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OCHRATOXIN A DETECTION IN RICE SAMPLES IN MAZANDARAN PROVINCE

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ABSTRACT

Background: Known as one of the most poisonous mycotoxins, ochratoxin A (OTA) currently tops the challenges posed to clinical medicine and food hygiene in the world. OTA is increasingly albeit silently threatening public health and hygiene in all societies. The International Agency for Research on Cancer (IARC) has classified OTA under Group 2B of human carcinogens. The present study was conducted due to the significance of OTA health risks, particularly acute nephrotoxin in humans, and WHO-FAO proposals for a regular monitoring of OTA in rice.
Materials and methods: For this purpose, 220 samples from 10 categories of rice (homegrown and imported) consumed in Mazandaran Province were taken through simple random sampling. The percentage of OTA contamination was measured in ng/g by enzyme-linked immunosorbent assay (ELISA) test. Data analysis was conducted based on eight statistical tests.
Results: The percentage of contamination was measured at 20.45 in domestically grown rice and 13.63 in imported rice. In all samples together, the percentage was 17.73. An average OTA contamination of 3.51% (ng/g) and OTA frequency of 78.6% (ng/g) were detected in all rice samples.The OTA contamination range in all samples varied between unidentifiable levels to 1-10.91 ng/g. In 6% of the samples, OTA concentration of above 5 ng/g was observed. In 21.4% of Iranian rice samples, this concentration was below the limits set by Iran and European Union standards. The level of contamination in both Iranian and imported rice was not seen to exceed the standard 5% level ($P>0.05$). OTA contamination was significantly different in Iranian and imported rice categories ($P<0.05$), but the difference in OTA contamination in the Iranian and imported rice was not significant ($p>0.05$). The level of OTA contamination was diagnosed to be within limits in both Iranian and foreign samples of rice.
Conclusion: The level of OTA contamination in the Iranian rice samples was significantly 0.72% (ng/g) higher than in the imported rice samples. In terms of OTA contamination, no significant difference was seen between the Iranian and imported rice samples.

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Introduction

OTA is a chlorinated isocoumarin derivative amide-bonded to the amino acid L-β-phenylalanine. OTA detection in rice is a must due to four reasons: 1. Toxicity for some animals 2. Thermal resistance (OTA does not disappear in an autoclave after 15-minute exposure to 121 degrees Celsius) 3. Most fungi producing this toxin are likely to grow and produce toxin below 10 degrees Celsius. 4. It is very toxic [1, 2). Recently more studies have concentrated on OTA, which is the main contaminant in stored food (rice). In North Europe and North America, due to climate, cereals and cereal products are the main source of OTA. OTA has been also detected in other food products like grape juice, coffee, pork, poultry and cow

