Determination of Zearalenone in Urine Samples from Guatemala: A Pilot Assessment

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Abstract:

Zearalenone (ZEA), one of the most prevalent estrogenic mycotoxins, contaminates crops, including maize, rice, and wheat. Previous studies have found high levels of mycotoxins, particularly fumonisin B₁ (FB₁) and aflatoxin B₁ (AFB₁) in maize from Guatemala. In addition, our group has reported high levels of serum aflatoxin B₁ (AFB₁) in this population. However, exposure to ZEA has not been examined in Guatemala. Thus, this pilot study assessed the presence of ZEA in urine samples from Guatemala City and vicinity (n=50). In addition, correlation between ZEA and AFB₁ was examined as well as correlations with maize and tortilla consumption. Ten of the 50 samples had detectable levels of ZEA, with a median level of 2.0 pg/uL. No correlations were found with AFB₁ levels, nor with maize or tortilla consumption. This is the first report on the presence of ZEA in urine samples from Guatemala. These results suggest that further research on ZEA in Guatemala is warranted.

Background:

Zearalenone (ZEA), a nonsteroidal mycotoxin with estrogenic properties, is produced by several species of *Fusarium* fungi that contaminate crops, such as maize, rice, wheat, barley, sorghum, and oats.¹ The estrogenic effects of ZEA cause a variety of reproductive disorders in animals, especially in swine.² ZEA has also been reported to increase the risk of liver and pituitary cancers in mice.³

There is limited data examining exposure to ZEA using biological samples. In rural China, a study that examined the presence of several mycotoxins in urine and plasma samples (n=260) found that ZEA was one of the most prevalent mycotoxins detected for both type of samples.⁴ In addition, a recent study from Spain (n=40) reported that 72.5% of urine samples had at least one of the mycotoxins examined, with ZEA being present in 40% of the samples.⁵ Correlations between ZEA and other mycotoxins (e.g. aflatoxin) have

not been examined in these studies, nor have correlations with dietary data been conducted to determine potential sources of contamination.

In Guatemala, a 2012 survey found high levels of mycotoxins, particularly fumonisin B₁ (FB₁) and aflatoxin B₁ (AFB₁) in maize samples across the country.⁶ In 2017, our group reported high levels of serum AFB₁-albumin adducts in a cross-sectional study of Guatemalan adults.⁷ To the best of our knowledge, ZEA has not been assessed in biological samples in this population. Therefore, the purpose of this pilot study was to determine the presence of ZEA in urine samples from individuals in Guatemala. In addition, the current study aimed to assess the correlation between urinary levels of ZEA and serum levels of AFB₁-albumin adducts, and to assess the associations between ZEA and maize and tortilla consumption levels.

Methods:

In 2019, urine and blood samples were obtained from 25 men and 25 women aged 18 years and older from Guatemala City and vicinity. The sample size was selected to capture a sufficient range of possible detectable levels of ZEA and AFB₁-lys albumin adducts. Demographic information such as age, sex, residence, as well as maize consumption was available. The determinations of AFB₁-lys adducts and ZEA levels were performed by isotope-dilution mass spectrometry in the laboratory of Dr. John D. Groopman at the Johns Hopkins University Bloomberg School of Public Health. AFB₁ adduct concentrations were normalized to total serum albumin and expressed as pg AFB₁-lys adduct/mg albumin.⁷

Descriptive statistics were used to characterize the individuals overall and stratified by sex. Spearman rank-order correlation coefficients were calculated to assess the correlation between urinary levels of ZEA and serum levels of AFB₁-albumin adducts. In addition, correlations between ZEA and maize and tortilla consumption levels were also computed. A two-sided p-value <0.05 was considered statistically significant. The statistical analysis was conducted using SAS v.9.4 (SAS Institute, Cary, NC).

Results:

The median age of the individuals was 34 years (Interquartile range [IQR]: 26,43). Over 50% of the samples were from persons who resided in Guatemala City and vicinity. The median maize consumption among men was 443.4 grams per day (IQR: 166.5, 755.2) and among women was 245.3 grams per day (IQR: 51.3, 559.6). In addition, the median tortilla consumption, the main foodstuff in the Guatemalan diet, among men was 375.0 grams per day (IQR: 150.8, 754.2) and among women was 71.5 grams per day (IQR: 51.3, 133.0) (**Table 1**).

Nearly all samples (n=49) had detectable AFB₁-lys adduct levels, with a median of 4.3 pg/mg (IQR: 2.0, 9.0). Ten of the 50 samples had detectable levels of ZEA, with a median level of 2.0 pg/uL (**Table 1**). No significant correlation was observed between urinary levels of ZEA and serum levels of AFB₁-albumin adducts (Spearman's rank correlation coefficient test= 0.0014; p value: 0.99) (**Figure 1**). In addition, no significant correlations between ZEA levels and maize or tortilla consumption were found (data not shown).

Conclusions:

This is the first report on the presence of ZEA in urine samples from Guatemala. These results suggest that further research on ZEA in Guatemala is warranted.

Conflict of interest:

The authors declare no potential conflict of interest.

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Table 1. Overall characteristics of the sample

Characteristics	Total (n=50)	Men (n=25)	Women (n=25)
Age (median [IQR])	34 (26,43)	30 (26, 39)	38 (26, 53)
Residence, n(%)			
Guatemala City and vicinity	29 (58%)	11 (44.0%)	18 (72.0%)
Outside Guatemala City	21 (42%)	14 (56.0%)	7 (28.0%)
Total maize consumption per day, grams, median (IQR)	302.9 (88.4, 658.9)	443.4 (166.5, 755.2)	245.3 (51.3, 500.0)
Tortilla consumption per day, grams, median (IQR)	134.4 (66.7, 437.5)	375.0 (150.8, 754.2)	71.5 (51.3, 130.0)
AFB₁-albumin adduct levels, (median [IQR])	4.3 (2.0, 9.0)	4.1 (2.3, 13.2)	4.3 (1.6, 7.1)
ZEA pg/uL (median [IQR])¹	0.20 (0.16, 0.29)	0.29 (0.20, 0.30)	0.16 (0.16, 0.16)
Albumin mg/ml (median [IQR])	44.7 (42.6, 47.6)	47.0 (45.1, 48.6)	42.9 (42.2, 44.0)
Creatinine mg/dl (median [IQR])	109.1 (68.9, 146.3)	115.6 (86.4, 210.0)	95.1 (68.9, 130.0)

¹10 samples above the limit of quantification (5 among men and 5 among women) and most of the samples (n=7) were from outside Guatemala City and vicinity.

Figure 1. Correlation between serum AFB₁-albumin adduct levels and urinary ZEA concentrations

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Figure 1.jpg available at <https://authorea.com/users/458783/articles/555253-determination-of-zearalenone-in-urine-samples-from-guatemala-a-pilot-assessment>

Spearman's rank correlation coefficient test: 0.001; p value: 0.99 (No correlation).

Note: Removal of the outlier in the upper right corner of the figure did not result in better correlation (Spearman's rank correlation test= -0.084; p value 0.57).