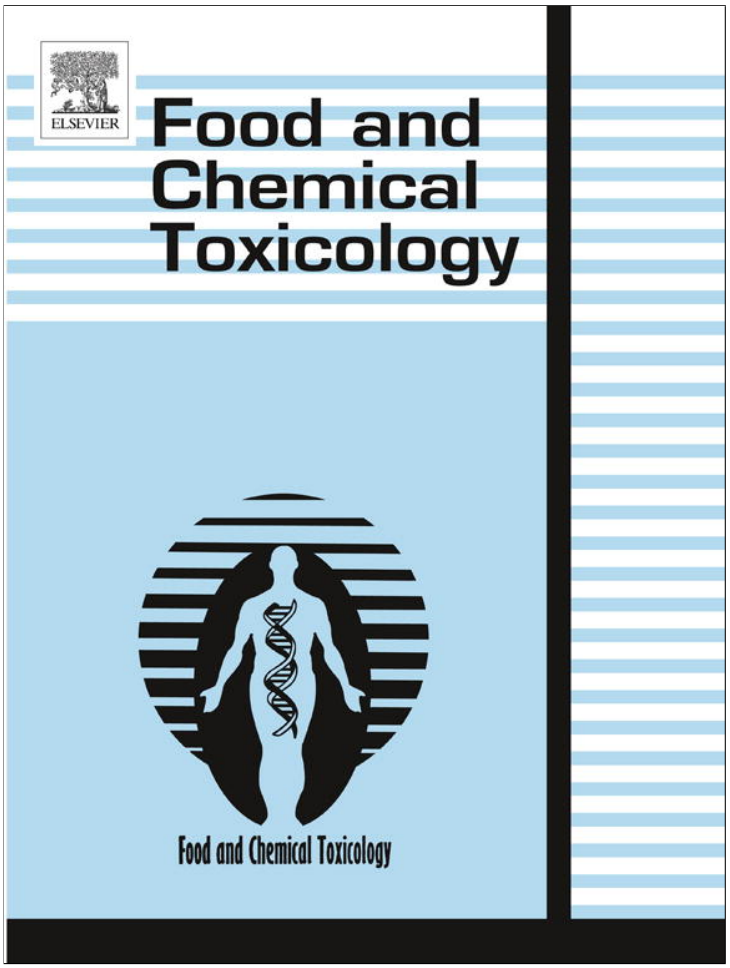

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Correlation between aflatoxin M_1 content of breast milk, dietary exposure to aflatoxin B_1 and socioeconomic status of lactating mothers in Ogun State, Nigeria

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ABSTRACT

Aflatoxin M_1 (AF M_1), a hydroxylated metabolite of AF B_1, is an important toxin that can contaminate the milk of lactating mothers. A correlation study was conducted to determine the relationship between AF M_1 content of breast milk, dietary exposure to AF B_1 and socioeconomic status of lactating mothers in the three Senatorial districts of Ogun State, Nigeria. Equal amounts of breast milk (20 ml) and food rations (40 kg) obtained from 50 volunteer lactating mothers and eighty-two frequently consumed food commodities in the preceding month were used for the study. The level of contamination of the foods by AF B_1 was low (0.16–0.33 μg/kg) and differed significantly ($p < 0.05$) across the state but did not exceed the EU limit of 2 μg/kg. The occurrence level of AF B_1 was however high (93.75–94.45%) and was more pronounced in Ogun East Senatorial district (94.45%). Eighty-two percent of the breast milk was contaminated with AF M_1 (3.49–35 ng/l) and 16% exceeded the EU limit of 25 ng/l while a 100% occurrence risk was recorded in Ogun Central Senatorial district. The socioeconomic status of the mothers also significantly influenced their dietary exposure and exposure risk of the sucklings to AF M_1.

1. Introduction

Milk is the lacteal secretion of animals that suckle their young (Galvano et al., 1996). It is a good source of protein that has high biological value in promoting the growth of children. Breast milk is considered to be the ideal food for infants and the importance of breast feeding for normal growth and development has been recognised as it provides the nutritional and immunological needs of infants (Picciano, 2001). However, breast milk may be contaminated with mycotoxins such as aflatoxins.