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milk. OTA has already been proven to be instrumental in producing SOS, SCE (sister chromatid exchange), DNA repair outside living organisms and DNA strand separation in the liver and kidney of rats [3, 4]. OTA facilitates the formation of OH in living organisms, treatment of harm from copper and plasmid [5] as well as formation of guanine, which is one of the four main nucleobases found in DNA [6]. Some studies have questioned the suggested role of oxidative metabolism in OTA genotoxicity [7]; however, firm evidence has emerged from recent studies that OTA phenoxyl radical serves as the mediator in the DNA reaction [8, 9]. In the human plasma samples taken in several European countries, OTA has been detected in the range of 0.02-2.3 ng/ml. Among the Balkans' residents [11 countries with a total population of 60 million), OTA levels of up to 100 ng/ml have been detected [10]. In this peninsula, OTA is blamed for the deadly Balkan endemic nephropathy, which is a form of interstitial nephritis. The Balkan residents also suffer from renal cell carcinoma (RCC), pelvic cancer, ureter cancer and bladder cancer [11]. OTA consumption in humans may vary from 0.7 to 4.7 ng/kg of body weight per day. The World Health Organization (WHO) has set the weekly standard level of OTA consumption at 100 ng/kg of body weight [12, 13]. OTA was discovered in Africa in 1965 and its chemical structure was then examined [14]. OTA is known as the most contaminating toxic for cereals (rice) and domestic poultry. It is mainly produced by Aspergillus ochraceus in rice and animal food [15-23]. The hydrolysis of OTA by microorganisms in rumen, cecum and large intestine yields non-toxic OTA (Otα). Acidic hydrolysis gives L-β-phenylalanine and Otα [24-27]. OTA's teratogenic, neurotoxic, genotoxic, immunotoxic and nephrotoxic impacts, particularly its role in renal fibrosis and formation of tumor in the urinary system, are already known [28]. Researchers focused on OTA mainly due to its toxicity for renal cells and the upper urinary tract. Studies conducted on animals show that OTA weakens the body immune system and is a potential cause of kidney cancer. According to IARC, OTA is classified under Group 2B which means possibly carcinogenic to humans [29-32]. Due to its specific characteristics, OTA may cause hormonal imbalance, cytotoxicity, stillbirth, sperm abnormality and male sterility, curb the generation of T and B lymphocytes, weaken immune system, slow antibody production, increase immune cell deaths and delay their replacement [33-36]. Liver and kidney diseases, weakened immune system and birth defects are among the major consequences of OTA in animals [37]. OTA is widely inclined to be bonded to plasma proteins, particularly albumin. It is mainly transmitted via blood all across the body especially to kidneys and it is scarcely accumulated in muscles and fats. OTA's half-life was examined in human volunteers and was reported to be 840 hours [38-41]. The OTA toxicity is so severe that metabolic studies on human beings are ethically banned and limited to in vitro circumstances. The most harmful and irreparable impacts of OTA in human beings are nephropathy, carcinogenesis and teratogenicity [42-44]. Human brain is very sensitive to OTA when it is taking shape during pregnancy. OTA is even able to affect neuron metabolism and cause such diseases as Parkinson's and Alzheimer's [45-46]. Cereal products, particularly rice, have been reported to be among the most important food items contributing to OTA transmission to humans. The reason is that this toxin is highly resistant when exposed to heat during food production and remains almost intact [47-48]. Such factors as the concentration of hydrogen ion, temperature and compounds exiting in the environment affect the level of destruction of OTA molecules; however, thermal processes like boiling, frying, cooking and fermentation do not reduce the level of destruction [49]. The European Union has set the maximum OTA level at 3.5 ng/g for cereals, 10 ng/g for dry fruits and 0.5 ng/g for baby food [50]. Numerous studies have so far been conducted by leading international agencies on the detection of rice contamination with OTA. They all show that the OTA level varies between below 10 ng and 200 ng [51-54]. The European Commission has set the maximum limit for OTA at 5 ng/g in cereals [55]. Iran National Standard Organization (INSO) has set the maximum limit for OTA levels in human and animal food and rice at 5 ng/g [56-57]. Rice is the dominant staple in Asian countries with a daily consumption of 158-178 grams per person, or an average of 165 grams per person, with an average weight of 60 kilograms [58]. Rice is the main source of energy for people and it has a share of more than 16% per capita. Cereals and cereal products are responsible for more than 70% of daily diets. Iran is the 14th largest consumer of rice with a per capita consumption of 40 kilograms a year [59]. According to Food and Agriculture Organization (FAO), per capita rice consumption is above 56.9 kg a year, which is reported at 67.9 kg a year for developing countries. Iran's per capita rice consumption is more than double the developed countries. It is ranked 24th in the world. Based on figures provided by Iran's National Nutrition and Food Technology Research Institute, rice accounted for 15% of Iranian people's per capita energy needs in 2004 [60-61]. Rice is the second most consumed food item in Iran and comes second to wheat in terms of nutritive value. According to FAO data, rice is the main source of nutrition for 4.2 billion people. Rice supplies 30% of food diet energy needs and 20% of protein intake in the world [62]. Since Mazandaran Province in northern Iran is a main source of rice cultivation and its ecological conditions (pH, temperature and humidity) are conducive to the growth of OTA-generating fungi and OTA-contaminated rice poses risks to health, this study was conducted within the framework of a research project approved by the Research Council of Mazandaran University of Medical Sciences under reference code 91-17 in order to examine 220 samples of rice distributed across the province.

Materials and Methods

This cross-sectional and observational study is a descriptive-analytic one conducted at the Food Quality Control Lab of Mazandaran University of Medical Sciences with the objective of measuring OTA levels in rice consumed in Mazandaran Province. The statistical population comprises homegrown and imported rice distributed across the province. The sampling units include domestically grown rice (Tarom, Tarom Hashemi, Fajr, Neda, Shiroudi and Behnam cultivated in Mazandaran Province) and rice imported from India, Pakistan, Thailand and Uruguay. In this study, the dependent variable is the OTA level and the independent variable is the categories of Iranian and imported rice.

