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Mycotoxin exposure in rural residents in northern Nigeria: A pilot study using multi-urinary biomarkers

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ABSTRACT

A pilot, cross-sectional, correlational study was conducted in eight rural communities in northern Nigeria to investigate mycotoxin exposures in 120 volunteers (19 children, 20 adolescents and 81 adults) using a modern LC–MS/MS based multi-biomarker approach. First morning urine samples were analyzed and urinary biomarker levels correlated with mycotoxin levels in foods consumed the day before urine collection. A total of eight analytes were detected in 61/120 (50.8%) of studied urine samples, with ochratoxin A, aflatoxin M_1 and fumonisin B_1 being the most frequently occurring biomarkers of exposure. These mycotoxin biomarkers were present in samples from all age categories, suggestive of chronic (lifetime) exposures. Rough estimates of mycotoxin intake suggested some exposures were higher than the tolerable daily intake. Overall, rural consumer populations from Nasarawa were more exposed to several mixtures of mycotoxins in their diets relative to those from Kaduna as shown by food and urine biomarker data. This study has shown that mycotoxin co-exposure may be a major public health challenge in rural Nigeria; this calls for urgent intervention.

1. Introduction

Mycotoxins, toxic secondary metabolites produced by various fungi on diverse agricultural commodities, induce a range of harmful effects (cancers, immune suppression and target organ toxicities) in many animal species (Bondy and Pestka, 2000; CAST, 2003; IARC, 1993, 2002). Aflatoxin B_1 (AFB_1) is classified as a group 1 human carcinogen IARC (2002) and represents the second leading cause of hepatocellular carcinoma globally. Aflatoxins induce adverse immune system and growth effects in animals (Bondy and Pestka, 2000), and are suggested to have similar effects in chronically exposed populations (Gong et al., 2002, 2003, 2004; Jiang et al., 2005, 2008; Jolly et al., 2011; Obuseh et al., 2011; Shuaib et al., 2010a,b; Turner et al., 2003, 2007). In ecological studies, fumonisin B_1 (FB_1) contamination levels in maize has been associated with the incidence of esophageal and liver cancer (Chu and Li, 1994; Sun et al., 2007, 2011; Yoshizawa et al., 1994) and is classified as a group 2B carcinogen (IARC, 2002). Fumonisin exposure has additionally been associated with the incidence of neural tube defects (Missmer et al., 2006). In animals, deoxynivalenol (DON) has been linked with gastroenteritis, anorexia, reduced weight gain and immune toxicity as well as interference with DNA and RNA synthesis and neurological processes (Pestka, 2010a,b). Ochratoxin A (OTA) and zearalenone (ZEN) are nephrotoxic and estrogenic, respectively (CAST, 2003; Gilbert et al., 2001; O'Brien and Dietrich, 2005). Human exposure to these natural toxins is predominantly through consumption of contaminated foods, though occupational exposures can include inhalation (Kuiper-Goodman, 1999; Oluwafemi et al., 2012). In sub-Saharan African countries like Nigeria, consumption of poor quality grains and other mycotoxin-prone foods as staples predominate and are the major sources of exposure; in part due to a lack of awareness of the problem, but significantly exacerbated by low income status which restricts dietary choice and variety (Bankole and Adebanjo, 2003; Bankole et al., 2006b).

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Several reports are available on the occurrence of mycotoxins in various foodstuffs in Nigeria, including those consumed by individuals with a low income. For example, aflatoxins have been reported in over 70% of stored maize and maize-based snacks, groundnut and groundnut products, rice, millets, sorghum and dried melon seeds (Atehnkeng et al., 2008; Bankole et al., 2006a, 2010; Ezekiel et al., 2012a, 2013; Kayode et al., 2013; Makun et al., 2009, 2010, 2011) while FBs, OTA, DON, ZEN amongst various other mycotoxins have also been found to contaminate agricultural products in Nigeria (Adejumo et al., 2007; Afolabi et al., 2006; Ezekiel et al., 2012b,c; Makun et al., 2013). About 50% of groundnut cake (*kulikuli*) and stored maize in Nigeria had co-occurrence of more than one of these 10 mycotoxins — AFB_1, alternariol, beauvericin, emodin, FBs, DON, moniliformin, nivalenol (NIV), OTA and ZEN (Adetunji et al., submitted manuscript; Ezekiel et al., 2012b). Despite the available data on mycotoxin occurrence in diverse foods in Nigeria, the risks posed to consumers are not clearly defined as exposure and risk assessment are complex, and the toxicology of complex mixtures of mycotoxins remains a major limitation. Few reports are available in Nigeria on the levels of dietary and occupational exposures to aflatoxins (Adejumo et al., 2013; Ibeh et al., 1991; Oluwafemi et al., 2012); whilst individual exposure estimates are lacking for other mycotoxins. Bio-monitoring of mycotoxins in biological fluids such as blood or urine will be useful to generate more reliable information on exposure incidence at the individual level compared to dietary assessments (Turner et al., 2011, 2012a; Warth et al., 2013b). Hence, the present study aimed at assessing mycotoxin exposure in rural dwellers in Nigeria using a recently developed multi-biomarker method which is capable of quantifying up to 15 analytes that represent measures of eight mycotoxins and selected mycotoxin metabolites (Warth et al., 2012a).

