Prevalence of Aflatoxin M1 and Factors Associated with AFM1 Contamination in Breast Milk of Mothers in Banke, Nepal

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Background

- Aflatoxin M1 (AFM1) are hydroxylated metabolites of aflatoxin B1 (AFB1) expressed in human breast milk (BM) when mothers consume foods contaminated with AFB1 or AFM1 toxins.
- AFM1's expression in breast milk is of significant public health concern due to its known carcinogenic effect, its effect on child growth outcomes, and more importantly since breast milk is the primary source of nutrition for infants under six months of age (1).
- Furthermore, young infants are more susceptible to adverse effects of AFM1 exposure due to low body weight, high metabolic rate, low detoxifying capacity, higher food intake based on body weight, and incomplete development of vital organs and tissues(2–4).
- The International Agency for Research on Cancer has classified AFM1 as 'possibly carcinogenic' for humans (5). The European Union has set 25 nanogram/Liter (ng/L) as the maximum AFM1 level allowed in infant formula and baby foods (6).
- Given the adverse effects of AFM1, susceptibility of infants to AFM1, and universal agreement on the need for reducing AFM1 exposure, it is imperative that AFM1 prevalence and factors associated with AFM1 contamination are studied and addressed.

Objectives and Methods

- The aim of the study is to determine the prevalence of AFM1 and the factors associated with AFM1 exposure in the BM of mothers in Banke, Nepal.
- The data for this study are from the Aflacohort study, an observational birth cohort study which followed 1675 mother-infant dyads from pregnancy up to 2 years of age. This study used data from lactating mother when the infant was at 3 months of age.
- Ethical approval was obtained from Nepal Health Research Council (295/2014) and Tufts University Health Sciences Institutional Review Board (11535). Data used in this analysis was collected between July 2015 and June 2017.
- AFM1 in BM was analyzed using high-performance liquid chromatography. The detection limit of AFM1 in BM is 0.04 ng/L. The AFM1 values were log transformed as the distribution was right skewed.
- The study investigated factors such as mother's education, age, diet, body mass index, seasonality, wealth quintile, and household location as potential factors associated with AFM1.
- All statistical analysis was carried out using Stata SE 14.2 software. Bivariate analysis were used to test the relationship between logged aflatoxin M1 concentrations and the independent variables.
- Food items associated with AFM1 levels (at P-value <0.1) in parametric or non-parametric or both tests and which had a variation of at least 5% between consumption and non-consumption were included in multivariate OLS regression model and quantile regression models at 25th, 50th and 75th quantile regression models.

