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Assessment of deoxynivalenol exposure among Bangladeshi and German adults by a biomarker-based approach

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HIGHLIGHTS

- Deoxynivalenol (DON) biomarker analysis in urines of Bangladeshi adults document low exposure to this mycotoxin.
- Biomarker levels in German samples reveal more frequent and considerably higher dietary DON intake of German adults.
- Yet, presently DON intake in German adults is below the provisional maximal tolerable daily intake (PMTDI) value set for this mycotoxin.
- The new results are discussed in relation to data from other countries which indicate that the PMTDI for DON can be exceeded in parts of the population.

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ABSTRACT

Deoxynivalenol (DON) is a frequent mycotoxin contaminant in cereal crops worldwide and can cause adverse health effects in exposed animals and humans. Since DON contamination in Bangladeshi food is unexplored, we conducted a biomonitoring study to assess DON exposure in the Bangladeshi population and compare it with that of German adults. In total 214 urines were collected, n = 164 in Bangladesh and n = 50 in Germany. In Bangladesh rural and urban residents of Rajshahi district provided urines in two seasons (n = 69 in summer, n = 95 in winter, with 62 participants enrolled in both periods). Urinary DON and its de-epoxy metabolite DOM-1 were measured by a previously validated sensitive LC–MS/MS method. In Bangladeshi urines, DON was detectable in 27% (range 0.16–1.78 ng/mL) in summer and 31% (range 0.16–1.21 ng/mL) in winter season. There was no significant difference at the mean DON level between season (summer 0.17 ± 0.25 ng/mL and winter 0.16 ± 0.18 ng/mL) and region (rural or urban residents). The metabolite DOM-1 was not detected in any urine from Bangladesh. In contrast, DON and DOM-1 were detected in 100% (range 0.16–38.44 ng/mL) and 40% (range 0.10–0.73 ng/mL), respectively, of the German urines. The mean DON level in German urines (9.02 ± 6.84 ng/mL) was about 53-fold higher than that found in Bangladeshi samples. This indicates a low and high dietary DON exposure among the adult population in Bangladesh and Germany, respectively. The biomarker concentrations found and published urinary excretion rates for DON then served to calculate the daily mycotoxin intake in both cohorts: the mean DON intake in Bangladesh being 6 ng/kg b.w., and in Germany a mean of 268 and maximum intake of 975 ng/kg b.w., values lower than the provisional maximum tolerable daily intake of 1 μg/kg b.w. set by the WHO/JECFA.

1. Introduction

Deoxynivalenol (DON) is one of the most prevalent *Fusarium* mycotoxins that contaminates various types of grains worldwide, mainly wheat, maize, barley, and oat (Canady et al., 2001; EFSA, 2013 EFSA, 2013). In animals, ingestion of DON contaminated feed is associated with a number of adverse health effects, including feed refusal, decreased weight gain, gastroenteritis, and immune toxicity (Rotter et al., 1996; Pestka and Smolinski 2005; Alizadeh et al., 2015). Teratogenic effects are only seen at very high DON doses and therefore not as relevant as feed refusal, decreased weight gain, gastroenteritis and immunotoxicity. The acute effects of DON in humans as observed in food poisoning incidents (Bhat et al., 1989; Luo 1994) are similar to those observed in animals with typical symptoms such as nausea, vomiting, abdominal pain, diarrhea, headache, dizziness and fever. Although long-term

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effects in humans are not established so far, chronic dietary DON exposure in animals causes altered nutritional efficiency, impaired growth and immune function (Pestka and Smolinski 2005). At the cellular level, the primary toxic effect of DON is inhibition of protein synthesis, activation of a signaling pathway known as ribotoxic stress response and induction of apoptosis (Pestka 2010; Wang et al., 2014).

In light of the above and the prevalence of DON as contaminant, it is important to protect consumers against undesirable effects by limiting dietary exposure to levels lower than the 'provisional maximum tolerable intake' (JECFA, 2011). Thus, most developed countries have set a regulatory limit for this mycotoxin in grain and food commodities and conduct regular surveys (EFSA, 2013; BMEL, 2014). In contrast, many developing countries still need to establish regulatory strategies to control mycotoxin contamination in food and feed. In Bangladesh, one small survey detected DON in 10 maize samples (17% positive), with the highest level of 337 μg/kg in maize from the Northern part of the country (Dawlatana et al., 2002). The reported DON levels did not exceed US or EU regulatory limits, but this study focused on maize alone and did not include analysis of other possibly contaminated food commodities. In situations, when food contaminant data are scarce, as often the case in developing countries, analysis of biomarkers in human body fluids provides useful insights, since biomonitoring covers mycotoxin intake from all dietary sources and exposure by various routes (Degen 2011; Turner et al., 2012).