Sampling Method

In stage 1, pilot sampling was conducted with 36 samples selected randomly in counties located in the west, east and center of Mazandaran Province. The findings showed that OTA levels had approximately identical variance in both homegrown and imported rice. The OTA level had an approximately uniform distribution. Therefore, simple random sampling (SRS) has been used in this study.

Sample Size Determination

Since OTA level is a quantitative trait, the sample size determination formula for continuous data was used. Given the size of the population of homegrown and imported rice in the province (N) and the area of paddy fields in the province ($N\rightarrow \infty$), the approximate sample size formula was used. In the present research, we expected to have OTA standard error of the mean and true mean below 0.065 ($r = 0.065$) with a confidence level of 95% ($\alpha = 0.05$). Meantime, in the pilot sampling, the OTA mean and standard deviation in Mazandaran Province were respectively 4.36 and 2.21 ng/g ($\hat{s} = 2.13$, $\hat{\mu} = 4.36$). Calculations showed that 222 samples of rice were sufficient for concluding this research [63].

Sampling Implementation Method

Sampling has been done based on the area of land under cultivation by well-trained experts from rice distribution centers in the cities of Sari, Qaemshahr, Jouybar, Babol, Babolsar, Fereydounkenar and Mahmoudabad. Since the variety of homegrown and domestic rice was identical across the province, sampling in several cities would not significantly affect the bias-torch error. It was necessary to consider identical number of samples for each category of rice in order to reach maximum power in future statistical tests. The sample size was then reduced from 222 to (22x10) 220 , [64]. The criteria for homegrown rice in this study was its production in one of the aforesaid cities and a two to four-month time interval between rice milling and delivery to customers. Sampling was done from each bag of rice to be comprehensive enough for the study. Therefore experts took samples with their special tools from the upper, middle and lower parts of the bags. In order to avert extra costs, only one kilogram of each bag of rice was purchased and carried in nylon bags for sampling. The samples were transferred to the lab in temperature 2-9 degrees Celsius and were kept in -18 degrees Celsius until the test time.

Preparation of Samples

First, 50 grams of rice was grinded and five grams was thrown into a test tube. Then, 10 milliliters of phosphoric acid and 20 milliliters of dichloromethane (Germany's Merck KgaA) were added. It was centrifuged at 2000 rpm for five minutes. The upper layer (phosphoric acid) was removed. The suspension was filtered and 12 milliliters of the filtered liquid was transferred into the test tube to be exposed to mild nitrogen flow under 50 degrees Celsius. After evaporation and drying, 1.5 milliliters of extraction buffer and 2 milliliters of hexane were added to the tubes before five minutes of centrifugation at 2000 rpm. The upper layer (hexane) was removed and 50 microliters of the lower layer was diluted with 200 microliters of diluting buffer [65-68].

OTA Level Detection

In this study, given climatic conditions in Mazandaran Province the ELISA sensitivity to low OTA levels [69-77], for measuring the level of contamination in the rice samples, ELX800TM and OTA ELISA kit (r.biofarm radio screen made in German) and a sampler (Eppendrof) in the volumes of 20-250 microliters were used based on the instructions of the manufacturing company. Fifty microliters of standard solutions and prepared samples (for each standard and sample, a separate sampler splitter has been used) were added into the microplate wells. Twenty-five microliters of conjugate solution (ochratoxin-A-HRP) plus 25 microliters of antibody solution were added into the wells. The microplate was kept far from light under temperature 20-25 degrees Celsius. After the liquid leaves the microplate (by placing it upside down on moisture absorbing papers and knocking on it gently), all wells were washed with 250 microliters of PBS powder buffer twice. Every time after washing the microplate was placed upside down on several layers of tissue paper so that the remaining water would leave entirely (exit of materials not involved in the reaction). Then 100 microliters of substrate was added to each well. The microplate was incubated under temperature 20-25 degrees Celsius for 30 minutes. Some 100 microliters of stop solution was poured into the wells and the level of absorption by each sample was registered by ELISA reader (Stat Fax 2100, USA) for a wavelength of 450 nanometers. The data was recorded based on the level of absorption of each well. The level of absorption (OD) is subtracted from zero standard absorption and multiplied by 100 to give the percentage of absorption. Based on the percentage of absorption of standard samples and OTA levels in the rice samples, a standard calibration curve was sketched. In the final stage, each sample's percentage of absorption was compared with the calibration curve to calculate OTA concentration for each sample in ng/g [78-80].

