assessed by BMI only in the non-pregnant group. According to the results, there was a significant relationship between BMI and the excretion of AFM_1, as the highest levels of AFM_1 excretion were observed in women with BMI>30. Polychronaki et al observed a significant association between the excretion of AFM_1 and BMI, as the concentration of AFM_1 was very high in obese people (BMI >30) (35).

In various studies, different relationships between the excretion of AFM_1 and geographical location have been

Table 2. Correlation between AFM1 excretion in urine samples and socio-demographic factors

Socio-demographic Factors	Sample Tested (N)	Positive Samples N (%)	Min-max (pg mL^{-1})	Mean±SD (pg mL^{-1})
Pregnancy status				
Pregnant	42 ^{a*}	37 (88.1)	0–23.8	8.23±2.9
Non-pregnant	43^{b}	43 (100)	13.6–67.8	35.5±1.05
Age (year)				
20-30	54^{a}	49 (90.7)	0–54	14.4±1.2
30-40	15^{b}	15 (100)	0.4–54.6	29.9±1.8
40-50	16^{c}	16 (100)	13.6–67.8	37.7±1.6
BMI (kg/m^2)**				
<24.9	12^{a}	12 (100)	16.8–54	28.63±1.3
25-29.9	20^{b}	20 (100)	13.6–54.6	33.35±3.2
>30	11^{c}	11 (100)	23.8–67.8	46.99±1.5
Education level				
Primary	34^{a}	34 (100)	1.6–60.2	31.3±1.6
Secondary	32^{b}	29 (100)	0–53	14.2±1.3
University	19^{b}	17 (100)	0–67.8	18.4±1.8
Occupational status				
Employed	21^{a}	19 (90.5)	0–67.8	20.1±1.9
Housewife	64^{a}	61 (95.3)	0–60.2	22.6±1.6
Monthly income				
<195$	20^{a}	20 (100)	1.8–60.2	31.9±1.9
195–387 $	39^{b}	35 (89.7)	0–55.4	17.7±1.4
>387 $	26^{b}	25 (96.2)	0–67.8	20.8±1.6
Geographic region				
Azadshahr	20^{a}	20 (100)	1.8–59.4	24.7 ±1.7
Panbekaran	20^{b}	20 (100)	7.2–67.8	32.7±1.9
Maskan	22^{ac}	19 (86.4)	0–55.4	14.6±1.1
Safaieh	23^{c}	21 (91.3)	0–47.8	17.4 – 1.4

*In each section, different letters on the same column indicate that there is a statistical significant difference between each factor ($P<0.05$).
**Because of false weight gain during pregnancy, BMI was calculated only in non-pregnant women.

reported. Lei et al found no significant relationship between the excretion of AFM_1 and region of residence (23), while Polychronaki et al observed a significant correlation between the excretion of AFM_1 and geographical location in children of Egypt and Guinea (30). In the present study, a significant relationship between the excretion of AFM_1 and geographic region was found. The average excretion of AFM_1 in Panbekaran locale was higher than that of other three regions (Azadshahr, Maskan, and Safaieh). Based on the answers to the question about the way of procuring food, most residents of Panbekaran bought their foods in bulk and non-packaging form from traditional shops. Undesirable ventilation and lack of hygiene in the traditional shops provide the conditions for fungal growth and AF production on food products. Approximately, 50% of Panbekaran locals had incomes less than 195 dollars per month, and in comparison with Azadshahr, Maskan, and Safaieh regions, the highest rate of excretion of AFM_1, in each income level, belonged to Panbekaran locals. Research done in Malaysia showed that the rate of excretion of AFM_1 was higher in participants with low education levels ($P=0.4$). In the present study, the rate of excretion of AFM_1 was also higher in people with low education levels ($P<0.001$), where 40% of participants had primary education, 37.6% secondary education, and 22.4% university education. The mean level of AFM_1 excretion (41.2 pg mL^{-1}) in participants with primary education was more than that in those with secondary or university education. Therefore, it was found that the excretion of AF had a significant relationship with the level of education in both pregnant and non-pregnant groups. In both groups, the highest level of AFM_1 excretion was observed in participants with primary education. On the other hand, there was no significant association between occupational status and excretion of AFM_1. The level of AFM_1 excretion in housewives was 11% more than that in

Table 3. Correlation between AFM1 excretion in urine samples and consumption of foods in recent 72 hours

