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Public health risk associated with the co-occurrence of mycotoxins in spices consumed in Sri Lanka

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ABSTRACT

A quantitative risk assessment of mycotoxins due to the consumption of chilli (*Capsicum annum* L.) and black pepper (*Piper nigrum* L.) was performed in Sri Lanka. A food frequency questionnaire was administered in order to collect the data on consumption of spices by households in the Northern and Southern region ($n = 249$). The mean chilli consumption in the North was significantly higher ($p < 0.001$) compared to the South. Mean exposure to aflatoxin B1 (AFB1) in the North (3.49 ng/kg BW/day) and South (2.13 ng/kg BW/day) have exceeded the tolerable daily intake due to chilli consumption at the lower bound scenario, while exposure to OTA was small. Dietary exposure to other mycotoxins, fumonisin B1, fumonisin B2, sterigmatocystin and citrinin due to spices were estimated. Margin of exposure estimations at the mean exposure to AFB1 were remarkably lower due to chilli (45–78) than for pepper (2315–10,857). Moreover, the hepato cellular carcinoma (HCC) risk associated with the mean AFB1 exposure through chilli at the lower bound was 0.046 and 0.028 HCC cases/year/100,000 based on the North and South consumption, respectively. AFB1 exposure via chilli should be considered as a great public health concern in Sri Lanka due to both high mycotoxin concentration and high consumption.

1. Introduction

Human beings are exposed to a wide range of chemicals, the uptake of which by the human body is mainly through food, water, air and dermal contact. In this regard, mycotoxins, the secondary fungal metabolites are considered to be one of the potent natural and unavoidable chemical contaminants humans are exposed to in their daily lives. In the last decade, several studies focusing on mycotoxins contamination in foodstuffs, mainly cereals, nuts and spices have been reported from many countries especially from Asia and Africa (Marin et al., 2013; Shephard, 2008a).

Sri Lanka (also called as “Spice Island”) is a tropical nation in Asia where several highly valued spices are produced. The prevailing climatic condition in the island is very suitable for the cultivation of the spices but unfortunately it is also suitable for the mould infestations and thus contamination with mycotoxins. However, the moulds' ability to produce mycotoxins is greatly influenced by environmental factors, of which the most important are temperature, relative humidity, insect damage, drought and inadequate storage conditions (Miraglia et al., 2009; Prandini et al., 2009). Contamination of spices with aflatoxins (AFs) and/or ochratoxin A (OTA) has been reported in several countries (such as Turkey, Hungary, Malaysia, Spain, India, Pakistan) (Aydin et al., 2007; Fazekas et al., 2005; Jalili et al., 2010; Paterson, 2007; Reddy et al., 2001; Santos et al., 2010). Only a very recent study reports the co-occurrence of multiple mycotoxins in pepper (Yogendrarajah et al., 2014a) and chilli (Yogendrarajah et al., 2014b) from Sri Lanka, the two most widely used spices in Sri Lankan cuisine.

Regarding the toxicity of mycotoxins, the aflatoxin group (AFB1, AFB2, AFG1 and AFG2) is considered as an extremely potent genotoxic carcinogen in all the animal species investigated. The

Abbreviations: AFB1, aflatoxin B1; OTA, ochratoxin A; LB, lower bound; MB, medium bound; UB, upper bound; HCC, hepato cellular carcinoma; BW, body weight; TDI, tolerable daily intake; PTDI, provisional tolerable daily intake; PMTDI, provisional maximum tolerable daily intake; MoE, margin of exposure; SL, Sri Lanka; HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; HBGV, health based guidance value; FFQ, food frequency questionnaire; ND, non-detects; LOD, limit of detection; LOQ, limit of quantification.

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International Agency for Research on Cancer (IARC, 1993) classified aflatoxins as group 1 human carcinogens. It has been suggested that AFB1 in food should be reduced to as low as reasonably achievable (ALARA), since exposure at any level possesses risk for human health. On the other hand, OTA, STERIG and fumonisins have been classified as possible human carcinogens in group 2B due to the inadequate evidences of carcinogenicity in humans (IARC, 1993 and 2002). Thus, the occurrence of mycotoxins in agricultural commodities has been recognized as a potential hazard for the human and animal health and warrants the need for an exposure assessment.