Statistical Analysis

Descriptive statistics (tables and diagrams) has been used to describe the samples, the Kolmogorov-Smirnov test [81] to examine the normality hypothesis, Levene's test [82] to assess the equality of variances, One-Sample T-Test [83] to assess the existence of contamination in domestic and imported rice, Independent Sample T-Test [84] to compare the significance of OTA difference in the two categories of rice, One-Way ANOVA [85] to understand any significant difference in the OTA concentration between Iranian and imported rice, Brown-Forsythe Test [86] to examine the validity of model in terms of stability of variance of errors, Run Test [86] to examine the independence of errors as well as R-3.2.0 software package and IBM SPSS 24 for relevant statistical calculations.

Results

The findings from 220 rice samples showed that in 47 cases (21.36%) there was no OTA, while in the remaining 78.64%, OTA was seen at 10.91 ng/g (Average OTA level in 173 samples was equal to 4.54 ng/g while the level of this contamination varied from unidentified level to 10.91 ng/g). Average OTA level in domestic and imported rice was respectively 3.87 and 3.15 ng/g. (Tables 1, 2)

Table 1: OTA Levels in Rice Samples in Mazandaran Province (ng/g)

Rice		Frequency	Contamination (%)	Maximum Contamination	Average ($\mu g/kg$)	Standard Deviation	Frequency of Samples Exceeding Maximum Limit
Domestic	Tarom	22	9.09	7.98	3.12	2.22	2
	Tarom Hashemi	22	18.18	10.36	4.07	3.06	4
	Fajr	22	18.18	7.65	3.51	2.11	4
	Neda	22	27.28	9.89	4.27	2.64	6
	Shiroudi	22	27.28	10.91	4.34	2.64	6
	Behnam	22	22.73	8.22	3.88	2.21	5
	Total Domestic	132	20.45	10.91	3.87	2.49	27
Foreign	India	22	13.64	9.20	3.25	2.75	3
	Pakistan	22	13.64	8.39	3.18	2.33	3
	Thailand	22	18.18	8.41	3.40	2.48	4
	Uruguay	22	9.09	6.24	2.76	1.99	2
	Total Foreign	88	13.63	9.20	3.15	2.37	12
Total		220	17.73	10.91	3.51	2.43	39

Table 2: Frequency of OTA Level in Rice Samples in Distribution Centers in Mazandaran Province

Rice	OTA Level ($\mu g/kg$)	Frequency	Relative Frequency Percentage
Domestic	<LOD	24	18.2
	LOD ≤< 2	5	3.8
	2≤< 5	76	57.6
	5≤	27	20.4
Foreign	$< LOD$	23	26.2
	LOD ≤< 2	6	6.8
	2≤< 5	47	53.4
	5≤	12	13.6
Total	$< LOD$	47	21.4
	LOD ≤< 2	11	5
	2≤< 5	123	55.9
	5≤	39	17.7

OTA Levels in Iranian and Imported Rice in Distribution Centers in Mazandaran Province (**Figures**. 1,2)

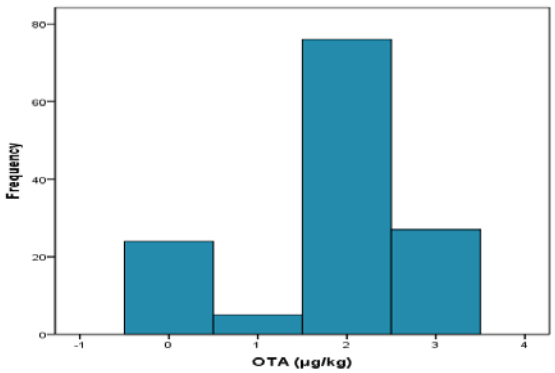

Fig. 1: OTA Levels in Iranian Rice Samples in Mazandaran Province

(On the horizontal axis: 5 = < :3 @ 5 >= >2: 2 @ 2 >= > LOD:1 @ LOD>☺O)

