Multiple mycotoxin exposure during pregnancy and risks of adverse birth outcomes: a prospective cohort study in rural Ethiopia

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ABSTRACT

Introduction: Mycotoxin exposure during pregnancy has been associated with adverse birth outcomes in low- and middle-income countries. The evidence, however, is inconsistent and mainly limited to the assessment of a single mycotoxin. We assessed biomarkers of exposure to multiple mycotoxins during pregnancy and their associations with adverse birth outcomes in rural Ethiopia.

Methods: We analyzed data from 579 pregnant women between 8 and 24 weeks of completed gestation enrolled in a prospective cohort study. Serum mycotoxin concentrations were determined using liquid chromatography coupled with tandem mass spectrometry. Multivariable linear probability models, adjusted for potential confounding factors and multiple comparisons, were fitted to assess the associations between mycotoxin exposure and small for gestational age and preterm birth. We applied principal component analysis to reduce the dimensionality of biomarker data from several taxonomic mycotoxin groups.

Results: All pregnant women were co-exposed to at least five mycotoxins, and one pregnant woman was co-exposed to 27 mycotoxins. Fumonisins (FB), i.e., FB1, FB2, FB3, and tenuazonic acid were the most frequently identified mycotoxins in 98.8, 95.3, 93.3, and 81.4% of the samples respectively. Deoxynivalenol was detected in 38.7%, nivalenol in 50.1%, ochratoxin α in 67.9%, and zearalenone in 50.9% of the serum samples. After adjustment, we found no statistically significant (all P ≥ 0.05) associations between mycotoxin exposures and birth outcomes.

Conclusions: Despite our study providing no evidence for relationships between mycotoxin biomarkers and adverse birth outcomes, our findings do indicate an extensive presence of multiple mycotoxin exposure among pregnant women. Public health policies and nutrition-sensitive interventions must ensure exposure to mycotoxins is reduced in rural Ethiopia.

1. Introduction

Mycotoxins are secondary metabolites produced by toxigenic fungi in plants. In sub-Saharan Africa, women and children are at higher risks of chronic (multiple) mycotoxin exposures, predominantly due to monotonous diets based on contaminated staple food crops (e.g., aflatoxins (AFs) and fumonisins (FBs) in rice and maize) (Andrews-Trevino et al., 2020; Kimanya et al., 2008; Gong et al., 2008). Fungal toxin contamination of food products might translate into detrimental acute or chronic health outcomes for consumers e.g., aflatoxin B1 (AFB1) is a cause of aflatoxicosis and a major risk factor for hepatocellular carcinoma (Wild and Gong, 2010; Kamala et al., 2018). Moreover, AFs...
are known to transverse the placental barrier, which might lead to embryonic or fetal exposure during critical developmental stages (Par- 
tanen et al., 2010; Stillerman et al., 2008). Mechanistic studies have 
indicated that in utero exposure to AFs might cause adverse birth out-
comes by inducing environmental enteric dysfunction, upregulating pro-
flammatory cytokines, downregulating anti-inflammatory cytokines, 
and increasing the toxicity to maternal and fetal organs (Smith et al., 
2012; Shuaib et al., 2010).

Numerous studies have indicated high prevalence of mycotoxin ex-
posures during pregnancy in low- and middle-income countries (LMICs) 
(Piekola et al., 2012; Leroy et al., 2015; Groopman et al., 2014). 
Consequently, there is heightened interest to quantify the effect of 
multiple mycotoxins on fetal growth restriction (Turner et al., 2007; 
Passarelli et al., 2020) and attributable post-natal child linear growth 
altering (Gong et al., 2004; Leroy et al., 2018; Chen et al., 2018). 
Newborns with adverse pregnancy outcomes, such as low birthweight 
(LBW), small for gestational age (SGA), and preterm birth (PTB), have 
increased risks of morbidity and mortality during the neonatal and post-
neonatal periods, impaired growth and neurodevelopment, and 
increased health and development risks throughout their lifetime (Pet-
rou, 2003; Katz et al., 2013; Christian et al., 2013). Moreover, Rasheed et al. estimated that AF-related stunting caused loss of between 3 and 
36% of disability adjusted life years in low-income African countries 
(Rasheed et al., 2021).

Nevertheless, previous observational research has provided incon-
sistent and low-quality evidence for causal relationships between 
mycotoxin exposures and higher rates of adverse birth outcomes in 
LMICs (Smith et al., 2015; Tesfamariam et al., 2020; Eze et al., 2018). 
To illustrate, several studies have indicated that higher relative AF expo-
sure was significantly associated with an increased risk of adverse birth 
outcome (e.g., LBW, SGA) (Abdulrazzaq et al., 2002, 2004; Andrews-
Trevino et al., 2019; De Vries et al., 1989; Lauer et al., 2019; Shuaib 
et al., 2010), whereas others have provided no or mixed evidence for 
such relationships (e.g., SGA, PTB) (Passarelli et al., 2020; Andrews-
Trevino et al., 2019).

