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Aflatoxin contamination of wheat flour and the risk of esophageal cancer in a high risk area in Iran

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ABSTRACT

Background: Golestan province in northeastern Iran has been known as a high-risk area for esophageal cancer (EC). This study was conducted to assess aflatoxin (AF) contamination of wheat flour (WF) samples in high and low EC-risk areas of Golestan province. *Methods*: Four WF samples were collected randomly from each of 25 active silos throughout the province in 2009. The levels of AFs were measured using the High-performance liquid chromatography method. Using the data of EC rates obtained from Golestan population-based cancer registry, the province was divided into high and low risk areas for EC. Student *t*-test and multivariate regression analysis were used to compare the levels of aflatoxins as well as the condition of silos between the two areas. *Results*: One hundred WF samples were collected. The mean levels of total aflatoxin and aflatoxin B1 was 1.99 and 0.53 ng g^{-1}, respectively. The levels of total AF ($p = 0.03$), AFG2 ($p = 0.02$) and AFB1 ($p = 0.003$) were significantly higher in samples obtained from high risk area. Multivariate regression analysis showed that humidity of silo was the most important source of difference between silos of the two areas ($p = 0.04$). *Conclusion*: We found a positive relationship between AF level of WF samples and the risk of EC. So, AF contamination may be a possible risk factor for EC in our region. We also found that humidity of silos was the most important determinant of AF contamination of WF. Intensive control of silos conditions including humidity and temperature are needed especially in high EC-risk areas.

1. Introduction

Cancers are among the most common causes of death throughout the world [1]. Esophageal cancer (EC) is the eighth most common cancer worldwide, with 481 000 new cases (3.8% of the total) estimated in 2008. It is the sixth most common cause of death from cancer with 406 000 deaths (5.4% of the total) in 2008 [2]. EC has a high incidence in countries such as France, Italy, China, South Africa, Uruguay and Iran [3]. This cancer is the fourth most common malignancy in Iranian population [2]. Golestan province, located in northeast of Iran, has been known as a high risk area for EC. The ASRs of EC in this area were 24.3 and 19.1 in male and females, respectively [4].

EC is a multi-factorial disease. Several risk factors have been proposed as possible etiologic factors for EC in Golestan province. The findings of several studies performed in this province have suggested Family history of EC, Opium consumption, Smoking (tobacco) [5], Socioeconomic status [6], drinking hot tea [7,8], dietary intake of benzopyrene [9], germline BRCA2 mutations [10] and tooth lost and lack of regular oral hygiene [11] as risk factors for EC. Environmental factors including silis contamination of flour [12], soil selenium level [13] and toxins [14] had also been suggested as possible risk factors for EC in this region.

Aflatoxins (AFs) are microbial toxins in food and produced by fungi of the genus Aspergillus [15]. The biological effects of this toxin include carcinogenicity, mutagenicity, teratogenicity and hepatotoxicity [16]. Therefore, this toxin is harmful to human health. Secondary toxic metabolites produced by fungi growing on food products, such as corn, peanut and wheat may have carcinogenic properties [17]. The results of studies have shown that food contamination by mycotoxins (AF) can lead to different types of cancer [18]. Aflatoxin B1 (AFB1) is the most toxic and carcinogenic member of AFs [19]. It is the most potent hepato-carcinogen known in mammals and is classified by the International Agency of Research on Cancer (IARC) as Group 1 carcinogen [20].

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Wheat flour (WF) is one of the most commonly used foods in different parts of Iran including Golestan province. It was suggested that contamination of wheat flour with aflatoxins may play a possible role in pathogenesis of EC in this area. So, we conducted this study to assess the relationship between aflatoxin levels of wheat flour and the risk of EC in this area.

2. Materials and methods

This cross-sectional study was conducted in 2010 (winter) in Golestan province. According to protocol number 2836 of the Institute of Standard and Industrial Research of Iran (sampling of agricultural products), a total of 100 samples were collected from 25 wheat flour silos throughout the province. The wheat used in all of the silos was locally cultivated. To determine the effect of environmental factors on aflatoxin formation, conditions of silos such as temperature, humidity and durations of storage (for wheat and WF) were recorded. Storage duration of wheat was defined as the time between entering wheat into silo until WF preparation. The time between entering WF into silo until its delivery to the consumers was defined as storage duration of WF.