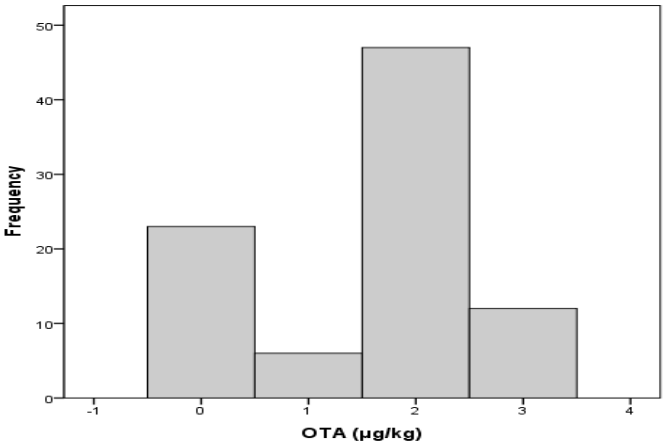

Fig. 2: OTA Levels in Imported Rice Samples in Mazandaran Province

The Kolmogorov-Smirnov test for OTA levels in the rice samples showed that for error rate of 5% (α=0.05), OTA distribution in the imported rice samples was normal (P>0.05). In the domestic rice samples, although the normality of data was rejected by the K-S test (P>5%), it could be concluded based on the Central Limit Theorem that the average OTA distribution in domestic rice samples is asymptotically normal [87]. Of course it could be observed from the P-P plot that the OTA distribution was approximately normal in all domestic and imported samples and the rejection of the idea of normality of domestic rice samples stemmed from the test's high sensitivity to large size samples [88]. (Table 3 , Figure. 3)

Table 3: Kolmogorov-Smirnov Test Results for Single Sample

Rice Type	Number of Samples	Z Test Statistic	Significance Level
Iranian	132	1.411	0.037
Imported	88	0.953	0.323

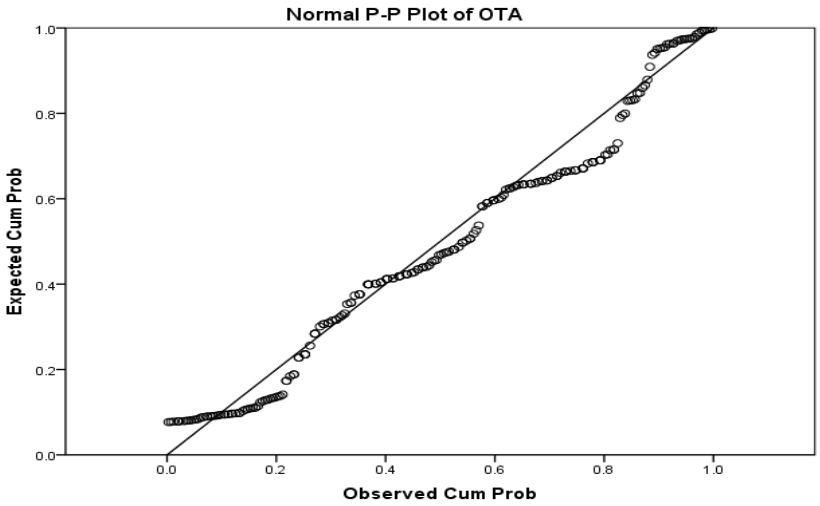

Fig. 3: Normal Probability for OTA Distribution in Mazandaran Province Rice Samples

OTA Contamination Significance in Iranian and Imported Rice

The results from Student's t-test (Table 4) with the hypothesis of normality for Iranian rice showed that at 5%, average OTA amount in Iranian rice samples was not even equal to illegal limits set by the European Union (P>5%). Given the normality of imported rice samples, Student's t-test showed results similar to Iranian rice. Average OTA level for imported rice samples was not observed beyond limits (P>5%).

Table 4: Standard OTA Hypothesis in Iranian and Imported Rice in Mazandaran Province

Student's T-test					
Rice	Null Hypothesis	Alternative Hypothesis	T Test Statistic	Degree of Freedom	Significance
Iranian	$\mu_1 < 5$	$\mu_1 \geq 5$	$\mathbf{-5/23}$	131	1.000
Imported	$\mu_2 < 5$	$\mu_2 \geq 5$	$\mathbf{-7/32}$	87	1.000

The results of Levene's test conducted to assess the homogeneity of variance of OTA levels in domestic and imported sables (Table 5) show that with a 95% confidence level, OTA variance does not differ significantly in domestic rice from foreign rice (P>5%).

Table 5: Levene's Test

Within-Subjects Variable	**F Test Statistic**	**Significance**
Iranian/Imported rice	0.154	0.695

Since the Independent T-Test findings (Table 6) confirmed the normality and homogeneity of variance, at a 4% error rate, no significant difference was observed in the average OTA level in domestic and imported rice samples (P<5%).