Fumonisin B1 (FB1) might be an important risk or contributing factor 
for epigenetic dysfunction-associated diseases (Sugiyama et al., 2021), 
including esophageal cancer in humans (Shephard, 2011). Moreover, 
limited research has also revealed that chronic maternal exposure to FBs 
during early pregnancy is associated with an increased incidence of 
nervous tube defects in their offspring (Misser et al., 2006; Marasas 
et al., 2004). However, mechanistic evidence is still lacking. Zear-
alenone (ZEN) mycotoxin has been shown to have estrogenic properties 
on animals; however, the evidence that they may pose a risk to humans 
is limited (Kuiper-Goodman et al., 1987).

Recent studies have indicated that humans are more frequently 
exposed to multiple, rather than to a single mycotoxin (Martins et al., 
2019; Heyndrickx et al., 2015), which has raised concerns about the 
potential combined effects of multiple mycotoxin exposure on human 
health. Nevertheless, most epidemiological studies assessing linkages 
between mycotoxins and adverse health outcomes have focused on the 
independent effects of single biomarkers (e.g., urinary aflatoxin M1 
(AFMI) or FB, and serum aflatoxin B1-lysine (AFB1-lysine)) (Chen et al., 
2018). However, to our knowledge, longitudinal studies evaluating the 
effects of multiple mycotoxin exposure, within taxonomic groups (e.g., 
AF, FB, ZEN, deoxynivalenol (DON)), on adverse birth outcomes are 
currently absent (Eze et al., 2018). Several classes of mycotoxins have 
been identified and characterized to date. These taxonomic groups are 
based on their association with human diseases.

In Ethiopia, 12–36% of neonates were born SGA, whereas 10% of 
children were born PTB in 2012 (Lee et al., 2017). Only a few studies 
have reported AF exposure among Ethiopian mothers (Estete et al., 
2021) and children (Ayele et al., 2017) based on biomarkers, rather 
than the contamination of staple cereals (Getachew et al., 2018; Ayele 
et al., 2018; Seepuuya et al., 2018; Mesfin et al., 2021). Nevertheless, 
associations between multiple mycotoxin exposures during pregnancy 
and rates of adverse birth outcomes have yet to be documented. Using 
data from a prospective cohort study in rural Ethiopia, we quantified 
maternal mycotoxin exposures, in blood samples collected during 
pregnancy, and assessed the relationships with adverse birth outcomes 
in their offspring.

2. Materials and methods

2.1. Study design and setting

We used data from the (ongoing) Butajira Nutrition, Mental Health, 
and Pregnancy (BUNMAP) cohort study collected between October 2017 
and November 2020. The BUNMAP cohort was established under the 
Butajira Health and Demographic Surveillance Site (BHDISS), which 
consists of nine rural and one urban administrative sub-districts, rep-
resenting the lowland, midland, and highland agro-ecological zones in 
the district. Khat (Catha edulis) and chili peppers are the key local cash 
crops, while maize, banana, and enset (Ensete ventricosum) are the main 
staples (Hassen et al., 2020).

The BUNMAP open cohort study planned to follow-up pregnant women 
and their newborns (up until 59 months of age) to evaluate the 
role of maternal nutrition, mycotoxin exposure, and mental health on 
prenatal and postnatal growth and development. Pregnant women 
were identified through active house-to-house surveillance in the BHDISS. 
All women aged 15–49 years who were between eight and 24 weeks of 
completed gestation and planning to deliver in the study area were 
enrolled in the study. Exclusion criteria included women with multifetal 
pregnancy, known pre-existing medical conditions, or a preceding 
pregnancy with complications (i.e., abortion, stillbirth, or neonatal 
death). For the current study, we analyzed data from 579 pregnant 
women from whom serum samples were collected at baseline. Birth 
outcomes were measured among 483 (83.4%) participants (Fig. 1).

The Institutional Review Board of Addis Ababa University, College of 
Health Sciences (099/17/SPH) approved the BUNMAP study protocol. 
Eligible mothers were asked to provide written informed consent of 
participation after an information session detailing the study objectives, 
voluntary participation, and rights to study withdrawal. Study partici-
pants who were anemic (hemoglobin < 11 g/dL) were referred to a local 
health center for follow-up. Our study is reported as per the Strength-
ening the Reporting of Observational Studies in Epidemiology-
Nutritional Epidemiology (STROBE-nut) guideline (Lachat et al., 2016).