The samples were refrigerated at 4 °C until laboratory investigation. AF was measured by high-performance liquid chromatography (HPLC) method using immunoaffinity column. Worldwide regulations for the levels of mycotoxins in food and feed (total aflatoxin = 4 ng g^{-1} and aflatoxin B1 = 2 ng g^{-1}) [21] and the regulation number 6872 of the Institute of Standards and Industrial Research of Iran (ISRI) (15 ng g^{-1} for total aflatoxin, 5 ng g^{-1} for aflatoxin B1) [22] were considered to identify the permissible limits of aflatoxins in wheat flour.

To determine the relationship between contamination of WF with AF and the risk of EC, Golestan province was divided into two areas using the age standardized incidence rate (ASR) of EC. The data about the ASR of EC was obtained from Golestan population-based cancer registry [2]. The first area (high risk area) was "Turkmensahra", a region in the northeast of Iran near the Caspian Sea, bordering Turkmenistan, the majority of whose inhabitants are from Turkmen ethnicity. The second (low risk area) was other parts of the province [3]. All data were entered into computer and SPSS-16 and STAT-8 software were used for statistical analysis. Comparison of AF levels in WF samples as well as characteristics of silos between the two areas was done by Student *t*-test. We used multivariate linear regression analysis to determine the most important source of difference between silos located in low and high EC-risk areas. *P*-values of less than 0.05 were considered as significant.

3. Results

Totally, 100 WF samples were collected from 25 active WF silos. The mean (SD) temperature and humidity of silos were 18.35(2.95) and 43% (10), respectively. The mean of storage duration of WF and wheat were 5 and 205 days, respectively. The mean (SD) of AFB1, AFB2, AFG1, AFG2 and total aflatoxins levels in all WF samples were 0.53 (0.88) ng g^{-1}, 0.31 (0.71) ng g^{-1}, 0.55 (0.93) ng g^{-1}, 0.6 (0.6) ng g^{-1} and 1.99 (1.96) ng g^{-1}, respectively.

Table 1 shows the mean of AF in high and low risk areas. We found significant differences in AF levels of WF samples between the two areas. The mean of AFs G2, B1 and total AF were significantly higher in high compared to low risk area for EC (Table 1). No significant difference was found in mean of AFG1 and AFB2 between the two areas.

Table 2 shows that the temperature and humidity of silos in high risk area was significantly higher than low risk area. Storage duration of wheat and WF did not show any significant difference between the two areas. The results of multivariate regression analysis showed that humidity of silo was the most important source of difference between silos of the low and high EC-risk areas ($p = 0.04$).

Table 1
Mean (SD) of aflatoxin (AF) levels (ng g^{-1}) in wheat flour samples in low and high risk areas of EC in Golestan province of Iran.

Aflatoxins	Low EC-risk area	High EC-risk area	*P* value*
AFG2	0.45 (0.58)	0.75 (0.64)	0.02
AFG1	0.49 (0.76)	0.62 (1.08)	0.53
AFB2	0.34 (0.88)	0.27 (0.50)	0.62
AFB1	0.26 (0.31)	0.80 (1.14)	0.003
Total AF	1.55 (1.67)	2.42 (2.14)	0.03

* Student *t*-test.

4. Discussion

We found a significant relationship between aflatoxin levels of WF and the incidence of EC. The results of our study showed that the level of total AF and AFB1 was significantly higher in high than low EC-risk area. Our results also suggested that the level of AFB1 (with highest carcinogenic effect) was higher than other types of AF in high-risk area. In studies conducted in China and South Africa, fungal contamination of food was suggested as an important contributor of carcinogenesis in human. Permanent and long-term exposure to food products contaminated with aflatoxins was reported to cause cancer in people. This mycotoxin, especially AFB1, has been known as a carcinogen, teratogenic and mutagenic factor [23]. Some studies have revealed that there is meaningful association between esophageal cancer and meals contaminated with mycotoxins including aflatoxins [24]. Toxigenic fungus was found in many commodities and was isolated from patients with EC [25]. Marjani et al. reported that the risk of EC is increased by consumption of aflatoxin contaminated wheat [26].