Foods Consumed in Recent 72 Hours	Sample N	Positive Samples N (%)	Min-max (pg mL^{-1})	Mean ± SD (pg mL^{-1})	*P* value
Milk					
Yes	73	69 (94.5)	0–67.8	23.1 ± 1.8	0.1
No	12	11 (91.6)	0–39.8	15.9 ± 1.1	
Meat					
Yes	71	67 (94.3)	0–67.8	21.9 ± 1.9	0.9
No	14	13 (92.8)	0–54.6	22.2 ± 1.5	
Traditional confection					
Yes	29	29 (100)	1–67.8	29.6 ± 3.4	0.002
No	56	51 (91.1)	0–60.2	18.1 ± 2.1	
Nuts					
Yes	53	49 (92.5)	0–67.8	29.8 ± 3.4	0.02
No	32	31 (95.5)	0–59.2	17.7 ± 2.1	
Traditional Halva					
Yes	17	17 (100)	1.2–67.8	35.7 ± 5.4	0.001
No	67	64 (93.3)	0–59.2	19.1 ± 1.6	
Rice					
Native					
Yes	23	19 (82.6)	0–37	15.8 ± 1.9	0.4
No	8	8 (100)	1-26.1	12.5 ± 1.1	
Imported					
Yes	29	29 (100)	3.2–60.2	28.6 ± 3.8	0.03
No	5	5 (100)	5.4–32	13.4 ± 1.1	
Both					
Yes	15	14 (93.3)	6.4–67.8	29.1 ± 4.7	0.1
No	5	5 (100)	0–47.8	14.9 ± 2.8	

employed women. According to a similar study conducted by Mason et al, there was no relationship between the demographic factors and excretion of AFM_1, while a significant difference between the excretion of AFM_1 and consumption of nuts as well as traditional confection was observed (26).

The correlation between the level of AFM_1 in urine samples and the type of consumed food has also been investigated. According to a study done by Jager et al, a significant relationship ($P<0.05$) was found between consumption of milk, dairy products, corn, white hominy, and bean with excretion of AFM_1 (31). In Haitian population, an association between the consumption of maize, peanut products, and milk with the excretion of AFM_1 has been reported, wherein the level of AFM_1 in urine was found to be significantly associated with peanut consumption ($P<0.05$) (36). The nuts, as rich sources of protein, fatty acids, fiber, and vitamins (37), are recommended to be taken during pregnancy (38,39). Based on the results of this study, there was a significant association between AFM_1 excretion and consumption of nuts. In several studies, it has been reported that some edible nuts in Iran are contaminated by AFB_1 (4,40). Rice is one of the most important foods widely used around the world including Iran, and it is used not only as a food, but also as an ingredient for a variety of foods, such as noodles, snacks, pasta, and chips (41). After bread, rice is the staple food in Iran. The average consumption of bread and rice in Iran is 107-286 grams per person per day (42). If it is assumed that the average total AF in rice is 9.56 µg kg^{-1}, and 30% of it is eliminated in cooking process, each person receives 11.9-31.8 ng kg^{-1} b.w of AF by daily consumption of rice (43). More than 50% of the rice consumed in Iran, especially imported rice, is contaminated with AFs (10,44). The results of the present research revealed that there was a significant association between the rice consumption ($P=0.01$) and, type of consumed rice ($P=0.02$) with the excretion of AFM_1. The excretion of AFM_1 in people who had consumed rice was higher than those who had not. The average excretion of AFM_1 in the people who had consumed imported rice was approximately 2-fold higher than those who had not ($P=0.03$), while the average excretion of AFM_1 in the people who had consumed Iranian rice was merely

Table 4. Occurrence of AFM1 in urine samples of pregnant and non-Pregnant women according to the socio-demographic factors