2.2. Mycotoxin exposure biomarkers: serum analysis

Potential multiple mycotoxin exposure was assessed from maternal 
serum according to an adapted methodology, which has been previously 
published (De Ruyck et al., 2020). Briefly, aliquot serum samples were 
acclimated at room temperature in Eppendorf tubes and then 
stored. One-hundred and fifty µL of the serum sample was then spiked 
with 10 µL of internal standards and mixed with 150 µL liquid 
chromatography-mass spectrometry (LC-MS) grade acetoneitrile (C3H8N) 
for protein precipitation. After centrifugation (4000 g, 10 min), 240 µL 
of the upper layer, the supernatant, was taken, and then evaporated to 
dryness under a gentle N2 stream with Turbodav at 40 °C for 15 min. 
The residue obtained was reconstituted by vortexing in 150 µL of injection 
solvent (H2O:MeOH, 60/40, v/v), filtered (0.22 µm, PVDF, Durapore®, 
Cork, Ireland), and transferred to a UPLC vial upon LC-MS/MS analysis. 
The analysis of the samples was performed on a Waters® Acquity UPLC 
system coupled to a Quattro XEVO TQ-S mass spectrometer (Waters®, 
Manchester, UK). All instrumental parameters are detailed in a previous 
study (De Ruyck et al., 2020). We used Masslynx™ version 4.1 and 
QuanLynx® version 4.1 (Waters®, Manchester, UK) software to acquire 
and process relevant data.

Data from six taxonomic mycotoxin groups and three individual 
mycotoxins were analyzed for the presence of 33 biomarkers of expo-
sure: AFs (n = 6), FBs (n = 4), trichothecenes (n = 10), ZEN (n = 5),
ochratoxins (OTA, n = 2), Alternaria (n = 3), enniatin B (Enn B), stergmatocystin (STER), and citrinin (CIT). Exposure to a mycotoxin was defined as a serum biomarker concentration above the limit of detection (LOD); the LODs of the various mycotoxins metabolites/biomarkers ranged in the low ppb level from 0.03 (AF-lysine), 0.29 (AFB1), 1.32 (DON), 3.06 (FB1) to 5.84 ng/mL (ZEN).

2.3. Birth outcomes

Our dependent variables were SGA and PTB. PTB is defined as a child being born before 37 completed weeks of gestation (Howson et al., 2013), whereas SGA is defined as a birthweight less than the 10th centile for a specific completed gestational age by gender. SGA was calculated according to the international newborn size standards developed by the International Fetal and Newborn Growth Consortium for the 21st Century (INTERGROWTH-21st) (Villar et al., 2014).

2.4. Covariates

Covariates included in our multivariable-adjusted models were chosen a priori based on relevant literature (Kiserud et al., 2017; Tamirat et al., 2021). To construct a household wealth index across the BUNMAP cohort, we conducted a principal component analysis (PCA) using 26 wealth index constructs adapted from the Ethiopian Demographic and Health Survey (Central Statistical Agency (CSA) [Ethiopia] and ICF, 2016). Our models included the following potential confounders: maternal age (years), height (cm), weight (kg), education, parity (n), depression (yes; no), household wealth index (tertiles), and food security status (food secure; mildly food insecure; moderately food insecure; severely food insecure). Furthermore, we controlled our analyses for each agro-ecological zone and season as a fixed effect covariate (lowland; midland; highland).

2.5. Data collection

Study participants were invited to travel to the nearest health center for a comprehensive baseline assessment, which included a sociodemographic questionnaire, maternal anthropometry, hemoglobin measurement (g/dL), ultrasound examination, and the collection of maternal blood samples. A team of enumerators was given a ten-day training prior to conducting interviews among pregnant women.

Maternal depression was measured using the Patient Health Questionnaire-9 (PHQ-9) (Kroenke et al., 2001), whereas the Household Food Insecurity Access Scale (HFIAS) was applied to capture the access component of household food security (Swindale and Bilinsky, 2006). Maternal height and weight were measured using a Harpenden Pocket Stadiometer and a Tanita HD-314 digital scale, respectively. Hemoglobin concentrations were determined from finger-prick blood samples using a portable hemoglobin analyzer (HemoCue® Hb 301, Angelholm, Sweden). Pregnant women with hemoglobin levels < 11 g/dL were considered anemic. Gestational age at baseline was estimated by an experienced sonographer using a portable diagnostic imaging and full-color, flow-mapping ultrasound system (SonoSite M-Turbo, FUJIFILM SonoSite Inc., Bothell, WA 98021 USA) (Roro et al., 2019).

A trained phlebotomist collected a venous blood sample (5 mL) from eligible women. A temporary field laboratory in the study-area was set up to allow for immediate centrifugation of whole blood samples and preparation of the aliquots. The whole blood samples were taken according to a standardized operation protocol, performed by health-care personnel. Within one hour of collection, the samples were centrifuged, and the supernatant was transferred into the sterile cryovials using a polyethylene pipette. Serum samples were transported in cold boxes.
containing frozen gel packs (−20 °C) to the Ethiopian Public Health Institute (EPHI) for laboratory analyses immediately after collection and stored at −40 °C. Samples were shipped in dry ice to the Centre of Excellence in Mycotoxicology and Public Health, Faculty of Pharma-
caceutical Sciences, Ghent University, Belgium and stored at −80 °C to laboratory analyses. Community-based field workers measured chil-
ren’s birth weights within 72 h of delivery to the nearest 0.01 kg using a
digital baby scale (Tanita BD 585, Tokyo, Japan). Data collection was
carried out electronically using the Open Data Kit software on Android
tables. Enumerators crosschecked the completeness of a questionnaire
and first submitted it to the supervisor for confirmation. The supervisors
checked the data quality before transferring to a central database at
EPHI.

