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Original Research Article

Prevalence and Quantification of Aflatoxin M1 in Human Milk Associated with the Diet and Kitchen Microenvironment of Lactating Mothers in Rural Rajasthan: A Descriptive Study

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Abstract

Introduction: Aflatoxin M1, a metabolite of Aflatoxin B1 is secreted in human milk when the lactating mother consumes animal milk and other foods contaminated with Aspergillus moulds. AFM1 can cause loss of intestinal integrity, hepatic cancer or steatosis, and growth faltering at a later date among infants who were breast fed. This fungus is an agricultural by product. Our objective was to find out the burden of AFM1 concentration in human milk of lactating mothers residing in the rural field practice area of our medical college. We also described the frequency of common foods in mothers' diet known to carry aflatoxins and their kitchen characteristics.

Methods: 31 lactating mothers with infants less than 6 months of age were subjected to test their milk samples for AFM1. A test kit procured from VETMED Scientific, Bhopal was used to perform competitive ELISA. A pre-tested, semi-structured questionnaire was administered to gather further information.

Results: All human samples were contaminated with AFM1. The range was from 39-5100 pg/ml; 5061. The Standard Deviation depicted large variations among samples. Most mothers (77.42%) consumed boiled animal milk every day, all fed on cereals daily. The women who consumed stale food, stored grains in damp kitchen, fed on direct or pasteurized animal milk, procured grains from Public Distribution System, with frequent kitchen turnover corresponded to very high median concentrations of AFM1.

Conclusion: All lactating mothers were showing AFM1 concentration higher than safe limit (25 pg/ml). Diet pattern and food handling (FSSAI guidelines) must be taught to combat this risk.

Keywords: ELISA, AFM1

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Introduction

Aflatoxins are generated from the species of fungus Aspergillus. This fungus contaminates agricultural by-products. Animals that feed on the fodder or inhale

the moulds containing this ubiquitous fungus transmit its metabolites through their milk and meat. Botanicals[1] like aflatoxins are long known as hepatotoxins. Several studies have reported their role in hepatic cancer, fatty dystrophy of liver, impaired growth parameters in children, and poor intestinal integrity[2]. This toxin is heat resistant, perpetuates in hot and humid climate and it cannot be removed from food even if it is boiled and steamed. Grains, peanuts, milk and dairy, sweet potatoes are predominantly contaminated. When aflatoxins enter the food chain, humans fall prey to its subtle effects like fatty dystrophy of liver that can become massive in due course of time. Out of 20 metabolites[3], B1, B2, G1, G2, M1 are associated with human health. According to International agency for research on cancer[4], these metabolites are cancer causing. AFB1 converts to M1 in animal or human milk. Until recently AFM1 was considered benign but now evidence suggests that it can be harmful as a carcinogen and retards growth in children[5]. When ingested or inhaled by lactating mothers, there occurs its transmission through breast milk to the infant. The effect on infants is dose dependent.

Through this research we want to understand the food habits and microenvironment where it might affect the lactating mothers and their feed for the infant in our field practice area. The burden or the problem statement with respect to aflatoxin M1 can be generated through this work.

Objectives

1. To assess and quantify the presence of aflatoxin M1 in mother's milk of exclusively breast-feeding mothers of less than 6 months old infants.
2. To describe the diet pattern and kitchen microenvironment pertaining to the lactating mothers.
3. To depict graphically the AFM1 concentration in mother's milk corresponding with different kitchen microenvironment scenarios.

Materials and Method

Study Design: A cross-sectional descriptive study was conducted with the measurement of Aflatoxin M1 concentration in breast milk influenced by the diet pattern and kitchen microenvironment of lactating mothers.

Study population: All lactating mothers exclusively breast feeding their infants less than 6 months of birth residing in villages Sankh, Sindoli, Rooppura and Bhishansinghpura which is the rural field practice area of RHTC, medical college.

Sampling method: Census sampling, all lactating mothers were included.

Inclusion criteria: Lactating mothers who were exclusively breast feeding their babies.

Exclusion criteria: Those mothers who did not give consent to participate in this research.

Time period: 27thAugust to 5th September 2022.

Ethical clearance: Institutional Research Ethics Committee permission was obtained before conducting this study.

