Original Research Article

Exposure assessments of internally displaced infants to Aflatoxin M_1 through breast milk feeding, in Damaturu Yobe State.

Abstract

Aflatoxin M1 is a biomarker for the detection of breast milk contamination and also a risk factor for early infant's exposure to the toxin. Exposure assessment of 50 internally displaced infants to aflatoxin M_1 through breast-milk feeding was carried out between (June 2016 to October 2016), High performance liquid chromatography (HPLC) was used to evaluate the level of AFM1 in mother's breast milk samples and the infant's urine samples respectively. Results obtained from the study showed that 96% of the breast milk samples have maximum concentration of 0.0879µg/L with mean value of 0.0582µg/L while, the minimum and maximum excretion concentration of AFM1 in urine sample of infants was 0.0400µg/L and 0.0651µg/L respectively with mean value of 0.05005µg/L at 88%.

The study indicates that the occurrence of AFM1 in breast milk samples of mothers with the types of food the consumed within 24- 48hrs prior to sample collection that predispose the infant's exposure to AFM1 showed 40% of the women consumed rice and 32% consumed local food (brabisko/biski) and 24% consumed corn meal with statistically significant with P value less than 0.05. From the study the 96% of the infants were exposed to the toxin while

18% of the infants were undetectable this possess a concern that internally displaced infants that were on admission in the selected facility where exposed to AFM1.

Keywords: IDP, breast milk, Aflatoxin M1, HPLC

Introduction

Breast feeding is the best and recommended form of infant feeding. Six month is the recommended period of exclusive feeding for infants. Breastfeeding promotes the mother-child relationship and ensures better growth and development of the neonates given them required nutrients, strengthen antibodies and leukocytes (Brasil, 2010; Ishikawa 2016). Aflatoxins are metabolites of a Fungus of *Aspergilus flavus* and *Aspergilus parasiticus* there are various types of Aflatoxin discovered meanwhile the commonest are Aflatoxin B1, B2, G1,G2, M1 and M2 are the secondary metabolites of B1 and B2 respectively (Bianco *et. al.* 2012). Aflatoxin M1 (AFM1) as a biomarker of Aflatoxin B1 (AFM1) can be used to evaluate aflatoxin exposure through diet for both humans and animals. As a metabolite human exposure to aflatoxin (AFs) occurs through the intake of contaminated agricultural products or the consumption of products from animals that were fed with contaminated feed. This contamination may occur by fungal growth during harvest or improper storage (Ghiasian, 2012). AFB1 is considering the most toxic among all other forms of Aflatoxin (Bhet *et al*., 2010).

Toxicological evaluation of aflatoxin over food intake is essential to any risk evaluation and important for determining the relationship observed in humans and exposure to aflatoxin (Leblanc et. *al.*2005: Shundo *et. al.,* 2009). AFB_1 is metabolized in animals and the human liver into AFM_1 by Cytochrome P450–associated enzymes, and then distributed in serum and excreted into milk and urine. In mammals, AFM_1 can be detected in milk within 12 h after the ingestion of AFB_1 (Battacone *et. al.,* 2003 and 2012).

The maximum limits for fluid milk and powdered milk should be 0.5 and 5 ng/g, respectively. A technical regulation on the Maximum Tolerated Limit for Mycotoxins in Food has not been defined regarding whether the fluid milk is raw or pasteurized. The European guidelines established the maximum AFM_1 levels in both raw milk (0.05 ng/l) and infant formulae (0.025 ng/l) (E C, 2010). Based on the potential hazard to infant (zero to 12 months old) health due to carry-over of the aflatoxin biomarker (AFM_1) into milk, the aim of this study was to evaluate the exposure of infants to AFM_1 through consumption of breast milk.

Materials and Method

Study Area: the study area comprises Yobe State Specialist Hospital located in Damaturu in the north eastern of Nigeria.

Ethical Approval and Parents Consent

Ethical clearance was obtained from the ethical and scientific research committee of the ministry of health. A consent form was given to obtain permission from mothers whose babies were on admission. Only those who gave consent were recruited in the study.

Sample size and Study Population

This study was conducted on 50 internally displaced lactating mothers whose infants were on admission in Yobe State Specialist Hospital Damaturu, Yobe State.

Breast Milk Collection:

Breast milk samples were collected by hand expression into glass tubes; the milk samples were stored at 15^oC. Samples were thawed gradually to 4^oC and then centrifuged at 10^oC at

15rpm.Aflatoxins are water-soluble; hence, the upper creamy layer was discarded and the lower phase was used for the quantitative test (Adejumo 2013).