2.6. Statistical analysis

Descriptive statistics were presented as mean ± SD or median
[interquartile range (IQR)] for continuous variables, and as frequen-
cies and percentages for nominal variables. Exposure to a mycotoxin
was estimated as the n (%) of positive samples. We followed a complete
case analysis, where subjects without birth outcomes data collected and
those without blood samples at baseline were not included in our analysis. We
fit linear probability models with robust variance estimators to estimate the associations between mycotoxin exposures and birth
outcomes (i.e., SGA and PTB). Firstly, we evaluated the univariate
associations between each mycotoxin exposure and birth outcomes.
Secondly, multivariable-adjusted regression models were used to assess
the (independent) associations between mycotoxin exposures and birth
outcomes. To avoid over adjustment and multi-collinearity across
the individual mycotoxin variables, a data reduction approach was applied
to summarize mycotoxins under their predefined taxonomic classifi-
ycation. For this purpose, we applied PCA with varimax rotation to generate
latent factors explaining the variance within each taxon of mycotoxins
(O’Rourke and Hatcher, 2013). After identifying the factors which accounted for most of the variance, based on scree plot and eigenvalues
(≥1), these latent factors were used to build the models. Thus, Model 1
β-coefficients were adjusted for other principal components (PC) only,
whereas Model 2 β-coefficients were additionally adjusted for potential
confounding factors. To avoid type-I error inflation due to multiple
hypotheses testing, we used the Benjamini-Hochberg method to control
the false discovery rate. False discovery rate controlling procedures have
been adapted for multiple comparisons.

Data management and statistical analysis was performed using Stata
version 17.0 (StataCorp LLC, College Station, TX, USA). A two-sided
significance level of \( P < 0.05 \) was applied for all analyses.

3. Results

A flow chart of the study is presented in Fig. 1. From the 776 eligible
women enrolled in the BUNMAP cohort, 196 (25.3%) mothers were
excluded due to missing blood samples. Subsequently, we excluded an
additional 96 (12.4%) women due to missing birth weight data, occur-
rence of an adverse birth outcome, or a multifetal pregnancy. We
followed-up the remaining 483 mothers for a median duration of 22
weeks.

3.1. Maternal characteristics and pregnancy outcomes

Baseline characteristics of 579 pregnant women are shown in
Table 1. On average, pregnant women were 25.8 ± 4.58 years old at
enrollment and their median (IQR) parity was 2.00 (1.00, 4.00).
Approximately 48% of mothers were from a food insecure household.
Moreover, maternal BMI was on average 21.5 ± 2.85 kg/m², whereas
gestational age at enrollment was 16.8 ± 4.58 weeks. About one-third of
the participants received no formal education. Baseline hemoglobin
concentration was 13.1 ± 1.44 g/dL and 5.70% of pregnant women were
anemic. Gestational age at birth was 38.9 ± 2.44 weeks and children’s
birth weight was 2935 ± 459 g. Notable is that 27.2% (n = 131) of the
newborns were SGA, 11.6% (n = 56) were LBW, and 19.1% (n = 92)
were born PTB.

Table 1
Mothers’ characteristics and birth outcomes.

| Maternal characteristics (n = 579) | Value \(^{1}\) |
|----------------------------------|-------------|
| Woman’s age at enrollment (years) | 25.8 ± 4.58 |
| BMI (kg/m²)                      | 21.5 ± 2.85 |
| Gestational age at enrollment (weeks) | 16.8 ± 4.52 |
| Household food security           |             |
| Food secure                      | 301 (52.1)  |
| Mildly food insecure             | 68 (11.8)   |
| Moderately food insecure         | 181 (31.3)  |
| Severely food insecure           | 29 (4.80)   |
| Educational status               |             |
| No formal education              | 203 (35.1)  |
| Read and write                   | 41 (7.09)   |
| Primary school                   | 273 (47.2)  |
| Secondary school                 | 50 (8.65)   |
| College                          | 11 (1.90)   |
| Depression                       | 196 (34.0)  |
| Hemoglobin at baseline (g/dL)    | 13.1 ± 1.44 |
| Number of pregnancies            | 2.00 (1.00, 4.00) |
| Birth outcomes (n = 483)         |             |
| Gestational age at delivery, weeks | 38.9 ± 2.44 |
| Birth weight, g                  | 2935 ± 459  |
| SGA (<10th percentile)\(^{3}\)   | 131 (27.2)  |
| LBW (<2500 g)                    | 56 (11.6)   |
| PTB (<37 weeks of gestation)     | 92 (19.1)   |

1 Mean ± SD, median (p\(^{25}\), P\(^{75}\)), or n (%).
2 Depression was measured using the Patient Health Questionnaire-9 (35).
3 SGA was calculated using the international newborn size standards devel-
oped by the International Fetal and Newborn Growth Consortium for the 21st
Century (31) Abbreviation: BMI: body mass index LBW: low birthweight PTB:
preterm birth SGA: small for gestational age.

