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Levels of aflatoxin M_1 among breastfeeding mothers and infants attending Yobe State Specialist Hospital Damaturu Yobe State, North-eastern Nigeria

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Abstract

This study was carried out to determine the level of aflatoxin M1 contamination among breastfeeding mothers whose new born babies, between the ages of 0-6 months, were on admission in Yobe State Specialist Hospital, Damaturu. A total of 200 urine samples were collected and evaluated for aflatoxin levels using the High-Performance liquid chromatography (HPLC). The results revealed that 93% of breastfeeding mothers were exposed to aflatoxin. The urinary discharge rate of the toxin was 100% in unemployed compared to employed mothers. Informal education and elementary school certificate holders had 100% discharge rate of AFM1 in the urine. AFM1 excreted in the urine of lactating

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mothers within 72hrs of food consumption shows 84% of mothers that took milk were not exposed; meat 100% were exposed; cornmeal 93.4% exposed; dates 93%; 'Brabisko/Biski' 30.6%; imported rice 77.7%; native rice 93.4% event taken p< 0.05. In connection to socio-demographic factors, the highest concentrations of the toxin were among unemployed mothers within the age category of 18-25 and 34-41 years with 0.05µg/l. The infants' age category between 5-6 months had concentration of 0.07µg/L, and ≤ 2 months had 0.04µg/L concentration respectively. The discharged rate of AFM1 in urine of infants was 68%. The concentration levels of the toxin among infants indicates a short time exposure to the toxin.The consumption of certain nourishing items by lactating mothers if not strictly regulated exposes babies to AFM1 prompting its discharge in the breast milk that new-born children devour in their initial lives.

Keywords: Aflatoxin M_1; lactating mothers; infants; Urine; HPLC.

Introduction

Human exposure to mycotoxin is a significant issue in developing countries where warm and humid atmospheric conditions may permit fungal proliferation and growth, and in a situation where farm products are stored under poor conditions. Mycotoxins are poisonous metabolites produced by unique parasitic strains of fungi and found in food substances (Ghiasian and Maghsood, 2012).

Mothers are exposed to many toxins which will at a point get to the neonates through breastfeeding. One of this lethal toxins is aflatoxins which is produced majorly by the fungus *Aspergillus flavus* that colonizes the grains most often in tropical locales of high temperature and humidity (Ali *et al.,* 2017). Aflatoxins are poisonous, mutagenic, teratogenic and cancer-causing toxins (Hall and Wild, 1994; IARC, 2002). One of these aflatoxins is aflatoxin B1 that is converted by the enzyme (cytochrome P450) and discharged in the bosom milk as aflatoxin M_1 (AFM1) (Ghiasian and Maghsood, 2012). Aflatoxins have been detected in human sera and cord blood from women promptly after birth. Thus, the transplacental exchange of AF by the fetoplacental unit has been established (Denning *et al.,* 1990).

Exposure to this toxin in West African children and its impacts on the development of the kids have been assessed by Gong *et al.* (2004). Additionally, the centralisation of AFB1 has been connected with decreased birth weight and jaundice in neonates (Abdulu *et al.,* 1998). The immunity and various parts of kids' wellbeing may fundamentally be affected by aflatoxins, resulting in a decline in salivary IgA as observed in Gambian children exposed to aflatoxins (Turner *et al.,* 2003). Kumar *et al*. (2008) reported that aflatoxin-producing species of *Aspergillus* are common and widespread in nature. They can contaminate many staple food at different stages. Poor harvesting practices, improper drying, handling, packaging, storage and transport conditions add to the contagious development and increases the danger of mycotoxin production. Aflatoxin has a high presence in tropical and climatic zones where humidity and temperature conditions are optimal for toxin production (Kumar *et al.,*2008).