Structured Questionnaire:

Structured questionnaire was design to determine the kinds of food lactating mothers consumed within 24-48hrs that associate them to Aflatoxin exposure.

High performance liquid Chromatography (HPLC), Analysis for Aflatoxin M1

The concentration of AFM1 in breast milk was estimated by HPLC configured with LC-10AD pumps, coupled with tungsten detector RF-10Axl. Excitation and emission wavelengths were set at 350 and 440 nm respectively. The stationary phase was a Gemini Column. The mobile phase was isocratic mixture of methanol/acetonitrite/water (25:25:50 v/v/v), with a flow rate of 1ml/min and chromatographic run time of 10 min. The values obtained for recoveries and relative standard deviations of the methods of analysis were in agreement with Commission Regulation (EC) No. 401/2006 for methods of analysis of mycotoxins in foodstuffs (European Union Commission, 2006).

An injector with 50 µl loop was used for the determination of AFM1. A calibration curve was constructed for AF M1 using different levels of toxin concentrations with an average of 10 consecutive automated injections of standard solutions of AF M1 purchased from SUPELCO solutions within USA Involving series of dilutions (Adejumo, 2013).

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

The extraction and purification of urine samples for AFM1 determination were performed as follows: A 30 ml volume from the urine sample was filtered through a glass microfiber filter paper. Later, 20 ml of filtered extract 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 corrected to 5.0 using an appropriate volume of a 0.1 M glacial acetic acid solution whenever

necessary. The mixture was directly passed through an immunoaffinity column at a flow rate of approximately 1.0–1.5 ml min^{-1}. After adding the mixture the column was washed with 40 ml of ultrapure water. The column was dried by applying positive pressure with a syringe and bound AFM1 was eluted with 2.0 ml of HPLC-methanol which was recovered in a 4 ml vial previously treated with acid. The eluate was evaporated under nitrogen gas and reconstituted with 500 µl of the mobile phase before liquid chromatography analysis. Detection and quantification of sample extracts were performed by high-performance-liquid chromatography (HPLC) with a liquid chromatography system equipped with a LC-10AT Shimadzu pump, a Shimadzu RF-10AXL fluorescence detector (excitation 250 nm and emission 350 nm), an injection volume of 100µl, and a reverse phase column (250- 4.6mm, particle size of 3 µm) and pre column kept at room temperature. The mobile phase consisted of an isocratic mixture of water and acetonitrile at a volume ratio of 75:25 and a flow rate of 1.0 ml min^{-1}. A calibration curve was prepared using standard AFM1 solutions in mobile phase at concentrations of 0.005, 0.01, 0.02, and 0.03 ng ml^{-1} the standard obtained (SUPELCO solutions within USA.) as purified crystalline AFM1 was dissolved in HPLC-grade acetonitrite and its concentration was determined by spectrophotometer.

Statistical Analysis of the Data

The Statistical Package for Social Science program (SPSS, Chicago, Illinois, USA) version 21 was used for analysis of data. Data was summarized as mean, standard deviation, for the analysis of the difference in quantitative data between the two groups. The X^2-test was used for the analysis of qualitative data. *P value* was considered significant if it was less than 0.05 Simple linear correlations (Pearson's correlation for quantitative data Tumerak, 2011).

Result

Analytical performance

The limit of detection (LOD) for AFM1 was estimated at 0.01µg/mL and the limit of quantification (LOQ) was 0.05µg/mL. The linearity of the curve was 0.01–0.05 µg/mL. The calibration curve for AFM1 had a linear equation of y = 4147.x - 230.3; Fig 1 gives the calibration curve with a correlation coefficient $R^2 = 0.993$ and retention time of 10.0 min.

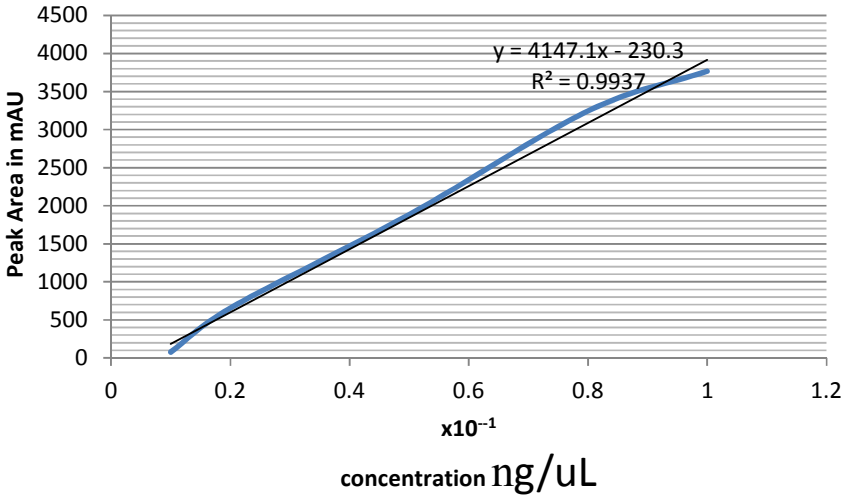

Fig 1 gives calibration curve of AFM1 with correlation coefficient.