3.2. Maternal multiple mycotoxin exposure

Prevalence estimates and maternal serum concentrations of 33
mycotoxin biomarkers are shown in Table 2. The highest concentra-
tion was recorded for tenuazonic acid (TeA; 33.8 ng/mL). In our Ethiopian
cohort, average AFB\(_1\)-lysine concentration was 0.30 ± 0.22 ng/mL. The
most widely prevalent mycotoxin group was FB, in particular FB\(_2\)
(98.8%), FB\(_3\) (95.3%), and FB\(_5\) (93.3%). In addition, most mothers
recorded a positive sample for AFB\(_1\)-lysine (85.4%) and TeA (84.1%).
Among the trichothecenes group, nivalenol (NIV; 50.1%) was the most
frequently observed biomarker, followed by deoxynivalenol-3-glucoside
(DON-3G; 43.2%), HT-2 toxin (HT-2; 42%), and 15-acetoxyscirpenol-
ol (15-ADON; 40.8%). Moreover, ochratoxin alpha (O\(\alpha\)) was detec-
ted in 67.9% of the serum samples. Overall, all pregnant women were
co-exposed to at least five mycotoxins (Fig. 2). Multiple exposure to at
least 15 distinct mycotoxins was identified in 61% of study participants.
One pregnant woman was co-exposed to 27 mycotoxins.

3.3. Relationships between maternal mycotoxin exposures and adverse
birth outcomes

The univariate associations between each mycotoxin biomarker and
children’s birth outcomes are presented in Table 3. Mycotoxins that
were significantly associated with a higher rate of SGA were aflatoxin G\(_1\)
(AFG\(_1\)), HT-2, and TeA. AFB\(_1\), beta zearalenol (\(\beta\)-ZAL), and O\(\alpha\)
were associated with a lower PTB rate. However, none of the observed re-
relationships were statistically significant (all \( P > 0.05 \)) after adjustment
for multiple comparisons.

Our PCA analysis indicated that the AFs group was represented by

**Table 2**

Mycotoxin exposures in serum samples of pregnant women (n = 579).

| Mycotoxin biomarker | Positive samples 1 (n %) | Median (range) ng/mL |
|---------------------|--------------------------|----------------------|
| Aflatoxins          |                          |                      |
| AFB1               | 43 (7.43)                | <LOD (<LOD – 0.2)    |
| AFB2               | 47 (8.12)                | <LOD (<LOD – 0.3)    |
| AFG1               | 284 (49.1)               | <LOD (<LOD – 0.9)    |
| AFG2               | 225 (38.9)               | <LOD (<LOD – 0.9)    |
| AFG3               | 49 (8.5)                 | <LOD (<LOD – 0.1)    |
| AFB1-lysine         | 493 (85.4)               | 0.3 (0.5-2.3)        |
| Fumonisins          |                          |                      |
| FB1                | 540 (93.3)               | 1.0 (<LOD – 1.0)     |
| FB2                | 572 (98.8)               | 1.0 (<LOD – 1.0)     |
| FB3                | 552 (95.3)               | 1.0 (<LOD – 1.0)     |
| HFB1               | 160 (27.6)               | 0.0 (<LOD – 4.5)     |
| Triothecenes        |                          |                      |
| 3-ADON             | 158 (27.3)               | <LOD (<LOD – 1.0)    |
| DON                | 224 (38.7)               | <LOD (<LOD – 2.4)    |
| DON-3G             | 250 (43.2)               | <LOD (<LOD – 4.6)    |
| FUS-X              | 225 (38.9)               | <LOD (<LOD – 0.3)    |
| NIV                | 290 (50.1)               | <LOD (<LOD – 2.1)    |
| DOM-1              | 234 (40.4)               | <LOD (<LOD – 2.7)    |
| NEO                | 170 (29.2)               | <LOD (<LOD – 3.7)    |
| DAS                | 100 (17.3)               | <LOD (<LOD – 2.0)    |
| HT-2               | 243 (42.0)               | <LOD (<LOD – 4.4)    |
| T-2                | 194 (33.5)               | <LOD (<LOD – 2.2)    |
| Zearealenone        |                          |                      |
| ZEN                | 259 (90.5)               | 0.1 (<LOD – 9.0)     |
| ZAN                | 196 (33.9)               | <LOD (<LOD – 15.6)   |
| α-ZEL              | 243 (42.0)               | <LOD (<LOD – 10.5)   |
| β-ZEL              | 378 (65.3)               | 2.1 (<LOD – 13.2)    |
| β-ZUL              | 374 (64.6)               | 1.1 (<LOD – 12.9)    |
| Ochratoxins         |                          |                      |
| OTA                | 183 (31.6)               | <LOD (<LOD – 9.1)    |
| OTA2               | 393 (67.9)               | 0.2 (<LOD – 7.6)     |
| Alternaria          |                          |                      |
| AOH                | 190 (32.8)               | <LOD (<LOD – 1.8)    |
| AME                | 280 (48.4)               | <LOD (<LOD – 23.3)   |
| TeA                | 487 (84.1)               | 6.6 (<LOD – 33.8)    |
| Em B               | 272 (47.0)               | <LOD (<LOD – 2.3)    |
| STER               | 316 (54.6)               | 0.2 (<LOD – 1.5)     |
| xCIT               | 216 (37.3)               | <LOD (<LOD – 0.4)    |