Method of data collection:

a. Enrolment of mothers in study- ASHAs in the field practice area were trained to accompany our Medical Social Worker to collect human milk samples from all the lactating mothers in RHTC catchment area. Cold chain was maintained through vaccine carriers. Sterile containers were used to collect the milk by our health team. These samples in a lot of 10-15 samples each time were subjected to competitive ELISA testing measuring Optical Density for the AFM1 quantification in the microbiology laboratory of JNUIMSRC, Jaipur, Rajasthan, India.

b. Our Medical Social Worker (MSW) followed-up the lactating mothers to survey

their diet pattern and kitchen microenvironment based on a pre-formed, semi-structured, pre-tested questionnaire with the help of a Google Form.

c. Sample Size: 31 lactating mothers, out of 42 mothers, 31 agreed to allow breast milk sampling and conduct the survey. Non-response rate was 26.2%

d. Procedure of testing: A self-procured competitive ELISA kit of Neogen Lab Germany, was utilized to test human milk samples for aflatoxins. It was made available by the vendor Vet Med Scientific Ltd, located in Bhopal. Around 96 samples could be tested by the kit.

Data analysis: Data was entered in Microsoft Excel spreadsheet. Descriptive statistics were applied in the form of mean, median and standard deviation to describe the levels the of aflatoxin M1 contamination in the human milk samples of the study group. Tabulation of data and line diagrams were constructed to describe the burden of AFM1 in the study group and frequencies of associated factors among them.

Budget: The researchers self-funded the cost of the ELISA kit but for the sample collection (35 sterile bottles, transportation (cold chain requiring 2 vaccine carriers) as well as reagents required in the microbiology laboratory, like methanol for the analysis of the samples, were funded by the institution.

Consent: An informed consent was duly signed by the lactating mothers who volunteered to participate in the study after explaining to them the procedure, purpose and confidentiality of this research.

Results

Table 1: Distribution according to age-wise exposure to Aflatoxin M1 in mother's milk

Age-group of infants in months	Number of infants	Maximum concentration (pg/ml)	Minimum concentration (pg/ml)	Range (pg/ml)	Mean ± SD
0-2 months	11	4965	39	4926	1639.36± 2173.51
2-4 months	06	5100	52	5048	919± 2048.35
4-6 months	14	4580	56	4524	1106.64± 1768.22
Total	31	5100	39	5061	1259.35±1927.66

The mean age of lactating mothers in this study was 24.5 years, range 20 to 32 years; 12 years. Median age of 31 mothers was 25 years. Monthly income of the rural families was within the range of Rs 35000 to Rs 5000; Rs 30000. Median monthly income was Rs 17000 and mean was Rs 18096.77 corresponding to lower middle class according to modified B.G Prasad scale 2021. Average size of the families was 6 members.

In this study we have followed the European Guidelines for infant food AFM1 concentration safe limits. We found all samples above 25 pg/ml. There was 100% presence of AFM1 in milk samples collected from the lactating mothers, all above the safe limits.

The mean concentration of AFM1 in mother's milk samples was found to be 1259.35 with standard deviation of 1927.66 indicating large variation among the samples. The range was 5061 pg/ml. Therefore, we have used the median values to describe the associated factors in this study. The median concentration of AFM1 was found to be 98 pg/ml.

As shown in Table 2, the high risk foods known to cause aflatoxin B1 contamination are classified according to the frequency of intake by lactating mothers. Most mothers

(77.42%) consumed boiled animal milk. Out of them, 64.52% took boiled milk on a daily basis. 6.42% mothers consumed direct animal milk without treatment regularly. Regular intake denoted frequent but not daily intake. 12.9% women consumed pasteurized milk on a daily basis. According to figure 5, the median AFM1 concentration was described among mothers consuming boiled milk was 93 pg/ml, maximum (757 pg/ml) consuming direct milk and high (475 pg/ml) median concentration in pasteurized milk consumers.

Curd as another dairy product was consumed by 64.52% mothers daily. In contrast, cheese or paneer was consumed sparingly (35.48%) and daily (19.35%). Quite a few, 22.58% consumed cheese regularly. All lactating mothers had cereals every day. 35.48% ate potatoes daily while 29.03% consumed regularly. Peanuts and other nuts were consumed sparingly. 51.61% consumed vegetables other than potatoes on a daily basis. Non-vegetarian food was consumed sparingly (once or twice in a month and beyond). 61.29% had bread, out of which 29.03% consumed sparingly, 19.35% daily and 12.9% regularly.

