RESEARCH COMMUNICATION

Fumonisin B1 Contamination of Cereals and Risk of Esophageal Cancer in a High Risk Area in Northeastern Iran

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Abstract

Introduction: Fumonisin B1 (FB1) is a toxic and carcinogenic mycotoxin produced in cereals due to fungal infection. This study was conducted to determine FB1 contamination of rice and corn samples and its relationship with the rate of esophageal cancer (EC) in a high risk area in northeastern Iran. Methods: In total, 66 rice and 66 corn samples were collected from 22 geographical subdivisions of Golestan province of Iran. The levels of FB1 were measured for each subdivision by thin layer and high pressure liquid chromatographies. The mean level of FB1 and the proportions of FB1 contaminated samples were compared between low and high EC-risk areas of the province. Results: The mean of FB1 levels in corn and rice samples were 223.64 and 21.59 µg/g, respectively. FB1 contamination was found in 50% and 40.9% of corn and rice samples, respectively. FB1 level was significantly higher in rice samples obtained from high EC-risk area (43.8 µgig) than those obtained from low risk area (8.93 µgig) (p-value=0.01). The proportion of FB1 contaminated rice samples was also significantly greater in high (75%) than low (21.4%) EC-risk areas (p-value=0.02). Conclusion: We found high levels of FB1 contamination in corn and rice samples from Golestan province of Iran, with a significant positive relationship between FB1 contamination in rice and the risk of EC. Therefore, fumonisin contamination in commonly used staple foods, especially rice, may be considered as a potential risk factor for EC in this high risk region.

Keywords: Fumonisin B1 - rice - corn - esophageal cancer - high risk region - Iran

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Introduction

Fumonisin, member of mycotoxin family, is a toxic and carcinogenic metabolite produced by fungal infections including fusarium species (Voss et al., 2001). Different subtypes of fumonisin have been known, but only fumonisin B1 (FB1), FB2 and FB3 are naturally found in contaminated foods (Marasas, 1996). FB1, the most toxic form, is one of the secondary metabolites commonly contaminates many kinds of cereals including rice, corn and wheat (Stockmann-Juvala and Savolainen, 2008). Experimental studies showed that FB1 has carcinogenic effects and has a role in etiology of renal, hepatic and esophageal cancers (EC) (Marasas et al., 1984; Sharma et al., 2003; Klaric et al., 2008). Epidemiological studies have also shown a correlation between consumption of FB1 contaminated foods and the risk of EC (Chu and Li, 1994; Yoshizawa et al., 1994; Wang et al., 2000). So, FB1 may be a risk factor for EC, especially in high risk areas.

Golestan province, located in northeastern Iran, has been known as a high risk area for EC (Roshandel et al., 2012). A number of risk factors have been reported for EC in this region, including opium consumption, drinking hot tea, poor oral hygiene, obesity, exposure to polycyclic aromatic hydrocarbons (PAHs), nutritional deficiencies and genetic factors (Kamangar et al., 2007). There is no data about the relationship between FB1 and incidence of EC in this high risk area. So, we conducted this study to determine FB1 contamination of rice and corn samples and its relationship with EC in Golestan province of Iran.

Materials and Methods

At first, using geographical information, Golestan province was divided into 22 divisions. Totally, 132 grain samples (330 grams each) were collected throughout the province. We considered the following process for sample collection. In each of the 22 divisions, we referred to the regional silos and obtained 3 corn as well as 3 rice samples, randomly from different parts. Finally, we collected 66 corn and 66 rice samples.

Each of the samples was mixed and packed in suitable zipped bags and stored in freezer until use. The samples collected from each of 22 divisions were combined so

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that fumonisin levels in corn and rice samples were determined and recorded for each division, separately. To avoid possible contamination, the samples were washed in hypochlorite (1%), rinsed with sterile distilled water and finally dried in laminar flow cabinet. They were separately cultured in petri dish containing potato dextrose agar with chloramphenicol (50 mg/l) to reduce the growth of bacteria and were finally incubated at 25 ºC for 72 h. Fifty Grains of each sample (5 per plate) were randomly cultured on potato dextrose agar for fungal colony growth. Each colony was subcultured in separate potato dextrose agar to assess single colony form. Fungi were identified according to colony characteristic, sporogenesis structure, macro and micro conidi arrangement (Nelson et al., 1993). Fumonisin was extracted from contaminated corn and rice by Rice et al. method (Rice et al., 1995). Briefly, five grams of fragmented corn and rice culture in mortar were mixed with 100 ml methanol/water (75:25, v/v) for 3 min and then centrifuged (1000 RPM) for 1 min at 4 ºC. Supernatant was filtered and the PH was adjusted to 6. Thin Layer Chromatography (TLC) was considered for detecting fumonisin. TCL plates of 25 × 25 cm covered with silica gel (60 Aº) were autoclaved for 15 min (121ºC). Then, they were spotted 3 cm of plate end with culture extract (10 μl) along with FB1 as standard was purchased from sigma and were floated in butanol/acetic acid/sterile distilled water (10:10:20 v/v) for TLC study. TLC plates were then exposed with iodine vapor and examined under longwave ultraviolet light. UV study showed contamination with mycotoxin in samples (Rottinghaus et al., 1992; Schaafsma and Hooker, 2007).