**Abbreviations:** 3-ADON: 3-acetyldeoxynivalenol; 15-ADON: 15-acetyldeoxynivalenol; AFB1: aflatoxin B1; AFB2-lysine: aflatoxin B2-lysine; AFB2: aflatoxin B2; AFG1: aflatoxin G1; AFG2: aflatoxin G2; AFM1: aflatoxin M1; AOH: alternariol; AME: alternariol monomethyl ether; CIT: citrinin; DAS: diacetoxyscirpenol; DOM-1: deoxynivalenol; DON: deoxynivalenol; DOM-3G: deoxynivalenol-3-glucoside; Enn B: enniatin B; FB1: fumonisin B1; FB2: fumonisin B2; FB3: fumonisin B3; FUS-X: fusarenon-X; HT-2: HT-2 toxin; HFB1: hydrolyzed fumonisin B1; LOD: limit of detection; NEO: neosolaniol; NIV: nivalenol; OTA: ochratoxin A; α-TOA: ochratoxin α; STER: sterigmatocystin; T-2: T-2 toxin; TeA: tenuazucar; ZAN: zearealenone; α-ZAL: alpha zearelanol; β-ZAL: beta zearelanol; ZEN: zearealenone; α-ZEL: alpha zearalenol; β-ZEL: beta zearalenol.

1 Values above or equal to the LOD were regarded as positive.

Our multivariable-adjusted models, including other PCs and the potentially confounding factors, identified that the second PC factor from the AF group was significantly associated with a higher rate of PTB (β (95% CI): 0.04 (0.00, 0.07); P = 0.027). In contrast, the second PC factor from the triothecenes group was associated with a lower rate of SGA (β (95% CI): −0.06 (−0.12, −0.01); P = 0.024). The first PC factor of the ZEN group was negatively associated with PTB (β (95% CI): −0.06 (−0.11, −0.01); P = 0.022) (Table 4). Nevertheless, we found no statistically significant (all P ≥ 0.05) associations after adjustment for multiple testing of hypothesis.

4. Discussion

To our knowledge, this is the first epidemiological study that comprehensively assessed maternal multiple mycotoxin exposures in serum (i.e. 33 biomarkers) during pregnancy and associations with adverse birth outcomes. Our findings show the presence of multiple mycotoxin exposure among mothers living in resource-poor settings in Ethiopia, and indicate a high prevalence of mycotoxin co-exposure.

After adjustment for potential confounders (including other mycotoxin PC factors) and testing of multiple hypotheses, our findings provide no evidence for statistically significant associations between maternal mycotoxin exposure and SGA or PTB rates.

In the BUNMAP cohort, FB1, FB2, and FB3 were the most frequently detected biomarkers in pregnant women’s serum samples (all > 95%). Our findings are similar to prevalence estimates of urinary FB1 among children in two Tanzanian studies (i.e., 80 and 98% positive samples, respectively) (Chen et al., 2018; Shirima et al., 2015). Research has shown that FB1 might be an important risk factor or contributing factor for epigenetic dysfunction-associated diseases (Sugiyama et al., 2021), including esophageal cancer (Sheppard, 2011) and neural tube defects in animals and humans (Mismser et al., 2006; Marasas et al., 2004).

