Environment International 115 (2018) 29–37

Contents lists available at ScienceDirect

Environment International

journal homepage: www.elsevier.com/locate/envint

Exposure to aflatoxin and fumonisin in children at risk for growth impairment in rural Tanzania

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ARTICLE INFO

Handling Editor: Lesa Aylward

Keywords:
Aflatoxin
Fumonisin
Dietary exposure
Biomarkers
Child growth

ABSTRACT

Growth impairment is a major public health issue for children in Tanzania. The question remains as to whether dietary mycotoxins play a role in compromising children's growth. We examined children's exposures to dietary aflatoxin and fumonisin and potential impacts on growth in 114 children under 36 months of age in Haydom, Tanzania. Plasma samples collected from the children at 24 months of age (N = 60) were analyzed for aflatoxin B_1-lysine (AFB_1-lys) adducts, and urine samples collected between 24 and 36 months of age (N = 94) were analyzed for urinary fumonisin B_1 (UFB_1). Anthropometric, socioeconomic, and nutritional parameters were measured and growth parameter z-scores were calculated for each child. Seventy-two percent of the children had detectable levels of AFB_1-lys, with a mean level of 5.1 (95% CI: 3.5, 6.6) pg/mg albumin; and 80% had detectable levels of UFB_1, with a mean of 1.3 (95% CI: 0.8, 1.8) ng/ml. This cohort had a 75% stunting rate [height-for-age z-scores (HAZ) < -2] for children at 36 months. No associations were found between aflatoxin exposures and growth impairment as measured by stunting, underweight [weight-for-age z-scores (WAZ) < -2], or wasting [weight-for-height z-scores (WHZ) < -2]. However, fumonisin exposure was negatively associated with underweight (with non-detectable samples included, $p = 0.0285$; non-detectable samples excluded, $p = 0.005$) in this cohort of children. Relatively low aflatoxin exposure at 24 months was not linked with growth impairment, while fumonisin exposure at 24–36 months based on the UFB_1 biomarkers may contribute to the high growth impairment rate among children of Haydom, Tanzania; which may be associated with their breast feeding and weaning practices.

1. Introduction

Growth impairment is one of the key indicators of child malnutrition, and is an underlying cause in 2.9 million deaths in children under age 5 worldwide in 2011 (Black et al., 2013). Beyond the indication of malnutrition, childhood stunting, underweight, and wasting have been associated with increased vulnerability to infectious diseases, cognitive impairment lasting well beyond childhood, and potentially reduced adulthood achievement (Blössner and Onis, 2005; Schaible and Kaufmann, 2007; Guerrant et al., 2008; Victora et al., 2008). Most of these cases of child growth impairment occur in resource-poor regions of the world in Africa, South Asia, and Central America (Black et al., 2013).

There are an estimated 130 deaths of children under age five every day in Tanzania (URT and UNICEF, 2016). Growth impairment may play an important role in these deaths. Tanzania has the third highest rate of childhood stunting and underweight among African nations, after Ethiopia and the Democratic Republic of Congo (United Republic

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https://doi.org/10.1016/j.envint.2018.03.001
Received 10 November 2017; Received in revised form 1 March 2018; Accepted 1 March 2018
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of Tanzania (URT) and United Nations Children's Fund (UNICEF), 2016). In 2016, the prevalence of stunting (height-for-age z-score < −2), underweight (weight-for-age z-score < −2) and wasting (weight-for-height z-scores < −2) for children under five years old was 34%, 14%, and 5%, respectively (MoHCDGEC et al., 2016). Although multiple factors are involved in child growth impairment, there has been a growing interest in understanding the role of dietary and environmental toxins in increasing the risk.

Aflatoxin is a dietary mycotoxin produced by the fungi *Aspergillus flavus* and *A. parasiticus*, which commonly infect food crops in warm climates worldwide (Kensler et al., 2011) The main sources of aflatoxin exposure in humans are maize and groundnuts, which are consumed in significant amounts by many populations worldwide; particularly in low- and middle-income nations in tropical/subtropical climates (Villers, 2014; Wu and Guclu, 2012). "Naturally occurring mixes of aflatoxins" are classified as a Group 1 human carcinogen by the International Agency for Research on Cancer (IARC, 1993). Aflatoxin exposure causes hepatocellular carcinoma (HCC), or liver cancer, which is the third-leading cause of cancer deaths worldwide. Most of these aflatoxin-related cancer cases occur in sub-Saharan Africa, China, and Southeast Asian countries where people subsist largely on cereal and cereal products (Liu and Wu, 2010). Moreover, extremely high doses of aflatoxin may cause acute aflatoxicosis in animals and humans; symptoms include internal hemorrhaging, acute liver damage, edema, and death (Khlangwiset et al., 2011). In the last two decades, interest has grown in the potential role of aflatoxin in child growth impairment and various epidemiological studies have shown associations between aflatoxin exposure and growth impairment among children in Africa and the Middle East, where maize and nuts are dietary staples (Gong et al., 2002, 2004; Okoth and Ohingo, 2004; Turner et al., 2003, 2007; Shuaib et al., 2010). However, two recent studies did not find an association between aflatoxin exposure and impaired growth of children in Nepal and Tanzania (Mitchell et al., 2017; Shirima et al., 2015).