Previously described High-Pressure Liquid Chromatography (HPLC) method with fluorescence was used to determine the level of fumonisin in samples (Rice et al., 1995). Briefly, an aliquot of purified extract was derived with o-phthaldialdehyde (OPA) solution and injected into the reversed-phase C18 column of HPLC system (Waters 590 Milford, MA) equipped with scanning fluorescence detector (Waters 470). Methanol (0.1 M) together with potassium dihydrogen phosphate (75:25, pH 3.35 adjusted with o-phosphoric acid) at 3 ml/min flow rate used as mobile phase. To quantify fumonisin level, area under the curve was used. Recovery in samples spiked at 1041 μg/kg and accuracy test were finally done (Akiyama et al., 1994; Duncan et al., 1998; Yazdanpanah et al., 2006). Analytical values were not corrected for recovery. The detection limits of methods were 0.010 mg/kg (Rice et al., 1995).

The results of fumonisin assessment were recorded and entered to computer using SPSS-13 software. To assess the relationship between fumonisin contamination of cereal samples and the risk of EC, Golestan province was divided into low and high EC-risk areas according to the aged standardized incidence rates (ASR) of EC. We considered a previously reported method for this classification (Semnani et al., 2010). We used both qualitative and quantitative methods for data analysis. The Mann-Whitney U test was considered to compare the mean of fumonisin levels in samples taken from high and low EC-risk areas. We used Chi-square test to assess the differences in proportions of contaminated samples between the two areas. P-value of less than 0.05 was considered as significant.

Table 1. The Proportion (%) of Fungal Contamination in Cereal Samples from Golestan Province of Iran

Sample	Aspergillus			Fusarium		Mucor
Type	Niger	Fumigatus	Flavuus	Verticilloids	Solani	
Corn	50.6	4.54	7.03	31.81	4.34	2.34
Rice	47.82	2.03	7.04	3.56	3.12	1

Table 2. Mean (SE) (µg/g) of Fumonisin Level of Cereal Samples from High and Low EC[a]-Risk Areas of Golestan Province of Iran.

Type of samples		Mean	SE	P-value[b]
Rice	High risk	43.75	13.97	0.01
	Low risk	8.93	4.98	
Corn	High risk	167.14	87.47	0.79
	Low risk	150	75.91	

[a]Esophageal cancer, [b]Mann-Whitney U test

Table 3. Proportion of Fumonisin Contamination in Rice Samples from High and Low EC[a]-Risk Areas of Golestan Province of Iran.

Type of samples		Contaminated	Non-contaminated	P-value[b]
Rice	High risk	75%	25%	0.02
	Low risk	21.40%	78.6	
Corn	High risk	57.10%	42.90%	0.79
	Low risk	46.70%	53.30%	

[a]Esophageal cancer, [b]Chi-square test

Results

The mean (SE) of FB1 levels in corn and rice samples were 223.64 (58.18) and 21.59 (6.84) μg/g, respectively. FB1 contamination was found in 50% and 40.9% of corn and rice samples, respectively. Table 1 shows the proportion of fungal contamination in studied samples. FB1 level was significantly higher in rice samples obtained from high EC-risk area than those obtained from low EC-risk area (p-value=0.01) (Table 2). No significant difference was seen between the mean of FB1 between high and low EC-risk areas (Table 2).

The proportion of FB1 contaminated rice samples was significantly higher in high than low EC-risk area (p-value=0.02) (Table 3). We found no significant difference in the proportion of corn samples contaminated by FB1 between the two areas (Table 3).

Discussion

We assessed FB1 contamination in corn and rice samples collected from a high risk region for EC in northeastern Iran. The level of FB1 in rice and corn samples in this study was much more than previous reports from Iran (Shephard et al., 2000) as well as other parts of the world (Sanchis et al., 1994; Abbas et al., 1998; Chelule et al., 2001). These levels of fumonisin were higher than the permissible level for Iranian people (Ghiasian et al., 2005).