Table 6: OTA Average Difference in Iranian and Imported Rice Samples

Independent T-Test						
Source of Change	Number	Average	Variance Deviation	T-Test Statistic	Degree of Freedom	Significance
Iranian	132	3.87	2.49	2.131	218	0.034
Imported	88	3.15	2.37			

OTA Level in Iranian and Imported Rice

The results from Levene's test (Table 7) conducted on domestic rice showed that the OTA variance was approximately the same in the six types of homegrown rice (P>5%). The test produced similar results for imported rice samples, which indicated that OTA dispersion in the four categories of imported rice assessed in this test had no significant difference at 5% error rate (P>5%).

Table 7: Variance Homogeneity in Domestic and Imported Rice in Mazandaran Province

Levene's Test				
Rice	**F-Test Statistic**	**Degrees of Freedom**		**Significance**
		df2	**df1**	
Iranian	0.380	126	5	0.862
Imported	0.213	84	3	0.887

Table 8 shows the findings of one-way analysis of variance (ANOVA) in Iranian and imported rice samples. At 5% error rate, there was no significant difference in the OTA content of Iranian and imported rice (P>5%). The results were similar for imported rice (P>5%).

Table 8: Average OTA Difference in Domestic and Imported Rice in Mazandaran Province

ANOVA				
Rice	**F-Test Statistic**	**Degrees of Freedom**		**Significance**
		df2	**df1**	
Iranian	0.780	126	5	0.566
Imported	0.282	84	3	0.838

Model Reliability Assessment

The results of Brown-Forsythe Test showed that the variance of errors for Iranian and foreign rice samples was quite stable (P>5% for domestic and imported rice). Moreover, Run's test confirmed independence between error terms in the model (P>5%). Changes in average were assessed for the Iranian and imported rice samples (Figure. 4 , 5).

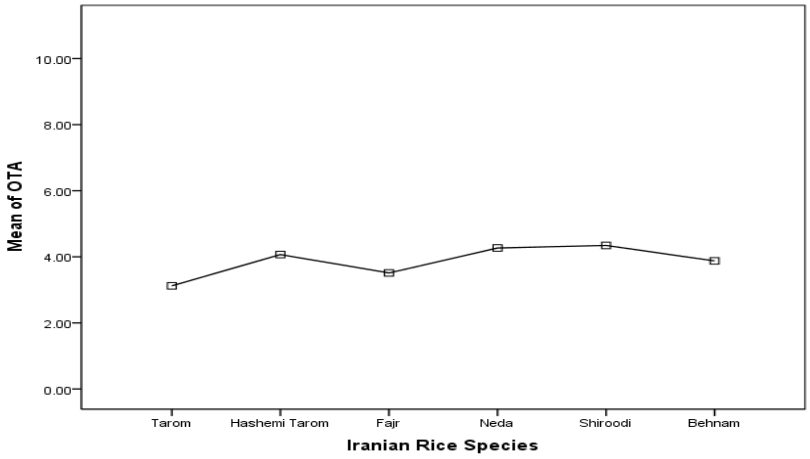

Fig. 4

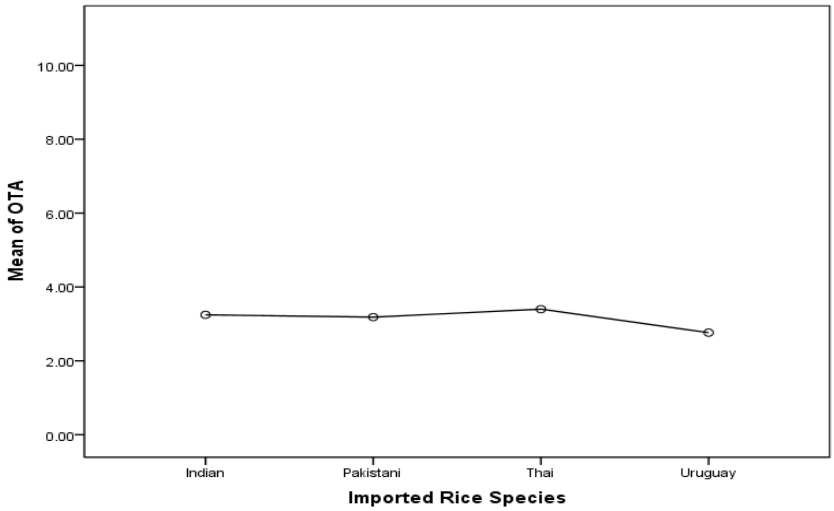

Fig. 5

Based on these results, average OTA contamination was diagnosed by ELISA to be within limits in both Iranian and imported rice; however, the level of this contamination was significantly higher in Iranian rice than in imported rice. In terms of OTA contamination, no significant difference was seen between Iranian rice types (Tarom, Tarom Hashemi, Fajr, Neda, Behnam and Shiroudi) and rice imported from India, Pakistan, Thailand and Uruguay.