A study by Gong *et al.* (2003) showed that regional differences in agro- ecology and food consumption affected aflatoxin exposure in rural areas which is higher than the urban. Leroy *et al*. (2015) showed that serum aflatoxin levels were 5 to 7 times higher among the poorest women as compared to the least poor in a fairly homogenous group of women from poor rural households in Kenya's Eastern Province. The socioeconomic status of the mothers significantly influenced aflatoxin exposure. There was a correlation between AFM_1, AFB_1

and socioeconomic status of the mothers (Ali *et al*., 2017). Adejumo *et al.* (2013) reported that the degree of contamination by AFM_1 and $AFB_1$of lactating mothers was low in Ogun State. There was a higher occurrence of AFB_1 contamination in Ogun east senatorial district. 18% of the breast milk had AFM_1 below the detectable limit, while 16% exceeded it.

A few biomarker-based (milk, urine, and blood) studies coupled to dietary study offer proof of inside nation variety in AF exposure, due differences in agro-ecology (Turner *et al.,* 2012), food storage practices (Hell *et al.,*2000), and other post-harvest practices (Turner *et al.,* 2005). Wild *et al*. (2000) discovered that human subjects in the Gambia had a higher geometric mean of AF-albumin adducts in rural regions than peri-urban regions and that introduction was seasonal. Maternal consumption of mycotoxin-contaminated food such as grain product, milk, and milk products, vegetables, meat, fish, corn oil, dried natural products, and nuts during breastfeeding my resulting in massive build-up of aflatoxin and their metabolites in breast milk and their subsequent discharge in urine.

The International Agency for Research on Cancer classifies aflatoxins as carcinogenic in humans with sufficient evidence for AFM1, AFB1 and AFG1 (IARC, 2012). AFM1 are transferable to sucking babies leading to a myriad of toxic effects such as genotoxic carcinogenesis, liver necrosis, haemorrhage, stunted growth, underweight, reduced food/feed efficiency, immune system suppression, reduced response to vaccines and death (Anthony *et al.,* 2016). Therefore, this study was carried out to assess the level of aflatoxin M_1 exposure among breastfeeding mothers with relation to the foods consumed within 72 hours.

Materials and methods

Study Population

The study utilised a cross-sectional design. The study participants were breastfeeding mothers of different age groups from Buni-yadi LGA whose children are seeking therapeutic services that required the use of urine samples at Yobe State Specialist Hospital Damaturu from July to September, 2018. Written informed consent was gotten from the study participants and ethical clearance was sought from the Yobe State Ministry of Health.

Dietary intake assessment and urine sample collection

The consumption of various kinds of food per individual daily was evaluated based on a food frequency questionnaire involving 100 individuals. Based on one day recall survey method; participants were asked about the foods they consumed. All responses to the dietary recall survey were recorded and documented.

Sample collection and Handling

Urine samples were taken from mothers and their babies within the ages 0-6 months (±2 weeks) before the introduction of any supplements (Tomerak *et al.,* 2011). Morning urine samples (50mL) were collected in a disposable container from lactating mothers as well as their new born babies and transported promptly to the Chemistry Research Laboratory, Yobe State University, Damaturu, where they were stored at - 20 °C (Manson *et al.,* 2015).

Chemical and reagents

Standard solution AFM1 was obtained from Sigma Aldrich, Steinheim, Germany. A daily working solution was set up in acetonitrile/water of 0.004µg/mL (25/75v/v) and this was used to spike the samples according to the method of Cammilleri *et al*. (2018). Deionised water was obtained by utilising a milli-Q purification system (Milford, MA, USA).

Acetonitrile (ACN) and methanol HPLC grade were bought from VRW (Milan Italy).

Preparation of standard solution of AFM1

The stock solution of AFM1 was set up in pure methanol at a final concentration of 0.5 g/mL and kept in dark at 20°C. Working solutions of AFM1 used for calibrating the HPLC and obtaining the calibration curve was prepared by making appropriate dilutions of the stock solutions in methanol and kept in the dark at 20°C.