Results

Figure 1. AFM1 levels (ng/L) in breast milk samples

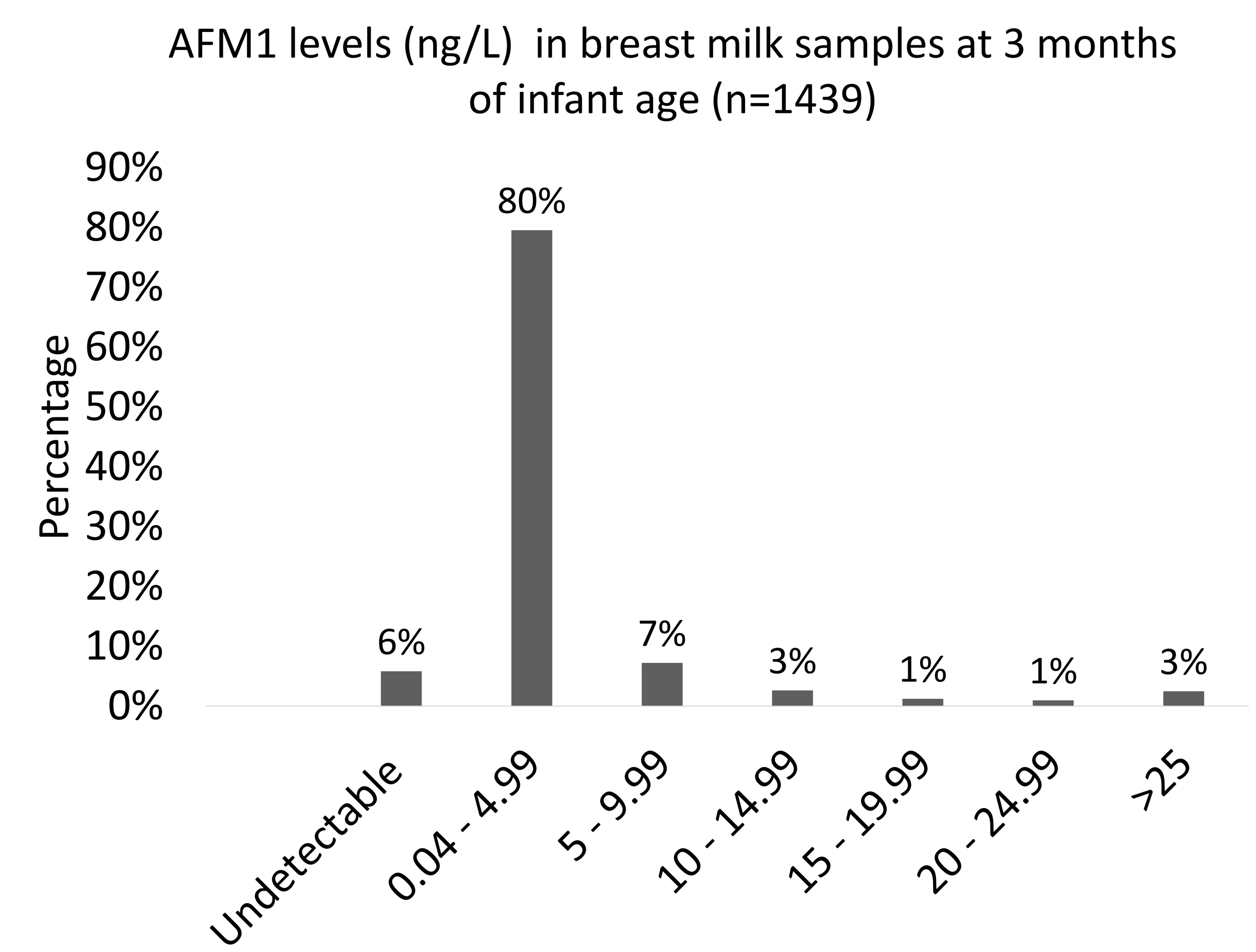

Table 1: Multivariate regression models for factors associated with AFM1

	OLS		Quantile regression at 25th quantile		Quantile regression at 50th quantile		Quantile regression at 75th quantile	
	β	P- value	β	P- value	β	P- value	β	P- value
Mother's age								
15-19 (REF)								
20-24	-0.12	0.39	-0.03	0.80	-0.14	0.44	-0.21	0.10
25-29	0.11	0.48	0.09	0.54	0.31	0.09	-0.11	0.39
30-34	0.15	0.51	0.15	0.43	0.15	0.56	-0.17	0.45
35+	-0.16	0.50	-0.09	0.57	0.24	0.54	-0.42	0.02*
BMI of mother	0.01	0.37	0.01	0.71	0.01	0.54	0.05	0.00**
Nepal's six seasons								
Spring (Basanta) (REF)								
Summer (Grishma)	0.87	0.00**	0.96	0.00**	1.06	0.00**	0.79	0.00**
Rainy (Barsha)	0.53	0.02*	0.29	0.15	0.92	0.00**	0.34	0.20
Autumn (Sharad)	0.36	0.08	0.39	0.00**	0.57	0.02*	0.24	0.34
Prewinter (Hemanta)	0.73	0.00**	0.55	0.00**	1.04	0.00**	0.80	0.00**
Winter (Shishir)	0.71	0.00**	0.49	0.01*	1.07	0.00**	0.87	0.00**
Schooling								
None (REF)								
Primary (1-5 years)	-0.10	0.48	-0.29	0.02*	0.07	0.69	0.07	0.57
Secondary (6-10 years)	0.15	0.32	0.01	0.95	0.53	0.00**	0.25	0.04*
More than secondary (>10 years)	0.35	0.12	0.24	0.19	0.72	0.01*	0.46	0.04*
Wealth quintile								
Poorest (REF)								
Poor	0.10	0.51	0.14	0.40	0.34	0.08	0.09	0.53
Middle	0.19	0.23	0.10	0.51	0.42	0.01*	0.26	0.09
Rich	0.15	0.33	0.23	0.09	0.21	0.25	0.28	0.05
Richest	0.07	0.70	-0.01	0.96	0.12	0.55	0.21	0.18
Consumption of Food items								
Curd in past week	0.26	0.04*	0.20	0.08	0.24	0.09	0.42	0.00**
Milk in past 24 hours	0.21	0.07	0.17	0.14	0.28	0.05	0.14	0.15
Legumes in past week	-0.25	0.03*	-0.36	0.00**	-0.37	0.00**	-0.20	0.03*
Ripe pumpkin in past week	0.39	0.02*	0.42	0.02*	0.45	0.07	0.27	0.12
Gourd in past week	-0.05	0.65	-0.13	0.17	-0.13	0.32	-0.03	0.73
Okra in past week	-0.16	0.22	-0.09	0.43	-0.25	0.07	-0.20	0.06
Hydrogenated oil in past week	0.43	0.04*	0.66	0.05	0.34	0.03*	0.50	0.14
_cons	-1.77	0.00	-2.28	0.00	-2.25	0.00	-1.34	0.00**
Adjusted R2/ Pseudo R2	0.05		0.03		0.04		0.04	
N	1429		1429		1429		1429	

Note: All models were controlled for the location of the household due to the possibility of confounding (i.e. VDCs)

Discussion

- This study found detectable levels of AFM1 in about 94% of breast milk samples with an arithmetic mean of 4.46 ng/L (95% CI: 3.49, 5.43), median of 0.82 ng/L, and a range of non-detectable to 315.99 ng/L.
- Among the food items investigated, consumption of curd, hydrogenated oil, and ripe pumpkin was significantly associated with higher AFM1 levels in breast milk