However, mechanistic evidence is still lacking. Furthermore, we report that over 85% of mothers’ serum samples had detectable levels of AFB1-lysine, which is in concurrence with studies among pregnant women in Tanzania (92%) (Passarelli et al., 2020), Nepal (94%) (Andrews-Trevino et al., 2019), The Gambia (Turner et al., 2007) and Uganda (both 100%) (Lauer et al., 2019), and young children in Tanzania (72%) (Chen et al., 2018), Mexico (Leroy et al., 2018), and Kenya (both ~100%) (Hoffmann et al., 2018). AF exposure is hypothesized to result in damage of the intestinal mucosa and hence nutrient malabsorption and increased gut permeability, immunomodulation, DNA methylation, and alteration in the insulin-like growth factor axis caused by liver damage (Abu et al., 2008; Hernandez-Vargas et al., 2015; Xu et al., 2021). Lastly, our findings on widespread presence of emerging Alternaria toxins is of concern, as this taxonomic group has been shown to induce fetotoxic and teratogenic effects in animals (Pollock et al., 1982; Fraeyman et al., 2017).

In particular, TeA (84.1% positive samples) is considered the most toxic, and many in vivo studies have demonstrated that it causes severe pathophysiological effects, such as intestinal multi-hemorrhages and impaired liver and kidney functions (Fraeyman et al., 2017). In contrast to our results, a mycotoxin exposure assessment by De Ruyck et al. did not detect any serum Alternaria toxins (i.e., TeA, AME, and AOH) among adults from five European countries (De Ruyck et al., 2020). In our study population, all 33 mycotoxin biomarkers analyzed were detected in serum samples, with a co-exposure of at least five mycotoxins. Few studies have reported on co-exposure to mycotoxins, in part, due to the limited number of validated mycotoxin biomarkers (Vidal et al., 2018).

Nevertheless, similar to a biomonitoring study among adults in China (Huang et al., 2021), we report a high frequency of mycotoxin co-exposure in pregnant women in Ethiopia.

Our results indicate relatively low mycotoxin biomarker concentrations in Ethiopia as compared to e.g., AF levels among pregnant women in South Asia (Groupman et al., 2014) or The Gambia (Turner et al., 2020).
However, there are currently no thresholds of safe human mycotoxin exposure in biological specimens (De Ruyck et al., 2020). Moreover, the cumulative effect of co-exposure to multiple mycotoxins at low concentrations remains unknown, due to a lack of robust methodological approaches to analyze the combined, potentially highly correlated, effect of mycotoxin biomarker data. Nonetheless, preliminary evidence from in vitro models suggests that when single mycotoxin exposures at low doses are non-toxic, various combinations of toxins at equal doses can give rise to complementary or even synergistic toxicity (Wan et al., 2013). Although there remains limited mycotoxin toxicity and toxico-kinetic modelling data or validated biomarkers of exposure in biological matrices (e.g., urine, blood, hair), large human biomonitoring initiatives, such as the HBM4EU-program (https://www.hbm4eu.eu/), aim to assess actual mycotoxin exposure in distinct populations and their potential adverse health outcomes. Our findings from bivariate regression models are, at least qualitatively, comparable to (crude) associations estimated between prenatal AFB1-lysine exposure and pregnancy outcomes in five prospective cohorts. Our results indicated no statistically significant associations between mothers’ AFB1-lysine and SGA or PTB rates, although serum concentrations were relatively low in the BUNMAP study. In parallel, Passarelli et al. (2020) and Andrews-Trevino et al. (2019) showed no statistical relationships (i.e., unadjusted estimates) between maternal AF exposure and SGA, PTB and LBW rates in Tanzania and Nepal, respectively. Furthermore, Turner et al. reported that higher maternal AF exposure was not associated with children’s birth weight or length in The Gambia (Turner et al., 2007), whereas De Vries et al. showed significantly higher birth weight among boys (but not girls) born to AF-negative mothers in Kenya (De Vries et al., 1989). Lauer et al. reported significant inverse associations between AFB1-lysine and birth weight and head circumference, but not birth length or completed weeks of gestation in Uganda (Lauer et al., 2019).

Our multivariable-adjusted models; which included other mycotoxin PC factors, a priori defined confounders, or both, showed inconsistent directions, and thus likely spurious associations between mycotoxin biomarkers, across and within taxonomic groups, and SGA or PTB. Moreover, all observed relationships were non-significant after adjusting for multiple testing of hypotheses. Epidemiological studies on mycotoxin and children’s birth outcomes, which have mainly focused on AFs and provide mixed evidence, have important variations in study design, analytical techniques, biological specimens collected, concentrations of mycotoxin biomarkers (i.e., exposure), assessment methods of gestational age, timing of pregnancy exposure, and (measured) confounder adjustment (Tesfamariam et al., 2020).