Preparation of urine samples for high-performance liquid chromatography (HPLC)

Urine extraction and purification of urine sample for AFM1 determination was performed according to the method of Kussak *et al.* (1995) with few modifications. A 25 mL volume of the urine sample was filtered through a glass microfibre filter paper (Whatman Scheider and Schuell, Maidstone, England, item number 934-AH). Afterward, 20 mL of separated concentrate was transferred to a 50 mL capacity vial and 20 mL of sodium acetate buffer (pH 5.0) was added. The pH of the mixture was measured and adjusted to 5.0 using an appropriate volume of 0.1 M glacial acetic acid solution. The mixture was directly passed through an immuno affinity column (Neocolumn, Neogen Europe, UK) at a flow rate of approximately 1.0- 1.5 mL min^{-1}. Subsequently the column was washed with 40 mL of ultra-pure water (Milli Q, Millipore, and Bedford, MA, USA). The column was dried by applying positive pressure with syringe and bound AFM1 was eluted with 2.0 mL of HPLC-methanol which was recovered in a 4 mL vial previously treated with 0.1 M glacial acetic acid. The elute was dissipated under nitrogen gas and reconstituted with 500 µL of the mobile phase before liquid chromatography investigation. Detection and quantification of sample extracts were performed by HPLC with a liquid chromatography system equipped with a LC-10AT Shimadzu siphon (Kyoto, Japan), a Shimadzu RF-10AXL fluorescence detector (excitation 365 nm and emission 460 nm), an injection volume of 100uL, and a reversed phase column (250-4.6 mm, particle size of 3 µm) and pre-segment (Synergi-Fussion, Phenomenex Inc., Torrance, CA, USA) kept at room temperature. The mobile phase comprised of an isocratic mixture of water and acetonitrile at a volume proportion of 75:25 and a flow rate of 1.0 mL min

Analytical performances

The limit of detection (LOD) for AFM1 was assessed as 10 ng/mL and evaluation esteem (LOQ) was 50 ng/mL, the linearity of the curve was 10 to 50 ng/mL. The calibration curve for AFM1 had a linear equation of y = 4147x 230.3 and a correlation coefficient of 1.0. Recovery values for AFM1 were observed to be 98.5 ± 1.8% and retention time of 8.5 min. the outcomes were rectified for recuperation

Statistical analysis

The data obtained from the study was analysed using SPSS version 24 software. The data was summarized as mean and standard deviation, for the analysis of differences in quantitative data between the two groups. Aflatoxin levels in samples exceeding Nigerian and European Union regulated limit of aflatoxin (0.05 µg/L) were considered significantly high. Chi-square test was used for the analysis of qualitative data. A P-value of $p<0.05$was considered significant.

Results

Table 1 shows the association between AFM1 levels in urine of mothers and some socio-demographic factors. Unemployed and employed mothers had the highest exposure at 87%

and 46.1% respectively with p-value 0.001, which is highly significant. All other factors showed significance at 95% class interval, with p-value less than 0.05

Table 1: Association between AFM1 levels in urine of mothers and some socio-demographic factors

Factors	No examined N=100	No. positive (%)	Odds ratio (95% CI)	χ^2	p-value
Occupation					
Employed	13	6(46.1)	2.564(1.915-3.003)	20.451	0.001*
Unemployed	87	87(100)			
Age (yrs)					
18-25	55	51(92.7)			
26-33	39	36(92.3)		5.671	0.047*
34-41	5	5(100.0)			
42 above	1	1(100.0)			
Educational Level					
Informal	39	39(100.0)			
Islamic	43	37(86.0)		8.756	0.048*
Primary	12	12(100.0)			
Secondary	6	5(83.3)			

Table 2 shows the occurrence of AFM1 in the urine samples of infants in relation to age and sex. 95% of the infants were exposed to the toxin at the ages 5-6 months (χ2 =10.08; p=0.032), 3-4 month at 88% occurrence (χ2=3.541; p=0.089); males infants at 78.1% occurrence of AFM1 and there was no significant statistical difference between the genders (OR= 0,611; χ2=0.611; p=0.113).