Despite the wide range of publications on occurrence of mycotoxins in several food products in several countries, only limited data are available on mycotoxin exposure among different populations (Marin et al., 2013). There are considerable differences in mycotoxin occurrence between various regions of the world as well as year-to-year fluctuations within countries. There are also significant differences between countries and even within countries with regard to the intake of food commodities, thus making exposure assessments and therefore risk assessments country specific (Kuiper-Goodman, 1995). Compared to Africa and Europe, mycotoxin occurrence and subsequent risk assessment studies are rather limited in the Asian context. In China, only aflatoxin exposure was previously studied for spices (Zhao et al., 2013) and peanut (Ding et al., 2012), in Japan for several foods (Sugita-Konishi et al., 2010) and in Malaysia for nut and nut products (Leong et al., 2011). Nonetheless, there are no studies available on assessing the risk associated with multiple mycotoxins exposure in any of the other Asian populations.

Therefore, the present study aims to evaluate the dietary risk associated with the intake of spices namely chilli and black pepper contaminated with multiple mycotoxins in populations from the two regions in Sri Lanka using both the deterministic as well as the probabilistic approaches. Risk characterization of the genotoxic mycotoxins was carried out based on the margin of exposure approach using the bench mark dose lower confidence level (BMDL). Moreover, the population risk for hepato cellular carcinoma (HCC) attributable to the AFB1 intake was estimated. This is the first Sri Lankan study of its kind.

2. Materials and methods

2.1. Collection of spice samples and analysis of mycotoxins

In total, 168 spice samples were collected from different regions of Sri Lanka during the period of 2011–2012. This included 86 chilli (*Capsicum annum* L.) and 82 black pepper (*Piper nigrum* L.) samples. Further details on the sampling and the analytical results of mycotoxin contamination in these spices can be found in Yogendrarajah et al. (2014a and 2014b). The summary of mycotoxins contamination data of both black peppers (Supplementary Table S1) and chilli (Supplementary Table S2) used in exposure estimations are provided as supplementary material.

Briefly, the ground spice samples were extracted using a modified QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) based approach. A simple and straightforward extraction procedure was applied for the analysis of multiple mycotoxins using a method developed in-house. Liquid chromatography was performed using a Waters ACQUITY ultra-performance liquid chromatography (UPLC™) system. Mass spectrometry (MS/MS) was performed with a Quattro Premier™ XE tandem quadrupole mass spectrometer (Waters; Milford, MA, USA). The MS was operated at electrospray ionization in positive mode (ESI+). More details of the instrumental conditions and method performances can be found in Yogendrarajah et al. (2013).

2.2. Collection of spice consumption data in Sri Lanka

A spice consumption survey was performed during the period of 2012–2013 in households from Northern and Southern regions. The ethnic groups of these regions have different culinary practices in the usage of spices. Hence, a food frequency questionnaire (FFQ) was prepared in order to collect the usual spice intake from these two regions. Beforehand, the developed questionnaire was pre-tested among few (n = 20) households. The female head of the family was directly interviewed to gather the information on the amount of these spices used in curry preparations based on the portion size pictures. Portion size pictures were prepared for chilli powder, black pepper powder and whole pepper corns. Actual weights of each spice portion sizes were recorded. Use of portion size pictures in food intake estimation has been validated in a previous study (Huybregts et al., 2008). Regarding the intake of whole chilli pods the participant was directly asked to provide the information on the number of chilli pods added in curry per day. Participants have been requested to choose the appropriate portion size picture which could reflect the right amount of particular spice added in each curry preparations. The amount of spices added was obtained separately for different curry preparations, meat, seafoods and vegetables and summed up to find the total spice consumed by the family (Supplementary Figs. S1 and S2). Finally, the mean daily spice consumption of the individual was calculated based on the total number of family members (>15 years of age) assuming that the consumption of spices is equally shared among the family members. In total, randomly selected 249 households from Sri Lanka comprising 62 families (235 individuals) from the Northern region and 187 families (783 individuals) from the Southern region were interviewed, after obtaining their informed consent. Assuming that the consumption of spices by children is insignificant, they were excluded (54 (13%) from North and 31 (7%) individuals from the South) from the calculations of the individual spice intake. Therefore, the consumption data reported in this study were from 204 individuals from the North and 729 individuals from the South. The gender distribution of the sample was 44% of male and 56% of female from the Northern and 48% of male and 52% of female from the Southern region. The ethical clearance was obtained from both Belgian (2012/082) and Sri Lankan (710/13) authorities in order to carry out this survey.