Aflatoxins (AFs) are a group of naturally occurring toxic fungal metabolites produced by certain *Aspergillus* species. There are more than three hundred aflatoxins but the most investigated and monitored are only four with AF B_1 being the most toxic of the lots. Their chemical classification is based on their fluorescence behaviour under ultraviolet light: blue (B_1 and B_2) or green (G_1 and G_2) and their relative mobility during separation by chromatography (Bennett and Klich, 2003). Aflatoxins are teratogenic, mutagenic and carcinogenic to several species of experimental animals. They have been classified as a Group 1 carcinogen in IARC Monographs on the Evaluation of Carcinogenic Risks to Humans (IARC, 2002). Chronic exposures to aflatoxins have resulted in reduced immune activities (Turner et al., 2003), malnutrition (Tchana et al., 2010) and growth impairment (Gong et al., 2003). Consumption of foods containing high levels of aflatoxins may produce acute aflatoxicosis with severe pathologies which in extreme cases may lead to death (Azziz-Baumgartner et al., 2005).

AF M_1, the main monohydroxylate derivative of AF B_1, is an important toxin present in the milk of lactating animals and nursing mothers fed with AF B_1-contaminated feeds or foods. It is formed in the liver by means of cytochrome P450-associated enzymes (Zinedine et al., 2007). Aflatoxin M_1 have been detected in milk of lactating animals and in breast milk of women (Atanda et al., 2007; Polychronaki et al., 2007; Dashti et al., 2009; Sadeghi et al., 2009; Gurbay et al., 2010). Although AF M_1, a genotoxic

Abbreviations: AF, aflatoxins; HPLC, high performance liquid chromatography.

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carcinogen, is less toxic (about 10 times) than AF B_1 (Creppy, 2002), exposure of infants to AF M_1 is worrisome since they are considered more susceptible to its adverse effect than adults. This is due to their low body weights, high metabolic rate, lower ability to detoxify toxins and incomplete development of some vital organs and tissues, especially the central nervous system (Galvano et al., 1996).

There is limited information on the occurrence and risk of exposure of milk and milk products to AF M_1 contamination in Nigeria. However, Opadokun et al. (1979) found no detectable level of AF M_1 in 92 samples of milk in a dairy farm in Kano State while Ogunbanwo (2005), unpublished conducted a nationwide survey and found AF M_1 concentration in the range of 0.15–0.17 μg/l in yoghurt and ice cream. Furthermore, Atanda et al. (2007) found high levels of AF M_1 contamination in human milk (4.0 μg/l), cow milk (2.04 μg/l) and ice cream (2.23 μg/l) in Abeokuta and Odeda local governments of Ogun State. Thus, there is need to investigate the level of AF M_1 contamination of the entire state and the possibility of correlation with the mothers dietary exposure to AF B_1 and socioeconomic status.

2. Materials and methods

2.1. Study area

The study was conducted in Ogun State, South Western Nigeria which is divided into three Senatorial districts (Ogun Central, Ogun East and Ogun West). The state is situated between latitude 6.2°N and 7.8°N and longitude 3.0°E and 5.0°E and the mean annual rainfall varies from 1280 mm (southern part) to 1050 mm (northern part) while the average monthly temperature ranges from 23 °C to 32 °C.

2.2. Recruitment of volunteer lactating mothers

Fifty lactating mothers that attended maternity centres in Ogun Central (15), Ogun East (18) and Ogun West (17) Senatorial districts that agreed to participate in the study were recruited and ethical approval was given by the Ogun State Ministry of Health, Abeokuta, Ogun State, Nigeria.

Each volunteer was also asked to complete a questionnaire to provide information on his socioeconomic status (work status, age, type of residential location of family and educational level).

2.3. Collection of food samples

Donors that agreed to participate in the study were recruited from the maternity centres in each Senatorial district and were educated on food sampling procedures. A structured food frequency questionnaire (FFQ) was used to obtain information on the food consumption pattern of the lactating mothers over the last month preceding the collection of samples after which 40 kg of the most frequently consumed foods were collected randomly from the three Senatorial districts from June to October, 2010. Food samples collected were: rice, beans, cassava flour, semovita, yam flour, wheat flour, whole wheat, maize and "gari". A total of 82 food samples were collected thus: Ogun Central (30), Ogun East (25) and Ogun West (27) and immediately stored at 4 °C until extraction and HPLC analysis.

Twenty millilitres of breast milk was obtained from each lactating mother that agreed to participate in the study and was transported to the laboratory in ice-packed coolers and immediately stored at −20 °C until extraction and HPLC analysis. In total, 15 samples were collected from Ogun Central, 18 from Ogun East and 17 from Ogun West Senatorial districts respectively.

2.4. Chemicals and reagents

HPLC-grade solvents were from Merck (Darmstadt, Germany). Water, for the HPLC mobilephase was produced in a Milli-Q system (Millipore, Bedford, MA, USA). Organic solvents used for extraction phases and the phase chromatography were purchased from Merck (Germany) and AF M_1 and AF B_1 standards were from Sigma Chemical Company (St. Louis, MO, USA).