According to the worldwide [21] and Iranian [22] regulations, the levels of total AF and AFB1 in WF samples from Golestan province were within acceptable range. So, it may be concluded that there is no relationship between AF contamination of WF and the risk of EC in this region. But, we found a significant difference in AF level of WF samples between high (0.80 ng g^{-1}) and low (0.26 ng g^{-1}) EC risk areas. Such considerable difference between two neighbor areas (with just 20–30 km distance) seems to be an important finding. It should also be noted that the majority of participants of the high EC risk area of Golestan province are from Turkmen ethnicity with specific genetic characteristics. Maybe, the participants of the high risk area are more susceptible to the carcinogenic effects of aflatoxin B1 than other populations. If so, any levels of aflatoxin (even within acceptable range) may have carcinogenic effects on this population. Because of its ecological design, our study could not suggest causal relationship between aflatoxin and EC. In other words, our findings could not introduce aflatoxin as a major risk factor for EC in our area. We just considered them to make a hypothesis about the relationship

Table 2
Characteristics of wheat four Silos in low and high risk areas of EC in Golestan province of Iran.

Characteristics of silos	Low risk area	High risk area	*P* value*
Storage duration of wheat flour (days)	5.7 (4.6)	4.5 (4.9)	0.2
Temperature (°C)	17.6 (1.9)	19.1 (3.5)	0.01
Humidity (%)	38 (7.9)	47.3 (9.4)	<0.01
Storage duration of wheat (days)	204.3 (4.6)	205.5 (4.9)	0.2

* Student *t*-test.

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between aflatoxin and EC in this area. So, AF contamination of WF may be a possible risk factor for esophageal cancer in Golestan province of Iran. Of course, future individual based studies (e.g. case–control studies) are needed to accept or reject this hypothesis.

Our findings suggested that humidity was the most important source of difference between silos of low and high EC-risk areas. Regarding the higher levels of humidity in silos located in high EC-risk area (area with higher AF level), it can be concluded that humidity was the most important factor associated with the production of aflatoxin in WF samples in our study. Saleemullah et al. showed that grain storage in the warehouse during the heavy rain will cause increased formation of aflatoxin [27]. Kumar et al. reported that high humidity and long-time storage results in aflatoxin production in agricultural products [28]. The results of another study suggested that high level of humidity may make conditions favorable for growth of fungus and consequently aflatoxin production [25]. Borut et al. also reported a relationship between humidity and fungal growth and AF production in WF [29]. Based on our findings, humidity of silos is the most important factor for AF contamination of WF. So, interventions for controlling humidity of silos may result in preventing fungal growth and AF production and consequently be helpful in controlling EC in this region as well as other similar high risk areas. Health policy makers should be aware about the point and consider this factor in EC controlling programs.

Our study also showed that the temperature of silos was also higher in high EC-risk area (the area with high AF levels). High temperature was suggested as an important determinant of fungal growth and AF production in WF [25,28]. Temperature was shown to have a direct relationship with the incidence of fungal contamination [30]. Controlling temperature should be considered as an important activity for preventing fungal growth and AF production in all silos especially in areas with high risk of cancers.

Basilico showed that different factors including type of fungi, host and environment may affect on the type and quantity of toxin produced by the fungus [31]. Weather condition, water activity, level of oxygen, poor storage condition and inadequate drying were also known as determinants of aflatoxin production in silos [32–34]. So, further studies are needed to completely clarify the determinants of AF production in WF silos in our region.

According to reports from the World Food and Agriculture Organization (FAO), wheat is the most common cereal consumed in Iran [35]. Most of Iranian families and almost all residence of Golestan province consume WF in their daily diet. Therefore, contamination of WF with aflatoxins, especially carcinogen ones (AFB1), may have important and direct effects on their health status. The conditions of WF silos including humidity and temperature should be maintained within standard ranges to prevent AF production. Regarding the relationship found in our study between AF contamination of WF and risk of EC, interventions for preventing AF production in WF may be considered in EC controlling programs in our area as well as other similar populations.