Discussion

The primary finding of this research indicates that the level of contamination in Iranian and imported rice samples was within the 5% limits, which is in harmony with a study conducted by Azizi on OTA contamination in the rice sold at chain stores in Tehran [67]. The second finding laid bare a significant difference between Iranian and imported rice in terms of contamination at the 5% error rate, which is in harmony with Rahimi's study on the OTA contamination level in the rice supplied to the city of Isfahan [65]. The third finding was that the difference in OTA contamination in Iranian and imported rice was not significant at 5% error rate. The fourth finding was that the OTA concentration in more than 21.4% of rice samples assessed in Mazandaran Province was below limits set by Iran National Standard Organization and the European Union (5 ng/g) an 17.7% of samples (29% of all samples) had an OTA concentration above 5 ng/g. The fifth finding pointed out that OTA contamination in 220 samples of rice distributed in Mazandaran province was 17.7% and average OTA contamination was 3.51 ng/g. The range of contamination in contaminated samples varied between 5 and 10.91 ng/g. Compared with the results of the first study conducted in Iran to measure the OTA incidence in the rice sold at chain stores in Tehran, the new study shows lack of harmony as OTA levels in 69% of domestic and imported rice were higher than the device could diagnose (0.1 ng/g) while the OTA concentration in different categories of rice was 1.37±5.72 and varied between 0.15 and 46.79. OTA concentration was higher in imported rice than in domestic rice [15]. The results from this study were also compared with the findings of the second study conducted in Iran to measure OTA contamination in the rice distributed in Isfahan. In this study, 25 of 120 samples (20.5% of all samples) of domestic and imported rice were

contaminated with OTA. Moreover, in more than 90% of rice samples, OTA concentration was below the limit set by Iran National Standard Organization and the European Union (5 ng/g) and only in 4% of samples, OTA concentration was above 5 ng/g. In this regard, there was no convergence between the two studies. But there is harmony with that part of the study in Isfahan that stated OTA contamination percentage and level in domestic rice was respectively 23.3 and 3.8 ng/g and was significantly higher than contamination concentration in Iranian rice samples [65]. The results of the present study are in harmony with the findings of the third study conducted in Iran to determine OTA contamination concentration in 182 samples of rice sold in Mashhad, which showed that contamination stood at 6% with a mean concentration of 1.6 ng/g [89]. There is also harmony between the findings of this study and the results of a study conducted in Tunisia on the contamination of 40% of rice samples within a contamination range of 0.8 to 2.3 ng/g and a mean of 1.4 ng/g [90]. A survey conducted in England found that 7.5% of rice samples were contaminated with OTA with a concentration varying between 1 and 19 ng/g. These findings are also in harmony with the results of our study [91]. The findings of this study also agree with the results of a survey conducted in Morocco to show that in 110 rice samples collected from five cities, OTA contamination was at 26% and concentration varied between 0.08 and 47 ng/g [92]. Turkey also conducted a study to that effect on 100 samples of rice distributed in the country. In 3% of cases, OTA concentration was above the limit of 3 ng/g. Our study's findings agree with these results [93]. The present study's findings are also in accord with the findings of studies conducted in Spain, Vietnam and Chile on rice contamination with OTA. These studies found that in Spain, 7.9% of rice samples were contaminated with OTA while in Vietnam and Chile the percentage was respectively 35 and 43. Mean OTA concentration was within the range of 0.75-44 ng/g [94]. Among other findings of the present study is that OTA concentration in 33.4% of rice samples was above limits set by Iran National Standard Organization (5 ng/g) which agree with the findings of a study conducted in the south of Vietnam where OTA contamination was within the range of 21.3-26.2 ng/g. But there is disagreement between our study and the study conducted in Vietnam on the point that OTA concentration in the rice samples in southern Vietnam was around 27 ng/g and approximately 30% of surveyed samples were contaminated [95]. Our study showed that the maximum concentration of 10.91 ng/g was indicative of improvement. A study was conducted in England from 1997 to 2000, which found that OTA concentration in rice varied between 1 and 19 ng/g. That is lower than what we found in our study [96]. A study conducted in Turkey's Ankara on the retail rice showed that OTA concentration was below the limited range of 0.27-4.07 ng/g. The results of this study, conducted by ELISA, are in agreement with the present study's findings [97]. A study conducted on OTA concentration in organic and non-organic rice samples in Spain showed that mean OTA concentration in 7.38% of non-organic rice samples was 27.3 ± 3.4 ng/g and in 30% of samples of organic rice was 1 ± 1.7 ng/g. Moreover, in 26% of rice samples, OTA concentration was at its highest level (47 ng/g). The present study does not agree with this one [98]. In Tanzania, a study conducted on cereals found that OTA contamination in the rice samples was 28%. Mean OTA concentration was 44 and the contamination range varied between 10 and 150 mic/kg. In total, 28% of rice samples had OTA concentration above limits set by the European Union. These findings do not agree with the results of the present study and the concentration limits set by the EU [99]. Compared with similar surveys conducted in Italy, Germany and Indonesia, the study conducted on rice samples in Mazandaran Province showed that the range of OTA contamination was much larger than that of those countries. Like other studies, the present study also shows that environmental factors, climatic conditions, health principles during harvesting and storage of rice are all important in the incidence of OTA and its concentration.