2.5. Preparation of standard solutions of AF B_1 and M_1

The stock solution of AF B_1 and AF M_1 were prepared in pure methanol at final concentration of 0.5 μg/ml and kept in the dark at −20 °C. Working solutions of AF M_1 and AF B_1 used for calibrating the HPLC and obtaining the calibration curves were prepared by making appropriate dilutions of the stock solutions in methanol and kept in the dark at −20 °C.

Table 1
Socioeconomic status of lactating mothers in Ogun State, Nigeria.

Work status	% No. of positive respondent	Educational qualification	% No. of positive respondent
Teachers	10	Primary school certificate	32
Bankers	2	Secondary school certificate	28
Civil servants	8	National certificate of education	6
Petty traders	40	Ordinary national diploma	8
Hairdressers	8	Higher national diploma	8
Nurses	2	Bachelors degree	14
Fashion designers	6	Postgraduate diploma	2
Photographers	2	No formal education	2
Food sellers	2		
Farmers	2		
Unemployed	14		
Businessmen	2		
Store managers	2		
Age group of volunteers	% No. of positive respondent	Residential location of family	% No. of positive answer
20–29	50	Urban	92
30–39	46	Rural	8
40–49	4		

Table 2
Aflatoxin B_1 contamination of foods consumed by lactating mothers in Ogun State, Nigeria.

	Occurrence[x]		Mean contamination level (μg/kg)	Range of contamination (μg/kg)
	Percentage	Frequency		
Food				
Rice	90.48	19/21	0.14[a]	nd – 0.30
Beans	88.24	15/17	0.15[a]	nd – 0.89
Cassava flour	75.00	3/4	0.05[a]	nd – 0.07
Semovita	33.34	2/6	0.09[a]	nd – 0.17
Yam	85.72	6/7	0.14[a]	nd – 0.27
Wheat meal	33.34	2/3	0.04[a]	nd – 0.06
Maize	100.00	3/3	0.16[a]	0.11–0.20
"Gari"	72.23	13/18	0.25[a]	nd – 0.69
Total	79.27	65/82		
Senatorial district				
Ogun Central	93.75	15/16	0.33[a]	nd – 0.89
Ogun East	94.45	17/18	0.18[b]	nd – 0.64
Ogun West	94.18	16/17	0.16[b]	nd – 0.69

[a,b]Mean values with same superscript within a column are not significantly different ($p > 0.05$).
[x] Positive samples/total samples.

2.6. Extraction of AF B_1 and M_1

AF B_1 was extracted from the food samples according to the method described by Göbel and Lusky (2004) with slight modifications: Two and half grams NaCl was added to twenty-five grams of food in a blender followed by the addition of 50 ml of methanol: water (80: 20 v/v). The mixture was blended at high speed by an Ultra-turrax T25 Basic (Staufen, Germany) at 4000 rpm for 2 min and filtered through a fluted filter paper and ten millilitres of the resultant filtrate diluted with 40 ml distilled water in a stopped glass tube. The tube was mixed gently by hand inversion for 1 min and the solution further filtered through regenerated cellulose (0.20 μm) into a 100 ml beaker. Ten millilitres of the filtrate was passed through an Aflatest® immunoaffinity column (VICAM Watertown, MA, USA) fitted on a solid phase manifold (Superlco, Bellefonte, PA) at a flow rate of 1–2 drop/s. The column was washed with 10 ml water and the bound aflatoxin B_1 eluted with 3 ml of pure methanol into an amber vial at the rate of 1–2 drop/s. The eluate was dried in a centrifugal evaporator vacuum centrifuge (Savant Instruments Inc., Farmingdale, NY, USA), reconstituted in 200 μl of methanol and 20 μl portion injected into the HPLC for analysis.

AF M_1 was extracted from the breast milk according to the modified method of the International Standard Organization (1998) with slight modifications. Briefly, 40 ml distilled water was added to 10 ml of breast milk in a 100 ml beaker followed by the addition of 0.25 g NaCl and the solution properly mixed. The mixture was then centrifuged at 4000 rpm for 10 min. After separation, the skim portion (bottom layer) was filtered through a glass microfibre filter and 8 ml of the filtrate passed through an Aflatest® immunoaffinity column (VICAM, Watertown, MA, USA) fitted on a solid phase manifold (Superlco, Bellefonte, PA) at a flow rate of 1–2 drop/s. Eight millilitres of methanol: water (10:90) was used to wash the column and the bound aflatoxin M_1 eluted with 3 ml of methanol into an amber vial at the rate of 1–2 drop/s. The eluate was dried in a centrifugal evaporator vacuum centrifuge (Savant Instruments Inc., Farmingdale, NY, USA) reconstituted in 200 μl of methanol and 50 μl portion injected into HPLC for analysis.