We found also significant positive relationship between fumonisin contamination of rice samples and the risk of EC in Golestan province of Iran. Although no significant relationship was seen between corn contamination with fumonisin and EC, but the level of fumonisin in corn samples was also higher in high EC-risk area. Previous ecological studies similarly suggested positive correlation between fumonisin contamination of cereals and the risk of EC (Sydenham et al., 1990; Rheeder et al., 1992). The carcinogenic effects of fumonisin on esophagus had been reported in experimental studies (Marasas et al., 1984). So, fumonisin contamination of foods, especially in rice as the most commonly used cereal, may be considered as a risk factor for EC in Golestan province of Iran.

Fusarium verticillioides and aspergilus niger have shown to be the most frequent fungal infection in corn samples, while the most abundant ones in rice was aspergilus niger. The high rate of infection with fusarium species in the present study is in agreement with worldwide reports (Rheeder et al., 1993: 1994; Orsi et al., 2000). The rate of contamination with fusarium verticillioides was also reported to be high in other similar parts of Iran including Mazandaran, a region with high rainfall and relative humidity (Ghiasian et al., 2004). Making an analogy between our study and previous one may imply that meteorological conditions have been rather alike and this has led to similar high level of fungal infection in corn grains (Hennigen et al., 2000).

The presence of fungal infection, especially fusarium species, can be a potential risk for human and animal health. The results of a previous study showed a correlation between corn contamination by fusarium verticillioides and esophageal cancer (Marasas, 1995). These infections affect the human health by producing fumonisin, a toxic and carcinogenic metabolite (Gelderblom et al., 1988; IARC team, 1993; Jackson et al., 2000). Fumonisin is readily absorbed from gastrointestinal tract. It causes sphynganine and sphyngosine accumulation and cell membrane dysfunction. Sphyngoid free bases in return, functions as cancer promoter and mutations (Soriano et al., 2005). According to FAO/WHO common committee, the highest tolerable daily intake of fumonisin is 2 μg/kg body weight (Humphreys et al., 2001). It was also reported that average fumonisin intake is variable from 0.2 μg/g up to 2.4 μg/g body weight in European and African countries, respectively. Daily intake of fumonisin from various foodstuffs in different countries has been reported between 0.00024 up to 440 μg/g body weight (Joint FAO WHO Expert Committee on Food Additives Meeting, 2002).

It seems that some environmental factors in different geographical regions are strongly related to fumonisin contamination of foods. Humidity, draught stress, temperature, and rainfall are among the important factors that affect on contamination of agricultural products during harvesting period. Growth of verticillioides species is strongly temperature-dependent and is ideal if it is above 26 ºC. Many studies focus on the complicated and important role of rainfall and temperature on fumonisin level in foods (Marasas, 1995; Miller, 2001; Schaafsma and Hooker, 2007). The high rate of infection with fusarium species in Golestan province is in accordance with climatic situation of this province. This region has high mean rainfall and relative humidity. All of these conditions are suitable for cereals to be infected by fusarium. Therefore, controlling fungal growth and mycotoxin production is crucial in foraging industries and ranches. Fungal growth may be controlled by keeping foodstuffs in low humidity climate, using clean equipment and fungal growth inhibitors. Cereals and other dried foodstuffs should not be kept at humidity percentage of above 14. Harvesting delay and rainfall can increase mycotoxin production. Mycotoxin concentration would be highest in damaged, tiny and chopped cereals. So sorting out damaged ones can decrease mycotoxin concentration. Silos should keep the foodstuffs from rainfall and other water sources. Silos' ventilation is important to keep the cereals dried. Air penetration can help the growth of acid-resistant microorganisms and fungi together with pH increase. Finally, it should be noted that proper agricultural and sanitary measures are needed to ensure good quality of staple foods including corn and rice.

The main limitation of this study was the nature and type of this research. This was a population-level ecological study and the correlation found in this study may be considered just as a hypothesis not an approved relationship. In other words, we could not conclude causal relationship between fumonisin contamination and development of EC in our region. Further individual-level studies (case-control of cohort) are needed to approve this hypothesis.

In conclusion, we found high levels of fumonisin contamination in corn and rice samples from Golestan province of Iran. Our results also showed significant positive relationship between fumonisin contaminations is rice and the risk of EC. So, fumonisin contamination in most commonly used staple foods, especially rice, may be considered as potential risk factor for EC in this high risk region. Further studies are needed to approve this hypothesis.

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