Table 2: Distribution showing Dietary Characteristics of lactating mothers

Food items	Daily intake (%)	Regular intake (%)	Sparingly consumed (%)	Total (%)
Animal milk n=31				
Boiled	20 (64.52)	02 (6.45)	02 (6.45)	24 (77.42)
Direct	00 (0)	02 (6.45)	01 (3.23)	03 (9.68)
Pasteurized	04 (12.9)	00 (0)	00 (0)	04 (12.9)
Cheeze/paneer	06 (19.35)	07 (22.58)	11 (35.48)	24 (77.42)
Curd	20 (64.52)	05 (16.13)	02 (6.45)	27 (87.09)
Cereals	31 (100)	00 (0)	00 (0)	31 (100)
Potatoes	11 (35.48)	09 (29.03)	00 (0)	20 (64.52)
Peanuts	03 (9.67)	02 (6.45)	07 (22.58)	12 (38.71)
Other nuts	00 (0)	03 (9.67)	04 (12.9)	07 (22.58)
Other veg not potato	16 (51.61)	07 (22.58)	02 (6.45)	28 (90.32)
Non-veg	01 (3.23)	02 (6.45)	17 (54.84)	20 (64.52)
Bread	06 (19.35)	04 (12.9)	09 (29.03)	19 (61.29)

According to Table 3, maximum families procured grains from mandi (54.84%). Figure 1 describes that there was high median (280 pg/ml) concentration of AFM1 in mother's milk who consumed grains procured from the public distribution system compared to other sources like grocery stores, mandi (128 pg/ml) and others. Table 3 shows most (38.71%) kitchen turnover for grains within 1-3 months. Buying grains exactly every month was followed by 35.48%. There was high median concentration of AFM1(Figure 2) in food turnover of 1-3 months (553 pg/ml), and highest in less than 1 month (2237.5 pg/ml) turnover. This finding can suggest poor refrigeration (48.39%) or methods of storing food in gummy bags or uncovered bins in a damp environment.

Table 3: Distribution showing kitchen microenvironment and characteristics for food procurement, storage and consumption by lactating mothers

Kitchen characteristics	Number of responses (%)
Procurement of grains (n=31)	
Public Distribution System	06 (19.35)
Mandi	17 (54.84)
Grocery store	06 (19.35)
Others	02 (6.45)
Kitchen turnover for grains (n=31)	
1 month	11 (35.48)
1-3 months	12 (38.71)
Longer than 3 months	04 (12.9)
Less than 1 month	04 (12.9)
Kitchen in the open (n=31)	10 (32.26)
Dark kitchen (n=31)	10 (32.26)
Dampness (n=31)	08 (25.81)
Consumption of fresh food (n=31)	31 (100)
Consumption of stale food (n=31)	05 (16.13)
Presence of fridge (n=31)	16 (51.61)
Cooking methods n=31	
frying	25 (80.65)
boiling	22 (70.97)
fermenting	04 (12.9)
Deep-frying	07 (22.58)
Steaming	14 (45.16)