Table 1: Occurrence and concentration of AFM1 in breast milk consume by infants and excretion in their urine.

Samples	No examined	No. positive (%)	Min conc. (µg/L)	Max conc.(µg /L)	Mean conc(µg/L) (±SD)	Chi square	p-value
Breast Milk	50	96	0.0457	0.0879	0.0582 (±0.012)	2.204	0.02
Infant's Urine (0-6 months)	50	88	0.0400	0.0651	0.05005(±0.004781)		

SD.Standard deviation of descriptive quantities

Table 2: Occurrence of AFM1 in breast milk samples of mothers with the types of food consumed within 24- 48 hours prior to sample collection.

Consumed foods in 72 hrs	No examined N=50	No. positive (%)	Chi square	p-value
Brabisko/Biski (local food)				
Yes	16	(32.0)	8.245	0.045
No	0	(0.0)		
Rice				
Yes	20	(40.0)	9.123	0.034
No	0	(0.0)		
Cornmeal				
Yes	12	(24.0)	10.233	0.013
No	2	(4.0)		

The percentage occurrence and concentration of AFM1 in breast milk consumed by infants and excretion in their urine as seen in table 1 shows that 96% of the breast milk consumed by IDP infants have the toxin with minimum concentration of 0.0457µg/L and maximum concentration of 0.0879µg/Land the mean concentration of 0.0582µg/L meanwhile, the minimum and maximum excretion concentrations of AFM1in urine samples of infants gave 0.0400µg/L and 0.0651µg/L respectively with mean value of 0.05005µg/L at 88% exposed of the toxin from their mother's breast milk .

Occurrence of AFM1 in breast milk samples of mothers with the types of food the consumed within 24- 48hrs prior to sample collection that predispose the ID infants exposure to AFM1 has been illustrated in Table 2; 40% of the women consumed rice and 32% consumed local food (brabisko/biski) and 24% consumed corn meal with statistically significant with P value less than 0.05.

144 **Discussion**

145 Exposure assessment of internally displaced infants to aflatoxin M_1 through consumption of
146 breast milk in Damaturu was 96% with maximum concentration of 0.0879µg/L of the toxin.
147 The mean value concentration of AFM1 in the breast milk samples was 0.0582µg/L this may
148 be explained that the mothers were exposed to the toxin in their diet. Considerably, another
149 independent screening by Adejumo *et al* (2013) report that occurrence of AFM1 was 82% in
150 breast milk samples in Ogun state, Nigeria which is lower. While Abdulrazzaq *et al.* (2003)
151 and Sadeghi *et al.* (2009) in United Arab Emirate and Iran respectively reported 99.5% and
152 98.1% frequencies which is higher. The percentage occurrence in this study is higher than
153 that reported in of Sierra Leone, Cameroun and Egypt, 31%, 48%, and 56% respectively
154 (Jonsyn *et al*, 1995; Tchana *et al*., 2010; Polychronaki *et al*., 2007). This could be as a result
155 of differences in the type of food consumed, storage condition of foods and lifestyle of the
156 people as well as level of contamination with the secreting *Aspergillus species.*
157 Infant examined are between the age brackets 0-6 month with minimum concentration of
158 0.04µg/L and maximum concentration of 0.0651µg/L with mean excretion concentration of
159 0.05005µg/L. This high concentration implies that the infant were exposed to high level of
160 aflatoxin which is indicative from the levels seen in their mothers' breast milk samples a
161 major food source for the infant which are expected to be breast feed for six months. 88% of
162 the examined children shown exposure to AFM1 which implies that 18% were undetected in
163 the urine samples of the infant which is in line with the study reported in Brazil by Giolo *et*
164 *al.*2012.
165 This result can be compared with other researchers that there is a relationship between food
166 intake and urinary excretion of AFM1 (Mason *et al.,*2015). Redzwan *et al* (2012) reported
167 that there was significant relationship between consumption of AFM1 in milk and excretion
168 in the urine.

Conclusion

The mean concentration of examined breast milk samples was 0.0582µg/L. It should be noted that, the concentration of AFM1 in all the breast milk samples used were higher than the acceptable tolerance level of 0.025µg/L and 0.05µg/L for infants milk by the European Communities and Codex Alimentarius respectively. This possess a concern on internally displaced infants that were on admission in the selected facility where exposed to AFM1.This implies that AFM1 has the potential to be a public health problem in Damaturu.