2.7. HPLC analysis of AF B_1 and AF M_1

The concentrations of AF B_1 and AF M_1 in the foods and milk were estimated by HPLC (Shimadzu, Japan) configured with LC-10AD pumps, coupled with a fluorescence detector RF-10Axl. Excitation and emission wavelengths were set at 360 and 440 nm respectively. The stationary phase was a Gemini Column (C_{18} TMS end-capping, 3 μm particle size, 150 × 4.60 mm, pore size 110 Å, Phenomenex, USA). The mobile phase was isocratic, mixture of methanol/acetonitrile/water (25:25:50 v/v/v), with a flow rate of 1 ml/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). The precision of the method in terms of repeatability (intraday precision) and reproducibility (inter-day precision) was evaluated by calculating the Relative Standard Deviation (RSD). The RSDs of the intra-day were in the range of 3.5–8.9% while the RSDs of the inter-day were in the range of 3.8–9.1%. Stock solutions of 1.00 μg/ml dissolved in methanol were prepared and stored at a temperature of −20 °C in the dark for the determination of the Limit of Detection (LOD) and Limit of Quantification (LOQ) for each toxin. The signal to noise (S/N) ratio was calculated (peak to peak analysis with Analyst software v. 1.4) from the calibration curve of each toxin and used to determine the LOD and LOQ values.

An injector with a 20 μl loop was used for the determination of AF B_1 concentration. A calibration curve was constructed for AF B_1 using a series of dilutions containing different levels of toxins with an average of 10 consecutive injections of standard solutions of AF B_1. The recovery experiments for AF B_1 were carried out in triplicates by spiking 25 g known blank samples with a standard concentration of 5 μg/kg. An injector with a 50 μl loop was used for the determination of AF M_1. A calibration curve was constructed for AF M_1 using a series of dilutions containing different levels of toxins with an average of 10 consecutive injections of standard solutions of AF M_1. The recovery experiments for AF M_1 were carried out in triplicates by spiking five millilitres of breast milk with 0.08 μg/ml standard AF M_1 concentrations for samples contaminated up to 0.005 μg/ml and 0.25 μg/ml.