Table 2: Association between AFM1 in urine of newborn with respect to their age and sex

Factors	No examined N= 100	No. positive (%)	Odds ratio (95 % CI)	χ^2	p-value
Age (months)					
≤ 2	32	10(31.2)		3.541	0.089
3-4	25	22(88.0)			
5-6	43	31(72.9)			
Sex					
Male	32	25(78.1)	0.611(0.487-1.010)	1.127	0.113
Female	68	38(55.9)			

Table 3: Association between AFM1 level in urine of breastfeeding mothers and type of foods consumed within the last 72hrs.

Foods consumed within the last 72 hours	No examined N=100	No. positive (%)	Odds ratio (95 % CI)	χ^2	p-value
Milk					
Yes	67	65(97.1)	2.141(1.911-3.711)	8.247	0.046*
No	33	28(84.1)			
Meat					
Yes	43	43(100.0)	1.145(0.789-1.654)	3.123	0.081
No	57	50(87.7)			
Corn meal					
Yes	79	74(93.4)	0.654(1.112-1763)	10.231	0.014*
No	21	19(90.5)			
Date					
Yes	45	42(93.3)	2.789(1.002-3.456)	8.911	0.043*
No	55	51(92.7)			
Brabisko /Biski (local food)					
Yes	36	11(30.6)	1.156(0.711-1.811)	11.654	0.048*
No	64	60(93.8)			
Imported Rice					
Yes	22	17(77.3)	1.010(1.151-1.863)	7.521	0.063
No	78	76(97.4)			
Native Rice					
Yes	63	59(93.4)	2.500(1.001-3.457)	9.112	0.050*
No	37	34(91.9)			

Table 4. Relationship between AFM1 levels in urine samples of mothers and some socio-demographic factors.

Socio demographic factors	Sample tested N= 100	Positive sample	%	Mean AFM1 (µg/L)
Occupational status of mothers				
Employed	13	6	46.1	0.04
Unemployed	87	87	100	0.05
Age				
18-25	55	51	92.7	0.05
26-33	39	36	92.3	0.04
34-41	05	05	100	0.05
42-above	01	01	100	0.04
Educational level				
Informal	39	39	97.4	
Islamic	43	37	86.0	0.04
Primary	12	12	100	0.04
Secondary	06	05	83.3	0.04

Table 5 showed the concentration of AFM1 secreted by infants in relation to their age and sex, infants between 1day old and two months (<2) had mean concentration of 0.05 µg/L 10(32) with 31.32% positive. Infants between 3-4 months had AFM1 concentration of 0.06ug/L while 5-6 months infants recorded high concentration of 0.07 µg/L

Table 5: Concentration of AFM1 in infants in relation to their age and sex

Factors	No. of samples collected	Positive sample	% of positive	Mean AFM1 (µg/L)
Infants age (month)				
< 2	32	10	31.32	0.05
3-4	25	22	88.0	0.06
5-6	43	31	72	0.07
Infants sex				
Male	32	25	78.3	0.06
Female	68	38	55.9	0.05

Discussion

Of the 100 analyzed urine samples from breast feeding mothers, AFM_1 was present in 93% of the indicating that most of the food substances consumed by the mothers prior to the test were contaminated. The daily dietary exposure of mothers to AFB_1which is metabolized to AFM_1 by the enzymes cytochrome P450 may lead to secretion of the toxin in breast milk as well as its excretion in the urine. Excretion of AFM_1 in the urine among infants that suffered kwashiorkor was reported by Onyemelukwe *et al.* (2012) whose findings revealed that less than 84.3% of the infants under study and who were malnourished may be amenable to AFM_1 production.