References

Abdulrazzaq, Y.M.,Osman, N.,Yousif, Z.M., Al-Falahi, S.(2003). Aflatoxin M1in Breast Milk of UAE women. *Ann.Trop. paediat.*,23:173-179.

Adejumo, O., Atanda. O.,Raiola, A., Somorin, Y., Bandyopadhyay, R. and Ritieni, A., (2013) . Correlation between Aflatoxin M1 content of breast milk, dietary exposure to aflatoxin B1 and socioeconomic status of lactating mothers in Ogun State, Nigeria: *Food and Chemical Toxicology*, 56:171-177

Battacone G., Nudda A., Cannas A., Borlino A.C., Bomboi G., Pulina G.(2003) Excretion of aflatoxin M_1 in milk of dairy ewes treated with different doses of aflatoxin B_1. *J. Dairy Sci*. ;86:2667–2675.

Battacone G., Nudda A., Rassu S.P.G., Decandia M., Pulina G. (2012) Excretion pattern of aflatoxin M_1 in milk of goats fed a single dose of aflatoxin B_1.*J. Dairy Sci*.;95:2656–2661.

Barbieri, G., Bergamini, C., Ori, E. and Pasca, P. (1994).Aflatoxin M1 in parmesan cheese: HPLC determination. *J Food Sci.,* **56**(6):1313-31.

192 Bhat, R., Rai, R.V.andKarim, A. A. (2010).Mycotoxins in food and feed: present status and
193 future concerns. *ComprehensRev.FoodSci.FoodSafety*, 9:57–81.

194 Biancardi, A. (1997). Determination of aflatoxin M_1 residues in milk: A comparative
195 assessment of ELISA and IAC-HPLC methods. *In. Aliment*., 36:870–876.

196 Bianco, G., Russo, R., Marzocco, S.,Velotto, S., Autore, G. and Severino, L. (2012).
197 Modulation of macrophage activity by aflatoxins B1 and B2 and their
198 metabolites aflatoxins M1 and M2.*Toxicon*, 59(6):644-650

199 Brasil .Amamentação e uso de Medicamentos e Outras Substâncias. 2nd ed.
200 EditoradoMinistério da Saúde; Brasília, Brasil: 2010.

201 European Commission (EC) EUR-Lex; [(accessed on 17 August 2016)]. Commission
202 Regulation (EC) No. 165/2010 of 26 February of 2010 Amending Regulation
203 (EC) No. 1881/2006 Setting Maximum Levels for Certain Contaminants in
204 Foodstuffs as Regards Aflatoxins. Available online: http://eur-
205 lex.europa.eu/legalcontent/EN/TXT/PDF/?uri=CELEX
206 :32010R0165&from=EN.

207 Ghiasian, S.A. and Maghsood, A.H. (2012). Infants' Exposure to Aflatoxin M1 from
208 mother's Breast milk in Iran: *IranianJ. Pub. Health*, 41(3):119-126

209

210 Ishikawa A., T., Cássia R. Takabayashi-Yamashita, Elisabete Y. S. Ono, Artur K. Bagatin,
211 Fabiana F. Rigobello , Osamu Kawamura, Elisa Y. Hirooka and Eiko N.
212 Itano.(2016).Exposure Assessment of Infants to Aflatoxin M_1 through

213 Consumption of Breast Milk and Infant Powdered Milk in Brazil. *Toxins*, 8(9):
214 246

215 Leblanc J.C., Tard A., Volatier J.L., Verger P.(2005) Estimated dietary exposure to principal
216 food mycotoxins from the first French Total diet study. *Food Addit.*
217 *Contam*.;22:652–672.

218 Mason, S., Hajimohammadi, B., Ehrampoush, M.H., Khabiri, F., Soltani, M.(2015). A
219 Survey on relationship between diet and urinary excretion of aflatoxin M1: a
220 screening pilot study on Iranian population. *Journal of Food Quality and*
221 *Hazards Control*, 2 : 66-70

222 Redzwan, S. M., Rosita, J., Sokhini, A. M. M., &Aqilah, A. R. N. (2012). Association
223 between aflatoxin M1 excreted in human urine samples with the consumption
224 of milk and dairy products. *Bulletin of Environmental Contamination and*
225 *Toxicology*,*89*(6), 1115-1119.

226 Shundo L., Navas S.A., Lamardo L.C.A., Ruvieri V., Sabino M. Estimate of aflatoxin M_1
227 exposure in milk and occurrence in Brazil. Food Control.2009;20:655–657.