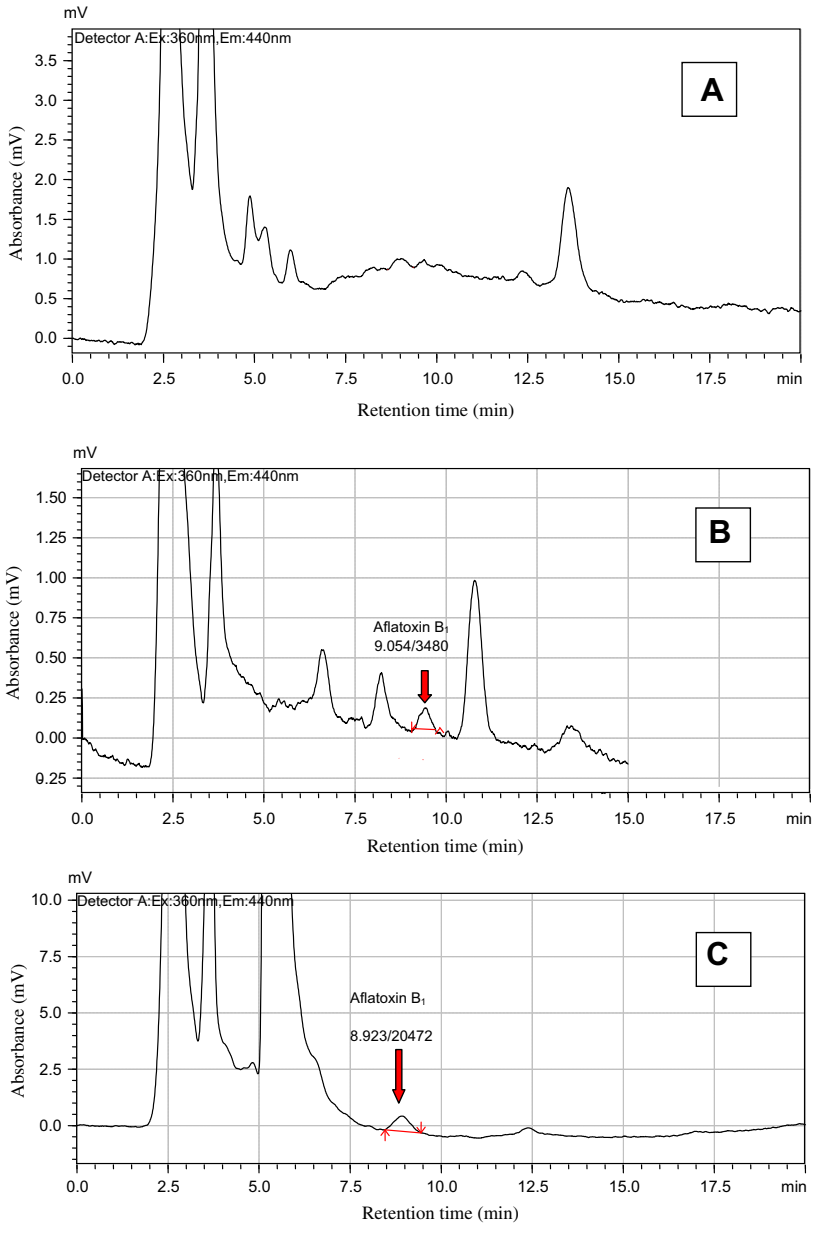

Fig. 1. Chromatograms of (A) uncontaminated maize (B) maize naturally contaminated with AFB_1 and (C) "gari" naturally contaminated with AFB_1.

2.8. Statistical analysis

The concentrations of AF B_1 and AF M_1 in the samples were reported as means of three replicates and subjected to analysis of variance (ANOVA) using SPSS 16.0 for Windows to determine whether there was significance at 5% probability level. The Duncan Multiple Range test was used to separate the means while the Spearman Correlation test was used to correlate the level of AF B_1 in foods with that of AF M_1. Each socioeconomic parameter was also pooled together with each response forming a distinct category within each pool and correlated with AF M_1 and AF B_1. The Chi Square test was used to assess the possible differences in the incidence of AF M_1 in the different groups that showed significant correlation.

3. Results

3.1. Analytical performances

The LOD for AF B_1 was estimated as 0.1 ng/ml and quantification value (LOQ) was 0.5 ng/ml, while the LOD for AF M_1 was estimated at 10 ng/l and LOQ was 50 ng/l. The linearity of the curve was 0.1–50 μg/ml for AF B_1 and from 10 to 50 ng/ml for AF M_1.

The calibration curve for AF B_1 had a linear equation of $y = 5758x - 332.3$ with a correlation coefficient of 0.999. Recovery values for AF B_1 were found to be between 57.2 ± 1.7% and 100 ± 2.5% with a mean of 85.5 ± 2.4% and retention time of 9.0 min. The results were corrected for recovery.

The calibration curve for AF M_1 had a linear equation of $y = 63{,}116x - 331.9$ and a correlation coefficient of 1.0. Recovery values for AF M_1 were found to be 98.5 ± 1.8% and retention time of 5.8 min. The results were corrected for recovery.

3.2. Occurrence of AF B_1 in foods and AF M_1 in breast milk

The socioeconomic status of the lactating mothers (Table 1) showed wide variation. In the present study, the highest academic qualification obtained by the lactating mothers was a Bachelors degree (14%) while 32% had only primary education and 2% had no formal education. Forty percent of the women were petty traders that lived in urban centres while 50% were in the age group of 20–29 years. In addition, 2% of the respondents were bankers and farmers while 14% were unemployed.

The level of contamination of the foods (Table 2) by AF B_1 was generally low (0.16–0.33 μg/kg) while the occurrence level was high (93.75–94.45%). Fig. 1 shows the chromatogram of the negative and positive AF B_1 samples. Maize had the highest occurrence of 100% followed by rice (90.48%) and beans (88.24%) while wheatmeal had the lowest occurrence of 33.34%. The overall occurrence of AF B_1 in the food samples was 79.27%. "Gari" had the highest contamination of 0.25 μg/kg by AF B_1 followed by beans (0.15 μg/kg) while wheatmeal had the lowest (0.04 μg/kg) and the range of contamination across the state was 0.06–0.89 μg/kg respectively (Table 2). The level of contamination of foods by AF B_1 in Ogun Central Senatorial district (0.33 μg/kg) was significantly higher ($p < 0.05$) than Ogun East (0.18 μg/kg) and Ogun West (0.16 μg/kg) Senatorial districts respectively. Conversely, the occurrence of AF B_1 was highest in Ogun East (94.45%), followed by Ogun West (94.18%) and Ogun Central (93.75%) Senatorial districts respectively. Rice was found to be the most frequently daily consumed food (84%) by the lactating mothers (Fig. 2) followed by "gari" (68%).