2.3. Exposure assessment

The most common approach to estimate the dietary exposure to mycotoxins is to integrate the contamination data obtained through the sample analysis and the consumption data generally obtained through national dietary surveys. Deterministic ("point estimations") and probabilistic ("simulated random sampling") methods were used to assess the risk associated with the mycotoxins exposure. However, some mycotoxins in spices had a low occurrence hence, no probabilistic approach could be conducted. Mycotoxin exposure assessment in this study incorporated the analysis of three different scenarios (lower, medium and upper bounds) related to the treatment of mycotoxin contamination data of the non-detects (NDs) and those below the limit of quantification (<LOQ) (Medeiros Vinci et al., 2012). Management of the left-censored contamination data is generally considered to be the main source of uncertainty in exposure models. Substitution of the NDs by the limit of detection (LOD) and zero for the upper bounds and lower bounds has been the most common approach in mycotoxin risk assessment studies (EFSA, 2010). Therefore, NDs were replaced by zero, half of the LOD and LOD, while <LOQs were replaced by half of the LOD, LOD and LOQ for the lower (LB), medium (MB) and upper bounds (UB), respectively. The exposure assessment was performed separately for chilli and pepper using the consumption data obtained from the population of the two regions in Sri Lanka. The mean body weight (BW) (n = 933) calculated (mean ± SD 59.85 ± 4.17 kg) based on the report of survey participants was used in the exposure calculations.

2.3.1. Deterministic exposure assessment

A deterministic exposure assessment was performed considering the above explained three concentration scenarios. The spice consumption data obtained from the population of the particular region was multiplied by the mycotoxin concentrations to determine the exposure associated with the particular mycotoxin of that region. Two deterministic approaches were performed for both the spices which could also help to identify potential acute toxicity associated with mycotoxins exposure. The exposure levels were estimated based on both the fixed mean mycotoxin concentration and the fixed mean consumption data using the minimum (min), mean, maximum (max) and the percentiles (P90, P95, P97.5 and P99) of the other exposure component in all the three scenarios considered.

2.3.2. Probabilistic exposure assessment

In addition to the deterministic approach, a probabilistic exposure assessment was carried out which considers the variances and uncertainties associated with the mycotoxins exposure determinants. Firstly, the fractions of the non-detects, < LOQ and >LOQ were calculated. For the risk output calculations, the fractions of >LOQ, < LOD + >LOQ, < LOQ+ > LOQ were utilized using an "if" logical function of the MS excel for the LB, MB and UB, respectively. Best fit distributions were determined for the three scenarios of the mycotoxin concentration data using the Chi-square statistics. The probability/probability (P/P) and quantile/quantile (Q/Q) plots were also assessed in order to determine the best fit distribution for both spice consumption and mycotoxins concentration data. The type of best fit distribution selected for the LB was also applied to the MB and UB of the concentration data. First order Monte Carlo simulations were performed considering 50,000 iterations. The simulations were repeated three times to ensure that the values remained stable. The simulations were performed with the add-in @risk® for Microsoft Excel version 6.1 (Palisade Corporation, USA). Probabilistic analysis was performed only for AFB1, OTA, STERIG in pepper and for AFB1 and OTA in chilli due to the low occurrence data of other mycotoxins. The probable dietary intakes of the mycotoxins (mean,