5. Conclusion

We found a positive relationship between AF level of WF samples and the risk of EC. So, AF contamination may be a possible risk factor for EC in our region. We also found that humidity of silos was the most important determinant of AF contamination of WF. Intensive control of silos conditions including humidity and temperature are needed especially in high EC-risk areas. The major limitation of this study was its ecological or population-level design. So, further individual-level studies should be conducted to clarify this relationship.

Conflict of interest statement

The authors declare that they have no conflict of interest.

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References

[1] Munoz N, Day NE. Esophageal cancer. In: Schottenfeld D, Fraumeni JF, eds. Cancer epidemiology and prevention. 2nd ed., New York: Oxford University press, 1996: 681–706.
[2] Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. GLOBOCAN 2008 v1.2, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 10 [Internet]. Lyon, France: International Agency for Research on Cancer; 2010. Available from: http://globocan.iarc.fr.
[3] Lu S, Lin P, Wang G, Luo X, Wu M. Comprehensive prevention and treatment for esophageal cancer. Chin Med J (Engl) 1999;112(10):918–23.
[4] Roshandel Gh, Sadjadi A, Aarabi M, Keshtkar A, Seyed Mehdi Sedaghat SM, Nouraie SM, et al. Cancer incidence in Golestan Province: report of an ongoing population-based cancer registry in Iran between 2004 and 2008. Arch Iran Med 2012;15(4):196–200.
[5] Islami F, Kamangar F, Aghcheli K, Fahimi S, Semnani S, Taghavi N, et al. Epidemiologic features of upper gastrointestinal tract cancer in northeastern Iran. Br J Cancer 2004;90:1402–6.
[6] Islami F, Kamangar F, Nasrollahzadeh D, Aghcheli K, Sotoudeh M, Abedi-Ardekani B, et al. Socio-economic status and oesophageal cancer: results from a population-based case–control study in a high-risk area. Int J Epidemiol 2009;38:978–88.
[7] Fagundes RB, Abnet CC, Strickland PT, Kamangar F, Roth MJ, Taylor PR, et al. Higher urine 1-hydroxypyrene glucoronide (1-OHPG) is associated with tobacco smoking exposure and drinking mate in healthy subjects from Rio Grand do Sul, Brazil. BMC Cancer 2006;6:139.
[8] Ghadirian P. Thermal irritation and esophageal cancer in northern Iran. Cancer 1987;60(8):1909–14.
[9] Hakami R, Mohtadinia J, Etemadi A, Kamangar F, Nemati M, Pourshams A, et al. Dietary intake of benzo(a)pyrene and risk of esophageal cancer in north of Iran. Nutr Cancer 2008;60(2):216–21.
[10] Akbari MR, Malekzadeh R, Nasrollahzadeh D, Amanian D, Islami F, Li S, et al. Germline BRCA2 mutations and the risk of esophageal squamous cell carcinoma. Oncogene 2008;27(9):1290–6.
[11] Abnet CC, Kamangar F, Islami F, Nasrollahzadeh D, Brennan P, Aghcheli K, et al. Tooth loss and lack of regular oral hygiene are associated with higher risk of esophageal squamous cell carcinoma. Cancer Epidemiol Biomarkers Prev 2008;17(11):3062–8.
[12] Jabbari A, Besharat S, Semnani Sh. Role of silis in esophageal cancer. World J Gastroenterol 2008;21(14 19):3106–7.
[13] Semnani S, Roshandel G, Zendehbad A, Keshtkar A, Rahimzadeh H, Abdolahi N, et al. Soils selenium level and esophageal cancer: an ecological study in a high risk area for esophageal cancer. J Trace Elem Med Biol 2010;24(3):174–7.
[14] Luo Y, Yoshizawa T, Katayama T. Comparative study on the natural occurrence of Fusarium mycotoxins (trichothecenes and zearalenone) in corn and wheat from high- and low-risk areas for human esophageal cancer in China. Appl Environ Microbiol 1990;56(12):3723–6.
[15] Moss MO. Recent studies of mycotoxins. Symp Ser Soc Appl Microbiol 1998;27:62S–76S.
[16] Speijers GJA, Speijers MHM. Combined toxic effects of mycotoxins. Toxicol Lett 2004;153(1):91–8.
[17] Leeson S, Diaz GJ, Summers JD. Poultry metabolic disorders and mycotoxins. Canada: Guelph University Books, 1995.
[18] Paget V, Lechevrel M, André V, Goff JL, Pottier D, Billet S, et al. Benzo[a]pyrene, aflatoxine B_1 and acetaldehyde mutational patterns in TP53 gene using a functional assay: relevance to human cancer aetiology. PLoS One 2012;7(2):e30921.
[19] Bennett JW, Klich M. Mycotoxins. Clin Microbiol Rev 2003;16(3):497–516.
[20] International Agency for Research on Cancer (IARC). Evaluation of carcinogenic risk of chemical to humans. Some naturally – occurring substance: food item and constituents. Heterocyclic Aromatic Amines and Mycotoxins. IARC Monographs 1993;56:359–62.
[21] Food and Agriculture Organization of the United Nations. Worldwide Regulations for Mycotoxins in Food and Feed in 2003. Rome: Food and Agriculture Organization of the United Nations, 2004.
[22] Institute of Standards and Industrial Research of Iran. Food and feed-mycotoxins: maximum tolerated level, First edition, Tehran: Institute of Standards and Industrial Research of Iran, 2002.
[23] Victor T, Du Toit R, Jordaan AM, Bester AJ, van Helden PD. No evidence for point mutations in codons 12, 13, and 61 of the ras gene in a high-incidence area for esophageal and gastric. Cancer Res 1990;50:4911–4.
[24] Sharma D. Carcinoma of esophagus etiological factors and epidemiology: an overview. J Ind Med Assoc 1999;97:360–9.