Fig. 3 shows the chromatogram of negative and positive AF M_1 breast milk samples. AF M_1 contamination was detected in (82%) of the milk samples (Table 3) and the highest risk of AF M_1 contamination was in Ogun Central (100%) followed by Ogun West (82.35%) and Ogun East (66.7%) Senatorial districts respectively. The range of contamination by AF M_1 within the state was between 4.65 and 92.14 ng/l and the lactating mothers can be categorised into four groups: no detectable AF M_1 (9), low (27), medium (6) and high (8). Furthermore, the level of contamination of Ogun Central Senatorial district by AF M_1 was significantly higher ($p < 0.05$) than those from Ogun West and Ogun East Senatorial districts.

There was a significant ($p < 0.05$) positive correlation ($r = 0.33$) between AF M_1 content of breast milk of the mothers and their dietary exposure to AF B_1 (Table 4) especially within Ogun West Senatorial district ($r = 0.56$) (Table 5). Consumption of beans ($r = -0.28$) and wheatmeal ($r = 0.30$) significantly ($p < 0.05$) affected the dietary exposure of lactating mothers to AF B_1 as well as the work status ($r = -0.29$) and educational qualification ($r = -0.33$). The concentration of AF M_1 in the breast milk of the mothers also showed a significant relationship with work status

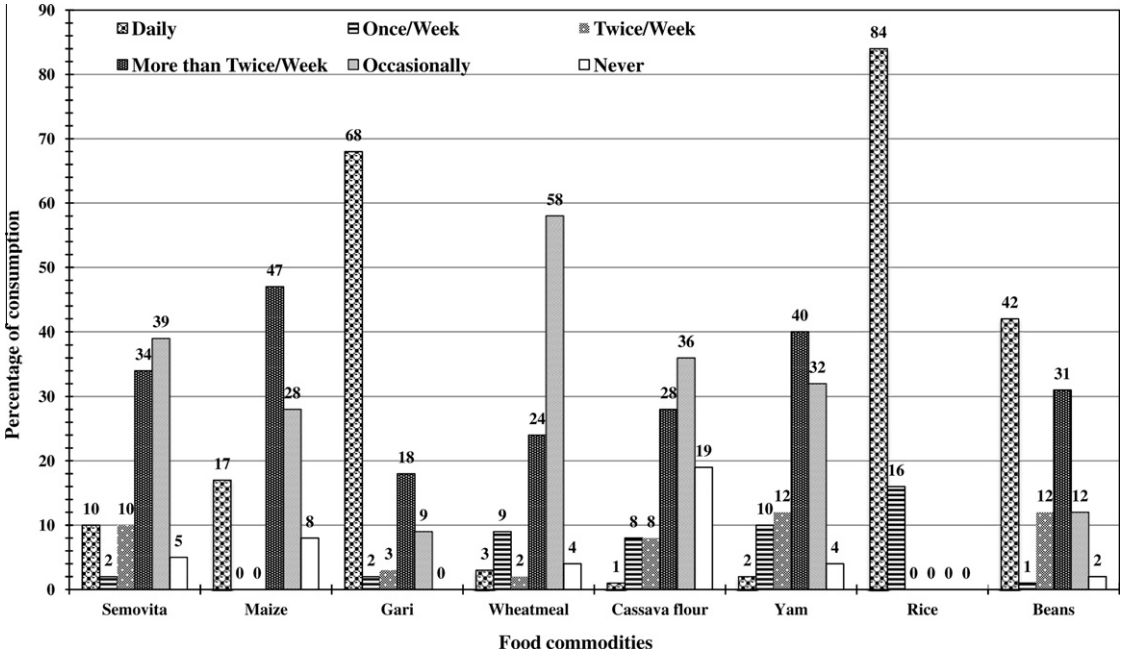

Fig. 2. Food consumption patterns of lactating mothers in Ogun State, Nigeria.