2. Methods

2.1. Study population

This study recruited rural residents of Nasarawa and Kaduna States situated in the northern part of Nigeria. The communities covered were: Agwatashi, Akwanga, Gako and Garaku in Nasarawa State; and Barde, Kurmin Bomo, Maitozo and Mararaban Rido in Kaduna State. The majority of the population in the rural communities depend on agriculture (e.g. large scale cultivation of staples including groundnuts, maize, millet and sorghum) for their income. The choice of States was based on data obtained from a previous survey carried out between July and August 2011 to determine the distribution and levels of mycotoxins in stored maize in Nigeria (Adetunji et al., submitted manuscript). Data obtained suggested populations in Nasarawa State were more exposed to dietary mycotoxins relative to those in Kaduna State, thus warranting further studies with more reliable conclusions based on individual exposure.

2.2. Study design and sampling

A cross-sectional survey which involved purposive selection of five families each from targeted sub-communities within Kaduna and Nasarawa was conducted in September and October 2012. From each of the five families, three participants only which included two adults (one male and one female; age: ≥20 years old) and one younger individual were recruited thereby constituting 120 participants. The younger individuals were categorized as either children (aged ≤8 years) or as adolescents (aged ≤19 years). Eight breastfeeding mothers and one partially breastfed male child were among the participants recruited in Nasarawa State. Individuals with previous medical record of kidney, liver or other metabolic problems were excluded from the study. A well-structured questionnaire was designed and administered by trained interviewers to each participant prior to sample collection in order to obtain basic information relating to demography (age, sex and education), food consumption pattern (frequency of weekly consumption of mycotoxin-prone staples such as groundnut, maize, rice and sorghum, and weight of meal consumed on previous day), socio-economic and general health status. Analyzed questionnaire data are reported in detail elsewhere (Ezekiel et al., manuscript in preparation); however, it was observed that maize, groundnut, sorghum and rice constituted about 39, 29, 12 and 9% of the overall diets consumed by participants.

2.3. Ethical considerations

The Ethics Committee of the Ministry of Health situated in the studied states in Nigeria approved the study. Informed written consent was obtained from all participants prior to inclusion in the study, and analytical measurements were conducted as blind analysis to participant's information. Parents gave informed consent on behalf of their children and adolescents.

2.4. Samples

About 40-ml of first morning urine sample was collected from each of the 120 recruited participants prior to consumption of food or water. A 25 g random portion of the meal consumed by the individuals in each family on the day prior to urine donation was also obtained. All urine and food samples were immediately frozen at −20 °C in Nigeria and sent on dry ice to Austria for analysis.

2.5. Reagents and chemicals

Methanol (LC gradient grade) and glacial acetic acid (p.a.) were purchased from Merck (Darmstadt, Germany), acetonitrile (ACN; LC gradient grade) from VWR (Leuven, Belgium), and ammonium acetate (MS grade) from Sigma-Aldrich (Vienna, Austria). The mycotoxin conjugates deoxynivalenol-3-*O*-glucuronide (DON-3-GlcA) and zearalenone-14-*O*-glucuronide (ZEN-14-GlcA) were synthesized by optimized procedures as described in detail by Fruhmann et al. (2012) and Mikula et al. (2012). Other mycotoxin reference standards were purchased from Romer Labs Diagnostic GmbH (Tulln, Austria) [DON, deepoxy-DON (DOM-1), NIV, T-2 toxin, HT-2 toxin, OTA, AFM_1, FB_1 and FB_2] or Sigma (ZEN, α- and β-zearalenol). Water was purified by an Elga Purelab ultra analytic system from Veolia Water (Bucks, UK). Solid standards were dissolved and combined to a multi-standard working solution for preparation of calibrants and spiking experiments as described in Warth et al. (2012a). Deoxynivalenol-15-*O*-glucuronide (DON-15-GlcA) was obtained from UHPLC separation and subsequent fractionation of a highly contaminated human urine sample which contained both, DON-3-GlcA and DON-15-GlcA. Details of this separation are published elsewhere (Warth et al., 2012b).

2.6. Equipment

Samples were analyzed using an ABSciex QTrap® 5500 LC–MS/MS system (Foster City, CA, USA) equipped with a Turbo V electrospray ionization (ESI) source interfaced with an Agilent 1290 series UHPLC system (Waldbronn, Germany). For data evaluation the vendors Analyst software (version 1.5.1) was used.

2.7. Mycotoxin exposure assessment

The concentration of 15 urinary analytes, either the parent mycotoxins or their metabolite(s), were measured simultaneously using a rapid “dilute and shoot” liquid chromatography tandem mass spectrometry-based method as described by Warth et al. (2012a). Several of the analytes are described in the literature as “validated” exposure biomarkers (AFM_1, OTA, FB_1 and total DON i.e. free DON + DON glucuronides), whilst other analytes at this time represent biological