[25] Chu FS. Mycotoxins: food contamination, mechanism, carcinogenic potential and preventive measures. Mutat Res 1991;259:291–306.
[26] Marjani A, Kabir MJ, Semnani Sh, Sedaghat SM, Moghaddami A. Females' esophagus cancer incidence in Golestan Province, Iran (2004). Internet J Med Update 2008;3(2):36–9.
[27] Saleemullah A, Iqbal I, Khalil A, Shah H. Aflatoxin contents of stored and artificially inoculated cereals and nuts. Food Chem 2006;98:699–703.
[28] Kumar V, Basu MS, Rajendran TP. Mycotoxin research and mycoflora in some commercially important agricultural commodities. Crop Prot 2008;27:891–905.
[29] Borut SY, Joffe AZ. *Aspergillus flavus*. Link and other fungi associated with stored groundnut kernels in Israel. Israel J Bot 1966;15:112–20.
[30] Gqaleni N, Smith JE, Lacey J, Gettinby G. Effects of temperature, water activity, and incubation time on production of aflatoxins and cyclopiazonic acid by an isolate of *Aspergillus flavus* in surface agar culture. Appl Environ Microbiol 1997;(63):1048–53.
[31] Basilico JC. Mycotoxinas in food. PhD thesis. Argentina: National University of Littoral; 1995.
[32] Cotty P, Jaime-Garcia R. Influences of climate on aflatoxin producing fungi and aflatoxin contamination. Int J Food Microbiol 2007;119:109–15.
[33] OBrain GR, Georgianna DR, Wilkinson JR, Yu J, Abbas HK, Bhatnagar D, et al. The effect of elevated temperature on gene transcription and aflatoxin biosynthesis. Mycologia 2007;99:232–9.
[34] Baliukoniene V, Bakutis B, Stankevicius H. Mycological and mycotoxicological evaluation of grain. Ann Agric Environ Med 2003;10:223–7.
[35] Food and Agriculture Organization of the United Nations. FAO Statistical Yearbook 2010. Rome: FAO, 2012.

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