Mulunda *et al.* (2013) also reported that approximately 95% of AFB_1 metabolite is excreted in breast milk as AFM_1which when consumed, has effects on the infant. Consummation of breast milk contaminated with AFM_1 was revealed to cause growth retardation in human children. Of the 100 infant urines tested in this study, AFM_1 was detected in 68.0%. Since this infants were on exclusive breast feeding, it suggests that they were exposure to the toxin through breast milk. The lower rate (68.0%) of AFM1 found in infants compared to their mothers might be responsible for the health status of the babies as a strong relationship was observed between aflatoxicosis and protein vitality unhealthiness (Nadia *et al.,* 2005).
The urinary excretion of AFM1 among the breast feeding mothers shows that $AFM_1$was detected in all (100.0%) of the unemployed mothers. This implies that employed mothers are more financially buoyant in such a way that they can afford foods that have fewer levels of aflatoxins contamination than unemployed mothers who may be financially unprivileged to afford such foods. So also, AFM_1 detected in all (100.0%) individual with elementary school education, this result is not in line with the result of Manson *et al.* (2015), who recorded that B.Sc holders had highest exposure of the toxin.

The highest occurrence of AFM1 in infants' urine (88.0%) was found in age group 3-4 months. The difference observed between the age group was statistical significance. This is in contrast to the lactating mothers which follows a pattern of increasing occurrence from the age group of 26-33 years. The study agrees with the reports in Nigeria and Brazil that age does not have any significance impact in determining the levels of AFM1 excretion in urine of infants (Sulaiman *et al.,* 2018; Gide *et al.,* 2019, Giolo *et al.,* 2012,).
The presence of AFM1 in urine was higher in male babies (78.1%), this could possibly be due to fact that male babies may have greater capacity to biotransform the AFB1- AFM1 in the liver resulting in quicker excretion in urine than female babies. It may also be due to the presence of Breast Cancer Resistance Protein (BCRP) in female infants. BCRP decreases aflatoxin uptake while at the same time increasing intestinal hepatobiliary and renal eliminations from the body.

The levels of AFM1 excreted in urine of lactating mothers in connection to foods consumed revealed a higher level of AFM1 in individuals that consumed milk, meat, corn meal, dates, and local rice within 72hrs prior to the study. However, higher level of AFM1 were found in those that didn't consume Brabisko/Biski and foreign rice. This could be that the sources of these foods were different as well as the method of processing these food materials. Therefore, the time interval of food consumption and the type of food may be associated with AFM1 exposure, even though meat and imported rice show no significant association. However, Rommero *et al.* (2010) investigated the presence of AFM1 in Brazilian population and reported that it isn't dependent on corn, nut, and milk consumption alone. This indicates that other idiosyncratic factors may play roles in exposure to this aflatoxin. From this study one of the major risk factors associated with exposure to AFM1 is the type of food taken by lactating mothers. This can be linked to the handling and processing method involved in prepare and processing these foods. This finding is in agreement with the findings of Iha *et al*. (2013).

The concentration of AFM1 in the urine of lactating mothers in relation to selected socio-demographic factors showed 0.05µg/L AFM1 among unemployed mothers and lower concentration (0.04µg/L) among the age of 42 years and above. This may be because elder mothers may be more careful on quality of food they consumed.

The concentration of AFM1 was found to be highest (0.07µg/L) in urine of infants between 5-6 months old while AFM1 level in urine was lowest among infants ≤ 2 months old (0.04 µg/L). This might be linked to increase in milk consumption (which correlates with increase in consumption of AFM1 in milk) with increase in age. This results corroborates with reports by other researcher who studied the relationship between type of food intake and urinary excretion of AFM1such as Mason *et al.*(2015) and Redzwan *et al.*(2012) who also reported a significant relationship between consumption of AFM1in milk and excretion in the urine.

Conclusion

The concentration of AFM1 in urine of mothers and their infants in this study indicates a short time exposure to the toxin. The consumption of certain nourishing items by lactating mothers if not strictly regulated or well processed may continue to expose infants to AFM1 in breast milk.

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