4.1. Strengths and limitations of the study

Most mycotoxin exposure studies have considered only a single (Mahfuz et al., 2021) or a limited number of mycotoxins (Chen et al., 2018) e.g., urinary FB1 or serum AFB1-lysine, or one mycotoxin group (Tessema et al., 2021) when assessing relationships with adverse birth outcomes. Our quantification of a suite of serum biomarkers should be regarded as both a study strength and limitation. Mycotoxin biomarkers have advantages over foodstuff (De Boevre et al., 2013) or dietary exposure assessments as they provide more objective data on mycotoxins exposure, and correct for the heterogeneous distribution of mycotoxins in food (De Ruyck et al., 2020). However, only urinary AFM1, aflatoxin-N7-guanine, and DON-glucuronides and blood AFB1-lysine have been validated in humans (Vidal et al., 2018). Hence, our findings using non-validated biomarkers (e.g., serum FBs) should be interpreted with caution. Although our study has a wide scope of the most adequate mycotoxins and their metabolites, a significant underestimation of mycotoxin exposure is plausible, especially for heavily metabolized mycotoxins (e.g., AFB1-lysine was found in 85% of the serum samples, while AFB1 was found in only 7.43%). To date, multiple metabolites are still not commercially available enabling quantitative measurements, and validated biomarkers for some mycotoxins have not been identified so far. However, the use of liquid chromatography–high-resolution mass spectrometry (LC-HRMS) in untargeted mode has allowed the identification of metabolites of mycotoxins (Lauwers et al., 2019). Nevertheless, considering the lack of commercial standards, LC-HRMS provides qualitative rather than quantitative benefits to mycotoxins analysis. In the present study, our focus remained on quantification. Thus, further research is required to harness the combination of LC-MS/MS and LC-HRMS, which enables detecting a broad range of mycotoxins, i.e., the
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Abbreviations: 3-ADON: 3-acyldeoxynivalenol; 15-ADON: 15-acyldeoxynivalenol; AFB1: aflatoxin B1; AFB1-lysine: aflatoxin B1-lysine; AFB2: aflatoxin B2; AFG1: aflatoxin G1; AFG2: aflatoxin G2; AFB1: aflatoxin B1; AFB1-lysine: aflatoxin B1-lysine; AFB2: aflatoxin B2; AME: alternariol monomethyl ether; CIT: citrinin; DAS: deoxynivalenol; DOM-1: deoxynivalenol; DON: deoxynivalenol; DOM-3G: deoxynivalenol-3-glucoside; Enn B: enniatin B; FB1: fumonisin B1; FB2: fumonisin B2; FB3: fumonisin B3; FUS-X: fusarenon-X; HT-2: HT-2 toxin; HT-2: HT-2 toxin; TeA: tenuazonic acid; ZAN: zearalanone; α-ZEL: alpha zearalanol; β-ZEL: beta zearalanol; ZEN: zearalenone; α-ZEL: alpha zearalenol; β-ZEL: beta zearalenol.

1 β coefficients (95% CIs) and P-values for the associations between mycotoxin concentrations and birth outcomes estimated using linear probability models with robust variance estimation. P-values were non-significant (P > 0.05) after adjustment for multiple comparisons using the Benjamini-Hochberg method.

4.2. Conclusions

In the BUNMAP prospective cohort study, pregnant women were exposed to multiple mycotoxins, for which evidence of human health effect is currently mixed or missing. Nevertheless, public health policies (e.g., food safety regulations and their enforcement) and nutrition-sensitive interventions must ensure exposure to mycotoxins is reduced. Despite the absence of statistically significant associations with adverse birth outcomes, our findings do indicate widespread prevalence of mycotoxin co-exposure among pregnant women living in rural Ethiopia. Mycotoxins not only pose major acute and chronic risks to both human and animal health, but also affect food and nutrition security by reducing access to safe and healthy food.

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Abbreviations: CIT: citrinin; Enn B: enniatin B; PC: principal component; PTB: preterm birth; SGA: small for gestational age; STER: sterigmatocystin.

Aflatoxins (PC1: aflatoxin B$_1$, aflatoxin G$_2$, aflatoxin M$_1$, and aflatoxin B$_1$-lysine. PC2: aflatoxin B$_1$ and aflatoxin G$_1$). Fumonisins (PC1: fumonisin B$_1$, fumonisin B$_2$, fumonisin B$_3$, and hydrolyzed fumonisin B$_1$). Trichothecenes (PC1: deoxynivalenol, 3-acetyldeoxynivalenol, deepoxy-deoxynivalenol, nivalenol, and neosolaniol). PC2: T-2 toxin and HT-2. PC3: deoxynivalenol-3-glucoside, and PC4: fusarenon and diacetoxyscirpenol).

Zearalenone (PC1: zearalenone, alpha zearalenol, beta zearalenol, and zearalenone). Ochratoxins (PC1: ochratoxin A and ochratoxin $\alpha$). Alternaria (PC1: alternariol and alternariol monomethyl ether. PC2: tenuazonic acid).

Association became non-significant ($P \geq 0.05$) after adjustment for multiple comparisons using the Benjamini-Hochberg method.

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Author contributions

CL and MDB: conceived the study; KT, SH, MBD, and CL: planned and implemented the study; and reviewed, or approved the final manuscript. All authors decllare no competing interests.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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