Fumonisin, another mycotoxin, is produced by the fungi *Fusarium verticillioides* and *F. proliferatum* species, which also commonly infect maize and maize-based products in warm climates worldwide. Fumonisin B_1, which is the most prevalent form of fumonisins, has been classified by IARC as a Group 2B possible human carcinogen (IARC, 2002). The Joint FAO/WHO Expert Committee on Food Additives (JECFA) established a provisional maximum tolerable daily intake (PMTDI) for fumonisins of 2 μg/kg bw/day on the basis of a no observed adverse effect level (NOAEL) for nephrotoxicity in male rats and an extrapolation factor of 100 (Bulder et al., 2012). Fumonisin has been shown to be associated with esophageal cancer in Asian and South African adult populations: higher fumonisin exposure from dietary sources correlated with higher number of esophageal cancer cases (Alizadeh et al., 2012; Shephard et al., 2002; Sun et al., 2007, 2011); and neural tube defects (NTDs) in human babies whose mothers were exposed to high level of fumonisins through consumption of maize-based food during the first trimester of pregnancy (Missmer et al., 2006).

Although dietary fumonisin intake is high among young children in rural areas of sub-Saharan African countries (Kimanya et al., 2010), only two epidemiological studies on the relationship between fumonisin exposure and child growth have been conducted. Kimanya et al. (2010) found that infants in Tanzania with relatively higher fumonisin intakes (exceeding the JECFA's PMTDI of 2 μg/kg bw/day, estimated from caregivers' dietary recall questionnaires) were significantly shorter and lighter than those whose fumonisin intakes were below the JECFA's guidance value (Kimanya et al., 2010). Shirima et al. (2015) reported that there was a negative relationship between fumonisin exposure and child growth among children from four villages in Tanzania, based on validated urinary biomarker levels of fumonisin exposure. By contrast, aflatoxin exposure did not have a significant impact independently on child growth in these cohorts; although co-exposure to these two mycotoxins was associated with impaired growth. It was concluded that fumonisin exposure is a significant risk factor in length and weight of the young children after the covariates were adjusted. However, other risk factors such as micronutrient status or exposure to infectious agents were not taken into account in these studies. The Etiology, Risk Factors, and Interactions of Enteric Infections and Malnutrition and the Consequences for Child Health and Development program (MAL-ED) is a multi-institutional project led by the Foundation of the National Institutes of Health (FNIH, 2018). It is a prospective longitudinal cohort study examining children from birth to 36 months of age, to identify risk factors compromising child health and development in eight low- to middle-income sites worldwide (MAL-ED Network Investigators, 2014). Traditionally, vitamin deficiencies, poor hygiene and lack of sanitation facilities (water scarcity and poor quality, lack of access to proper toilets), and infectious diseases (e.g. diarrhea, malaria) and dietary behaviors, including favoring boys over girls in food availability have been considered major risk factors in child growth impairment in low-income countries (Hadley et al., 2008; Katona and Katona-Apte, 2008; Levinson et al., 2004; Schmidt, 2014). However, after interventions were implemented to reduce growth impairment in some West African countries, including micronutrient supplementation and routine vaccinations, child stunting rates remain high (Ahmed et al., 2012). These results suggest the presence of other factors in stunting in these high-risk populations.

A contributing factor to poor growth may be dietary toxins. Mycotoxins are common contaminants of staple food that make up a large proportion of weaning foods of children. This study focuses on the potential role of dietary exposures to two of the most prevalent mycotoxins in maize - aflatoxin and fumonisin - on children's growth at the ages of 24 to 36 months of life in Haydom, Tanzania.

2. Materials and methods

2.1. Study design and description of study site

A detailed study design for the entire MAL-ED network of investigators is provided in the series of MAL-ED publications (MAL-ED Network Investigators, 2014). The Tanzania site within the MAL-ED network is Haydom, located in northcentral Tanzania approximately 300 km from Arusha. Haydom has a population of about 23,000 inhabitants of various ethnicities, and is a geographically diverse set of rural villages. This MAL-ED study area was chosen due to the comparatively high rates of growth impairment which is 55% among children aged 24 to 60 months according to a recent study (Psaki et al., 2014), the presence of Haydom Lutheran Hospital, and its clinics that support an efficient reproductive health system across the area (Ahmed et al., 2012; Mduma et al., 2014). In the present study, mother-infant dyads were recruited from communities within the Manyara Region of Haydom, Tanzania over a 2-year period beginning in November 2009. Determination of included study areas within the Manyara region is described in detail in Mduma et al. (2014). Location relative to Haydom Lutheran Hospital and child growth parameters were determining factors for inclusion of specific villages into the cohort. The Haydom cohort is a socioeconomically marginalized, rural community consisting primarily of subsistence farming households. Selection bias was minimized by defining a representative mother/child population and screening every mother for eligibility. Inclusion criteria included: mother aged 16 years or older, singleton pregnancy, intention to remain in the study area for at least 6 months following enrollment, and birth weight or enrollment weight of > 1500 g. Exclusion criteria included diagnosis of congenital disease or severe neonatal disease (MAL-ED Network Investigators, 2014).

IRB approval was obtained from the National Health Research Ethics Committee, which is part of the National Institute for Medical Research of Tanzania, and Michigan State University. In total, plasma samples collected at 24 months of age (N = 60) were utilized for measuring aflatoxin B_1-lysine (AFB_1-lys) biomarker concentrations and

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