Socio-demographic Factors	Pregnant			Non-pregnant		
	Min-Max (pg mL^{-1})	Mean ± SD Error (pg mL^{-1})	Correlation	Min-Max (pg mL^{-1})	Mean ± SD Error (pg mL^{-1})	Correlation
Age (year)						
20-30	0 – 23.8	8.07 ± 1.08	a*	13.6 – 39.8	26.5 ± 2.1	a
30-40	5.4 – 17.4	11.4 ± 6	a	16.8 – 54.6	33.8 ± 4.2	ab
40-50	-	-	-	18.2 – 67.8	44.1 ± 3.2	b
BMI** (kg/m^2)						
<24.9	-	-	-	13.6 – 35.2	25.7 ±1.8	a
25-29.9	-	-	-	16.8 – 60.2	37.18 ± 3.2	b
> 30	-	-	-	16.8 – 67.8	43.6 ± 4.1	b
Education level						
Primary	1.8 – 23.8	15.35 ± 2.8	a	13.6 – 67.8	41.2 ± 2.8	a
Secondary	0 – 19	8.4 ± 1.3	b	17.8 – 59.2	30.5 ± 3.3	b
University	0 – 14.6	4.1 ± 1.2	b	16.8 – 33.2	25.5 ± 3.1	b
Occupational status						
Employed	0 – 19	6.1 ± 2.1	a	16.8 – 67.8	35.37 ± 4.2	a
Housewife	0 – 23.8	8.9 ± 1.2	a	13.6 – 60.2	35.5 ± 2.4	a
Monthly income						
<195$	1.8 – 17.4	10.7± 1.6	a	24.2 – 60.2	45.4 ± 3.5	a
195–387 $	0 – 19	7.7 ± 1.52	a	13.6 – 55.4	30.1 ± 2.8	b
>387 $	0 – 23.8	7.2 ± 2.1	a	16.8 – 67.8	33.6 ± 3.5	b
Geographic region						
Azadshahr	0.4 – 17.4	8.06 ± 1.8	a	27.8 – 59.4	39.7 ± 3.1	abd
Panbekaran	7.2 – 23.8	16.02 ± 1.6	b	30.8 – 67.8	49.5 ± 3.8	bcd
Maskan	0.3 – 17.4	5.3 ± 1.8	a	13.6 – 55.4	25.54 ± 3.5	cd
Safaieh	0 – 11.5	4.2 ± 1.1	a	16.8 – 47.8	29.5 ± 2.5	acd

*In each section, different letters on the same column indicate that there is a correlation between factors.
**Because of false weight gain during pregnancy, BMI was calculated only in non-pregnant women.

about 26% higher than those who had not ($P > 0.05$). The results of a cohort study done in Bangladesh indicated that the urinary AFM_1 level had a direct correlation with the consumption of rice ($P = 0.09$) (32). Ferri et al observed a significant association between the excretion of AFM_1 and consumption of rice products (45). Interestingly, the present study not only showed no significant association between the meat consumption and the excretion of AFM_1, but also showed that the people who had consumed meat had excreted lower AFM_1. This finding is probably due to the existence of proteins and vitamins in meat. The previous studies on animals have shown that the diets which are rich of vitamins and proteins can reduce the toxic effects of AF (1). De Cássia Romero et al reported no significant relationship between the consumption of milk or milk-based products and the urinary excretion of AFM_1. The findings of the present study also showed no significant relationship between the excretion of AFM_1 and the consumption of milk and dairy products.

It was observed that the urinary excretion level of AFM_1 in participants who had consumed traditional confection of Yazd was significantly higher than those who had not. The main ingredients of Yazd traditional confections are sugar, wheat flour, walnut, pistachio, and almond. Due to the economic benefits, some Iranian confectioners prefer to use low-price nuts which usually have low quality and are more polluted with AFB_1 (46). In both groups of this study, approximately 34% of participants had consumed traditional confection, and the AF level in their urine samples was in the range of 1.2-67.8 pg mL^{-1}. Sesame is the main constituent of traditional Halva and Tahini. In some studies, it has been reported that AFB_1, which is the most toxic form of AFs and a risk factor for human health, exists in sesame seeds (47). The results of the present study revealed that the people who had consumed traditional Halva and Tahini excreted higher levels of AF than those who had not. Besides, it was indicated that the mean level of AFM_1 excretion in non-pregnant women was 4.3-fold higher ($P < 0.001$) than that in pregnant women.

The obtained results exhibited that overweight, age, and the consumption of traditional confections Halva and Tahini could be the most influential factors in the high

Table 5. Occurrence of AFM1 in urine samples of pregnant and non-pregnant women according to the type of foods consumed in recent 72 hours