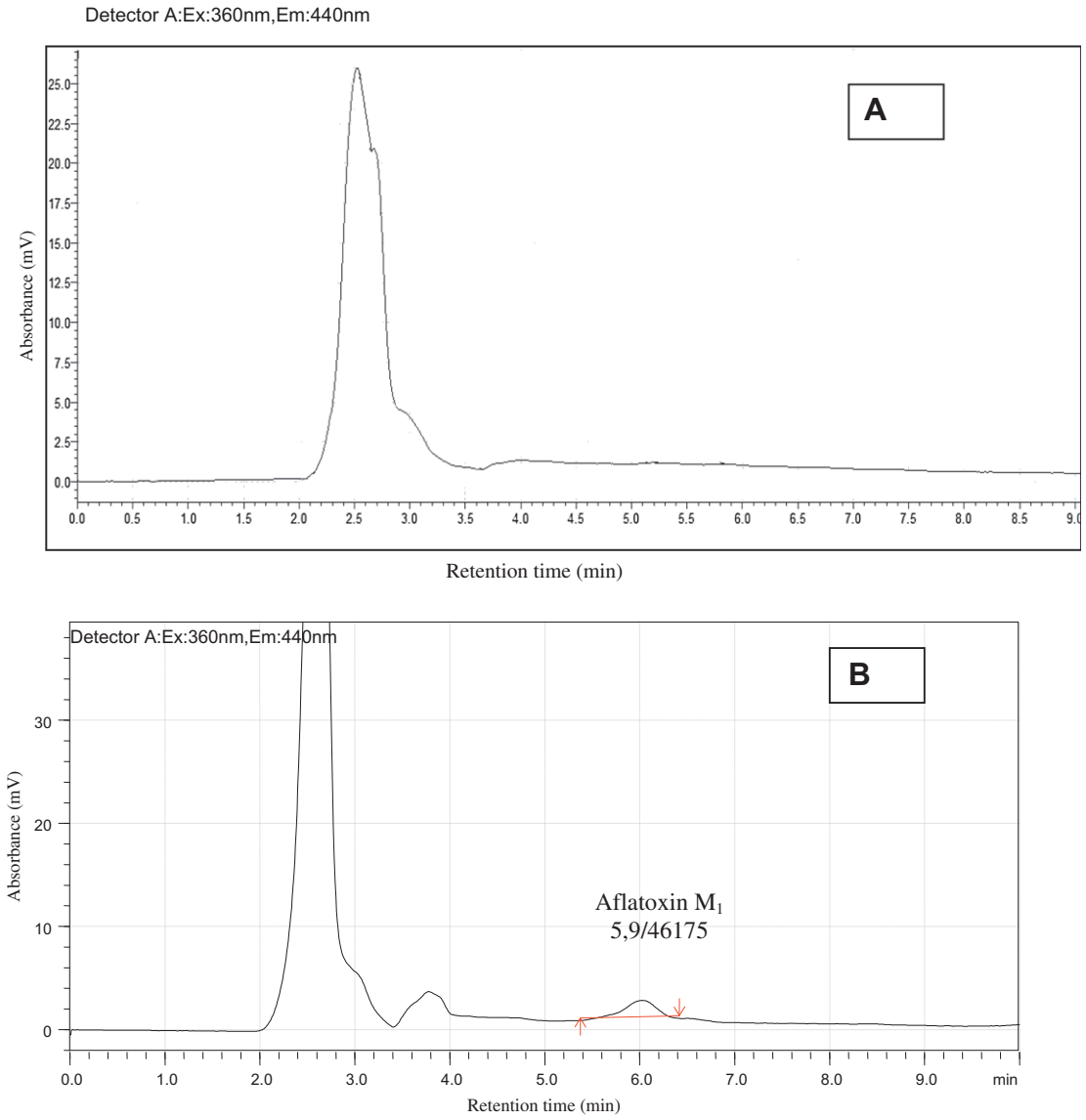

Fig. 3. Chromatogram of human breast milk (A) uncontaminated milk (B) milk naturally contaminated with AFM_1.

Table 3
Aflatoxin M_1 contamination of breast milk by lactating mothers in Ogun State.

Senatorial district	Occurrence[x]	Frequency distribution of AF M_1			Mean contamination level (ng/l)	Range of contamination (μg/kg)
		Low 1–10 (ng/l)	Medium 11–25 (ng/l)	High >25 (ng/l)		
Ogun Central	15/15	1	6	8	35.00[a]	4.65– 92.14
Ogun East	12/18	12	0	0	6.51[b]	nd – 18.58
Ogun West	14/17	14	0	0	3.49[b]	nd – 5.40

[a,b]Mean values with same superscript within a column are not significantly different ($p > 0.05$).
[x] Positive samples/total samples.

($r = -0.31$, $p < 0.05$), educational qualification ($r = -0.39$, $p < 0.01$) and "gari" consumption ($r = 0.40$, $p < 0.01$).

4. Discussion

Aflatoxin contamination occurred in different amounts in the food samples; in agreement with previous surveillance studies of aflatoxin contamination in maize – 22 μg/kg (Bankole and Mabekoje, 2004), rice – 37.2 μg/kg (Makun et al., 2007), beans – 59.29 μg/kg (Makun et al., 2010), "gari" (total aflatoxin) – 5.71 μg/kg (Ogiehor et al., 2007), wheat – 32.33 μg/kg (Makun et al., 2010) and yam flour – 32.33 μg/kg (Jonathan et al., 2011). However, the concentration of AF B_1 observed in the current study was much lower than those previously reported. This could be due to the several environmental and climatic conditions that affect aflatoxin production (Cotty and Jaime-Garcia, 2007). Fungal growth and mycotoxin contamination are closely dependent on climate and storage conditions and therefore vary with locations, agricultural and manufacturing practices (Ominsk et al., 1994).