Sensitive analytical methods have been developed and applied for the quantification of DON and its metabolites in human urine. Levels of unmetabolised or 'free' DON (aglycone) together with DON-glucuronides in urine reflect rather well dietary mycotoxin exposure, and the mean estimated fraction excreted within a day is rather high with about 68% (Warth et al., 2013) and 72% of the DON intake (Turner et al., 2010b). As DON is mostly present as glucuronide conjugate in human urine (Warth et al., 2012; Turner et al., 2011b), enzymatic hydrolysis of samples (deconjugation) is used in many single or multi-analyte methods for biomarker determination to increase detectability of total DON (Ali et al., 2015a; Solfrizzo et al., 2013). Main metabolites of DON identified in mammals (Maul et al., 2015; Šarkanj et al., 2013) are deoxynivalenol-3-glucuronide (DON-3-GlcA), deoxynivalenol-15-glucuronide (DON-15-GlcA), and the minor metabolite de-epoxy deoxynivalenol (DOM-1) that is also predominantly excreted as glucuronide. The far less toxic metabolite DOM-1 is formed by gut microbiota in animals (ruminants) and humans, yet to a different extent (Pestka and Smolinski, 2005). DOM-1 has been detected infrequently and at very low levels in urines of various cohorts (Turner et al., 2010a,b, 2011a,b; Brera et al., 2015; Föllmann et al., 2016), whilst a recent study reported relatively high urinary DOM-1 levels indicating that a certain fraction of DON may be detoxified also in humans (Gratz et al., 2014).

Up to now, DON biomarker occurrence has been analysed in urine samples from the general population in some African, Asian, and European countries (see Discussion section for more details). Data from such biomonitoring studies indicate quite variable human exposure to DON in various regions of the world, and likely reflect differences in DON contamination of food commodities and also food habits of the populations studied. This view is supported by a recent study where the observed levels of DON and its metabolites (DON-glucuronides) in human urine samples from Italy, Norway and the UK,were associated with the consumption of certain types of grain-based products (Brera et al., 2015). First analyses of DON biomarkers by a direct dilute-and-shoot approach found a low frequency of positive detects in urines collected in Germany (Gerding et al., 2014, 2015); yet this data is insufficient to reliably estimate DON exposure. Better biomonitoring data for Germany are thus of interest, also in light of a frequent and variable DON contamination in raw grains as documented in the annual German harvest report (BMEL, 2014) and in surveys of cereal-based food samples (Curtui et al., 2005).

A recent study conducted in the Dhaka district (central part) of Bangladesh reported the presence of DON biomarkers in 52% of urines from a pregnant women cohort (Ali et al., 2015a). Yet, data on DON exposure are still limited, and further biomonitoring studies in the general population from other parts of the country are of interest, considering also possible regional and seasonal differences in Bangladesh. Bangladesh has a subtropical climate with wide seasonal variations in rainfall, temperature and humidity, whilst regional climatic differences in this flat country are minor (http://en.wikipedia.org/wiki/Geography_of_Bangladesh). Three seasons are generally recognized: a hot, muggy summer from March to June; a hot, humid and rainy monsoon season from June to November; and a warm, dry winter from December to February. (http://en.wikipedia.org/wiki/Geography_of_Bangladesh). Moreover, since the climate, the main cereal crops and the food habitsin Bangladesh are different to those in Germany, a targeted biomarker analysis will provide new insights into DON exposure of inhabitants in both countries that reflects these differences. Also, contamination of grains with DON-producing *Fusarium* species is predominant in temperate regions of the world (Bryden 2007; Canady et al., 2001).

The present study thus investigated prevalence and levels of DON biomarkers in urines collected during two seasons (summer and winter) from rural and urban residents of the Rajshahi district (north-west part Bangladesh) to compare the new results first with those of our recent analysis in pregnant women residents of Dhaka district in Bangladesh. Biomarker analysis was also conducted in a German cohort in order to compare the situation between both countries in a broader context. Biomarker concentrations determined here and the known urinary excretion rates for DON were also used to calculate the daily mycotoxin intake in both cohorts and are then discussed in relation to the provisional maximum tolerable daily intake (PMTDI) of 1 μg/kg b.w. (JECFA, 2011).

2. Materials and methods

2.1. Reagents and chemicals

Methanol (LC–MS gradient grade) was purchased from Merck (Darmstadt, Germany). Standards for deoxynivalenol (DON), de-epoxy DON (DOM-1) and isotope labeled internal standard ([$^{13}C_{15}$] DON) were obtained from Romer Labs Diagnostics GmbH (Tulln, Austria). The enzyme β-glucuronidase/arylsulfatase (β-Gluc/ArylS) from *Helix pomatia* (with specific activity 5.5 U/mL β-glucuronidase, 2.6 U/mL arylsulfatase at 37 °C) was from Roche Diagnostics (Mannheim, Germany) and used with 10-fold hydrolysis buffer (13.6 g sodium acetate hydrate, 1.0 g ascorbic acid, 0.1 g EDTA in 100 mL deionised water, adjusted to pH 5.0 with acetic acid 96%) for enzymatic treatment of urine samples. Immunoaffinity DONTest™ columns (Vicam®, purchased from Ruttmann, Hamburg, Germany) were used for clean-up and enrichment of analytes.

2.2. Study population and sample collection

This study was carried out in Rajshahi district located in the north-western part of Bangladesh and in Dortmund in Germany. In Bangladesh a total of 164 urines were collected in summer (n = 69, May 2013) and winter season (n = 95, February 2014) from a rural (Mongol Para, Puthia) and an urban area (Rajshahi University region) of this district. There were 62 volunteers (n = 30 in rural and n = 32 in urban) who provided urines in both sampling rounds. In Germany, 50 urine samples were collected from IfADo employees