Foods Consumed in Recent 72 Hours	Pregnant			Non-pregnant		
	N (%)	Mean±SD Error (pg mL^{-1})	*P* value	N (%)	Mean±SD Error (pg mL^{-1})	*P* value
Milk						
Yes	36 (85.7)	8.4±1.1	0.4	31 (72.1)	37.6±2.5	0.7
No	6 (14.3)	6.9±1.7		12 (27.9)	30.1±3.1	
Meat						
Yes	35 (83.3)	7.7±1.2	0.2	32 (74.4)	35.8±2.5	0.9
No	7 (16.7)	10.5±2.4		11 (25.6)	34.6±3.7	
Traditional confection						
Yes	14 (33.3)	10.3±2.1	0.1	16 (37.2)	41.5±3.7	0.03
No	28 (66.7)	7.1±1.2		27 (62.8)	31.9±2.2	
Nuts						
Yes	24 (57.1)	11.1±1.5	0.007	15 (34.9)	42.4±4.1	0.01
No	11 (42.9)	6.1±1.3		28 (65.1)	31.8±2.2	
Traditional Halva and Tahini						
Yes	8 (19.1)	10.3±2.6	0.3	12 (20.9)	43.7±3.2	0.04
No	34 (80.9)	7.7±1.1		31 (79.1)	32.3±2.4	
Rice						
Native						
Yes	14 (78.2)	6.9±1.4	0.7	9 (64.3)	30.2±6.3	0.8
No	4 (21.8)	9.7±4.3		5 (35.7)	28.6±2.4	
Imported						
Yes	6 (60)	11.3±2.3	0.2	14 (73.7)	47.8±3.3	0.009
No	4 (40)	5.5±3.1		5 (26.3)	20.24±3.2	
Both						
Yes	6 (66.7)	8.5±4.1	0.5	7 (70)	35.7±3.8	0.6
No	3 (33.3)	6.4±3.4		3 (30)	33±1.7	

excretion of AFM_1 in urine. In addition to the possibility of their contamination with AF, the consumption of traditional confections Halva and Tahini can cause obesity because of their high oil and sugar content. Obesity is associated with increased risk of liver disease (48). As previously reported, all these factors (i.e., old age, obesity, and intake of AF) are the risk factors for liver cancer (34,49,50) and there is a significant correlation between excretion of AFM_1 in urine and liver cancer (51). Thus, these findings could be considered as a risk assessment.

In the present study, the heterogeneity of food contamination resulted from pregnant women's tendency to consume the best food with the best quality during pregnancy, the differences in type of consumed foods, and the differences in age were the impressive factors which could affect the excretion of AFM_1 in the two groups (pregnant and non-pregnant). As shown in the results, the excretion rate of AFM_1 increased with increasing age (the mean age of the pregnant and non-pregnant women was 25 and 37 years, respectively). The lower excretion of AFM_1 in pregnant women, as compared to non-pregnant ones, can be attributed to the transmittance of AF to the fetus through the umbilical cord (52,53) which causes a decrease in the excretion of AFM_1 in pregnant women. Moreover, the consumption of different types of vitamins (1,54) such as vitamins A, B, C, and D, and supplements during pregnancy may result in a decrease in the excretion of AFM_1 in pregnant women. Nonetheless, these findings need to be further investigated.

Based on the obtained results in the present research, it seems that some of the foods used in Yazd are highly contaminated with AFs, and many people of Yazd are exposed to high concentrations of AF and its associated health risks. This health problem needs the attention of government and health departments, as the government can play a key role in reducing the exposure to AFB_1. Furthermore, health officials should warn the public (especially vulnerable groups, such as children, elderly, and old pregnant women) about the consumption of AF-contaminated foods and the harmful effects associated with it. Also, detecting and introducing the sources of AF can help consumers to avoid consuming foods which are most likely to be contaminated. As complete elimination of AF from the diet is impossible, the toxic effects of AF can be reduced by taking vitamins and probiotic products. Because of the dire consequences of exposure to AF for humans and animals, it is of great importance to reduce exposure to AF as much as possible. AF exposure can be prevented or decreased by improving and enforcing safety regulations, changing crop storage practices, detoxification and modifying the dominant diet. For example, it has been shown that by increasing the consumption of probiotic products, the AF exposure can be reduced (55). Certain food preparation techniques, such as fermentation, may reduce the intestinal absorption of AFs (56).

Conclusion

This research aimed to determine the AF exposure of pregnant and non-pregnant women in Yazd, Iran, using urinary biomarkers. The mean level of AFM_1 in pregnant and non-pregnant women was 8.23 ± 2.9 and 35.5 ± 1.05 pg mL^{-1}, respectively. The results revealed that the excretion of AFM_1 in the urine samples had a significant relationship with some demographic factors and type of consumed foods. Besides, it was shown that some consumed foods in Yazd were highly contaminated with AFs. Hence, it can be concluded that many people are exposed to high concentrations of AF and its related hazards; so, as a part of cancer control program, some preventive strategies need to be suggested to reduce AF intake through food.

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Conflict of interests

The authors declare that there is no conflict of interests.

Ethical issues

The authors certify that all data collected during the study are as presented in the manuscript and no data from the study has been or will be published elsewhere separately.

Authors' contributions

All authors participated in the problem suggestion, experiments design, data collection, and manuscript approval.

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