Table 4
Statistical correlation between AF M_1, AF B_1 and socioeconomic status of lactating mothers in Ogun State.

Socioeconomic parameter	AF B_1 concentration	AF M_1 concentration
AF M_1 concentration	0.33*	–
AF B_1 concentration	–	0.33*
Work status of volunteers	−0.29*	−0.23
Age group of volunteers	0.14	0.28
Resident location of family	−0.22	−0.16
Educational qualification	−0.28*	−0.33*
"Gari"	0.06	0.40**
Cassava flour	0.01	−0.20
Yam	0.06	−0.11
Rice	0.25	0.26
Beans	−0.28*	−0.13
Semovita	−0.12	0.23
Maize	−0.09	−0.06
Wheat meal	0.30*	−0.14

* Correlation is significant at $p < 0.05$.
** Correlation is significant at $p < 0.01$.

Table 5
Correlation between AF M_1 content of breast milk and dietary AF B_1 in each Senatorial district of Ogun State, Nigeria.

Senatorial district	Correlation between AF M_1 and AF B_1
Ogun Central	0.02NS
Ogun East	−0.023NS
Ogun West	0.56*

NS – not significant.
* Correlation is significant at $p < 0.05$.

The uneven distribution of mycotoxins in food matrices may be another source of difference but in the present study, the sampling procedures have been selected to reduce its influence.

The Joint FAO/WHO Expert Committee on Food additives (JEFCAs) has not established a tolerable daily intake (TDI) for aflatoxins, but strongly recommends that the level of aflatoxins be as low as possible (Polychronaki et al., 2006). All the food commodities in this study had aflatoxin levels that were less than the European Union Commission limit of 2 μg/kg and the National Agency for Food, Drug Administration and Control (NAFDAC) recommended limit of 10 μg/kg for unprocessed foods in Nigeria. The low level of aflatoxins does not guarantee the safety of the mothers as it has been reported that maternal consumption of AF-contaminated foods during breastfeeding can result in the accumulation of aflatoxins and their toxic metabolites in breast milk (Polychronaki et al., 2006; Gurbay et al., 2010).

The higher concentration of aflatoxins recorded in Ogun Central Senatorial district may be due to the fact that the state capital is located within the Senatorial district and many traders and farmers bring their food items to markets in the state capital to sell. The food items are usually poorly stored and badly transported from the different locations thereby exposing them to risk of aflatoxin contamination. The consumption of beans (a major and readily available source of protein in Nigeria), "gari" (a cheap and staple food for majority of Nigerians) and wheat meal substantially contributed to dietary exposure of lactating mothers to aflatoxin contamination and are a serious source of concern.

The level of contamination of breast milk (82.0%) by AF M_1 in this study was lower than the 99.5% recorded in United Arab Emirate (Abdulrazzaq et al., 2003) and 98.1% in Iran (Sadeghi et al., 2009) but higher than the 31% in Sierra Leone (Jonsyn et al., 1995), 4.8% in Cameroun (Tchana et al., 2010) and 56% in Egypt (Polychronaki et al., 2007). Furthermore, Gurbay et al. (2010) reported a contamination range of 60.90–299.99 ng/l for breast milk in Ankara region of Turkey. The low level of contamination could be due to the low level of AF B_1 consumed by the lactating mothers as 18% of the breast milk sampled had no detectable level of AF M_1, while16% had aflatoxin levels that exceeded the European Union Commission limit of 25 ng/l (European Commission, 2006).

The educational qualifications of the lactating mothers were also found to be significantly associated with AF B_1 and AF M_1 concentrations. Mothers whose highest educational qualification was the primary school leaving certificate had higher risks of exposure to aflatoxin contamination compared to others with higher qualifications. Traders also had higher risks of dietary exposure to AF B_1 compared to bankers.

5. Conclusion

Although AF M_1 was found in breast milk of lactating mothers in Ogun State in low amounts, there was a significant positive correlation between AF M_1 contamination of the breast milk and the mothers dietary exposure to AF B_1. The socioeconomic status of the lactating mothers also significantly influenced their dietary exposure and exposure risk of the sucklings to AF M_1. The low amount of AF M_1 contamination buttresses the need for continuous breast feeding of infants because it is still the best feeding option from the food safety point of view. Apart from human breast milk, infants are also at risk of exposure to AF M_1 from meat, eggs, milk and other edible products from animals that consume AF-contaminated feeds. Thus there is need to continue to monitor the level of contamination of the lactating mothers in order to ensure infant protection.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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