- Legume consumption was found to have a significant association with lower AFM1 level across all multivariate models.
- Several non-dietary factors (other control factors) such as seasonality, mothers' BMI, mothers' age, mother's education, and household wealth were found to be significantly associated with AFM1 in this study.
- A strong seasonal association was seen across all multivariate models with summer, pre-winter and winter seasons having significantly higher mean AFM1 levels compared to the reference spring season.
- Quantile models were sought to investigate the association of individual factors at various levels of AFM1log distribution. At the 25th quantile of AFM1 log distribution, consumption of legumes, ripe pumpkin, education and seasonality was significantly associated. At the 50th quantile of AFM1 log distribution, consumption of legumes, hydrogenated oil, education, wealth and seasonality were significantly associated. At 75th quantile of AFM1log distribution, consumption of curd, legume, BMI, age, seasonality, and education was significantly associated.
- In this study, mother's BMI was significantly associated with higher AFM1 level in quantile regression at the 75th quantile.
- Mothers with secondary or more than secondary level of education level had higher AFM1 levels compared to mothers with no education level at 50th and 75th quantile. However, mothers with primary education had lower AFM1 level compared to mothers with no education at 25th quantile.
- At the 25th quintile, our study finds that mothers in middle wealth quintile have higher AFM1 compared to mothers in poorest wealth quintile

Conclusions

From a public health perspective, the modest level of AFM1 contamination is worrisome if not alarming as breast milk is the primary source of nutrition for infants. Furthermore, Nepalese mothers often continue breastfeeding their infants up to 2 years of age which implies that younger infants maybe exposed to aflatoxin during early life where rapid growth and development takes place.

Hence, this study recommends identification the major dietary sources of aflatoxin exposure followed by communication and implementation of proper aflatoxin mitigation measures among farmers, food traders and consumers in Nepal.

The study also recommends revision of regulatory standards and proper monitoring for aflatoxin levels in food items in Nepal.

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