Conclusion

The findings of this study showed that rice, as a cereal product, is likely to be contaminated with OTA. In the rice samples surveyed in the present study, the OTA level was lower than limits set by the European Union and Iran National Standard Organization. The level of contamination was 0.72% higher in Iranian rice than in imported rice. OTA is directly linked with porcine nephropathy and has been recognized as a factor contributing to human nephropathy in the Balkans. OTA is highly stable and does not disappear after rice is cleaned and even grinded, and it would be just distributed equally between rice flour and rice bran. Studies have shown that when samples of white powder are exposed to heat at 250 degrees Celsius for 40 minutes only 76% of their toxins will vanish. Unlike Aflatoxin B1, OTA is more resistant to heat and is very difficult to be uprooted. The most reasonable way to avoid OTA-related cancer is to limit human exposure to this deadly toxin by pursuing a suitable lifestyle (food diversity + suitable weight). The World Health Organization has set at 14 ng/kilogram of body weight the maximum daily intake of OTA. If we ignore the OTA intake from other food products and assume that rice is the only source of OTA for a mature person, a 60-kilogram person who consumes rice with mean OTA concentration (3.51 microgram/kg), he will be taking 8.36 ng/kg of OTA per day, which is below the amount recommended by WHO. Nonetheless, in the present study, it was shown that 23.4% of rice samples had OTA concentration above limits, but a low percentage of the samples contain life-threatening OTA concentration. In many cases, by regular monitoring of consumed rice and phasing out contaminated rice, possible threats could be averted.

Innovation in Present Study

Unlike studies previously conducted, simple random sampling was used to know the dispersion of OTA contamination in the rice sold across the province and estimate OTA mean and variance through pilot sampling. Furthermore, an identical number of samples of rice was reselected in order to maximize the power of variance analysis test.

Statistical Distinction

In this study, a high number of samples has been used while statistical analysis is based on a variety of tests in order to facilitate the credit scoring of the fitted model of data. Some of these tests are One Sample T-Test, Levene's Test, Kolmogorov-Smirnov Test, Brown-Forsythe Test, One-Way ANOVA, Independent Samples T-Test and Runs Test. These tests have not been used in similar studies. Furthermore, in order to make a decision about the contamination or non-contamination of Iranian and imported rice and compare OTA concentration in these two rice samples, One-Way ANOVA test was used to explore a possible significant difference between different types of Iranian and imported rice. In the end, contrary to rumors, no significant difference was seen between Iranian and imported rice in terms of OTA contamination.

Proposals for Future

1. Regular and precise monitoring of rice samples in Mazandaran Province via tandem mass spectrometry (LC/MS/MS); rice is a major component of food mix in Iran and OTA contamination will have long-term harmful impacts on consumers' health.
2. Monitoring rice production in Mazandaran Province
3. Observing preventive measures before and after cultivation like (GAP, GHP, GSP, GMP, HACCP) will be effective in reducing rice contamination with OTA
4. On-time consumption of rice
5. Sharing group and international experiences could help reduce contaminations emanating from the growth of OTA-generating fungi.

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