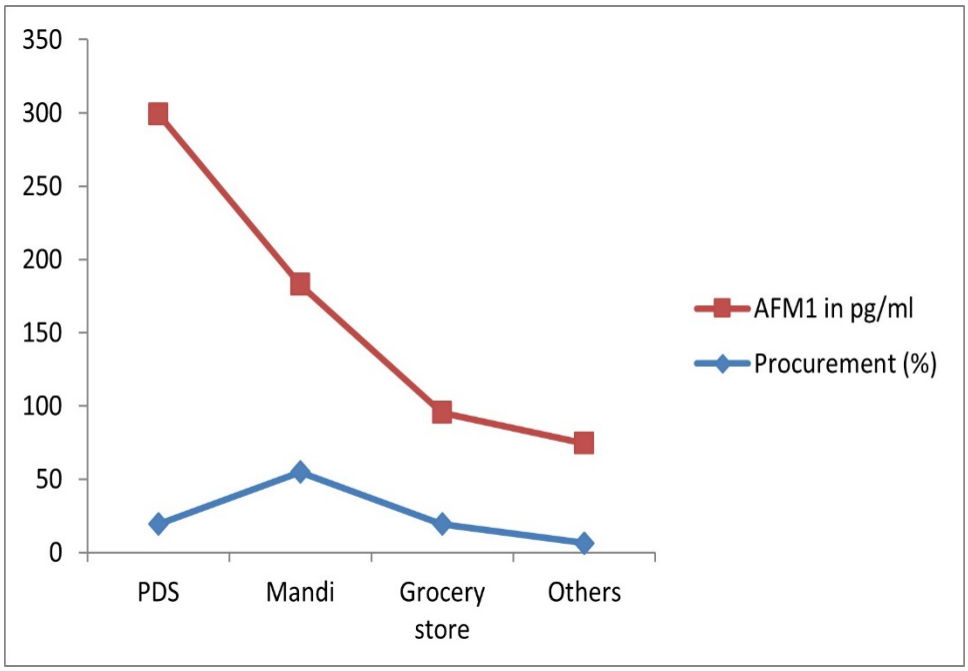

Figure 1: Line diagram describing the relation between place of procurement of grains and median AFM1 concentration in mother's milk

Figure 2: Line diagram describing the relation between kitchen turnover of grains in percentage and median AFM1 concentration in mother's milk

As depicted in figure 3, maximum median concentration of AFM1 was prevalent among mothers consuming stale food (3465 pg/ml). Damp kitchen also revealed a corresponding higher median AFM1 concentration (429 pg/ml) in breast milk of mothers. Table 3 shows 16.13% mothers consuming stale food and 25.81% of the mothers had food prepared and stored in damp kitchens. 51.61% women who stored food in the fridge corresponded to median AFM1 concentration of 80.5 pg/ml. Kitchen characteristics like open kitchen corresponded with 167 pg/ml of median AFM1 concentration compared to 113 pg/ml in dark kitchen utilization practices.

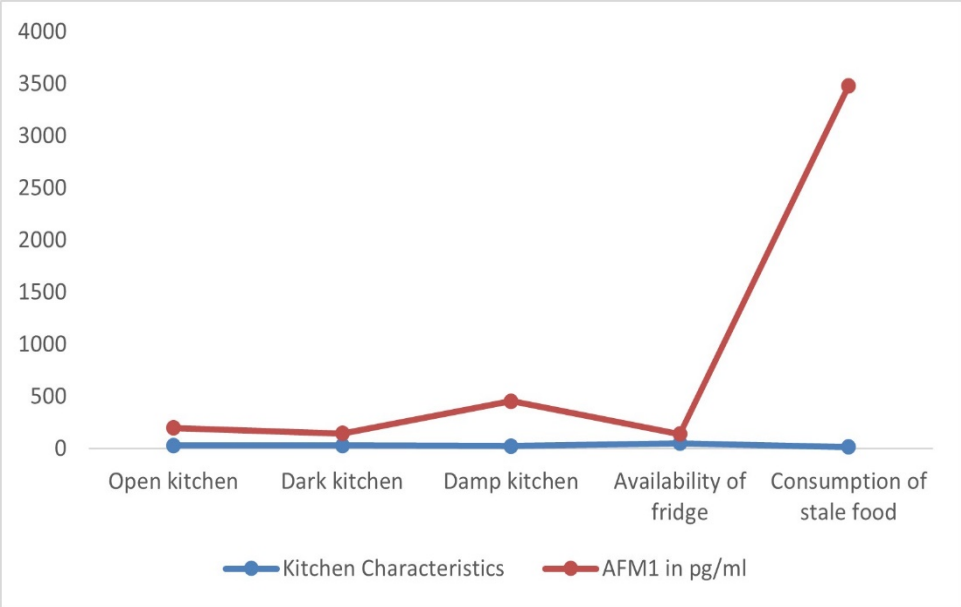

Figure 3: Line diagram describing the relation between kitchen environmental characteristics in percentage and median AFM1 concentration in mother's milk

Table 3 shows, frying (80.65%), steaming (45.16%) and boiling (70.97%) as the predominant cooking methods. There was a high median concentration of AFM1 (figure 4) in mother's milk who consumed food cooked through these methods; 98 pg/ml, 114.5 pg/ml and 99.5 pg/ml respectively. However, there was a very high median concentration among those who consumed food through fermenting (486 pg/ml) practiced among 12.9% of food preparation methods. Deep frying was corresponding to 128 pg/ml median concentration of AFM1. The figure 4 shows that the toxins are heat resistant as there are higher concentrations in each type of cooking method.

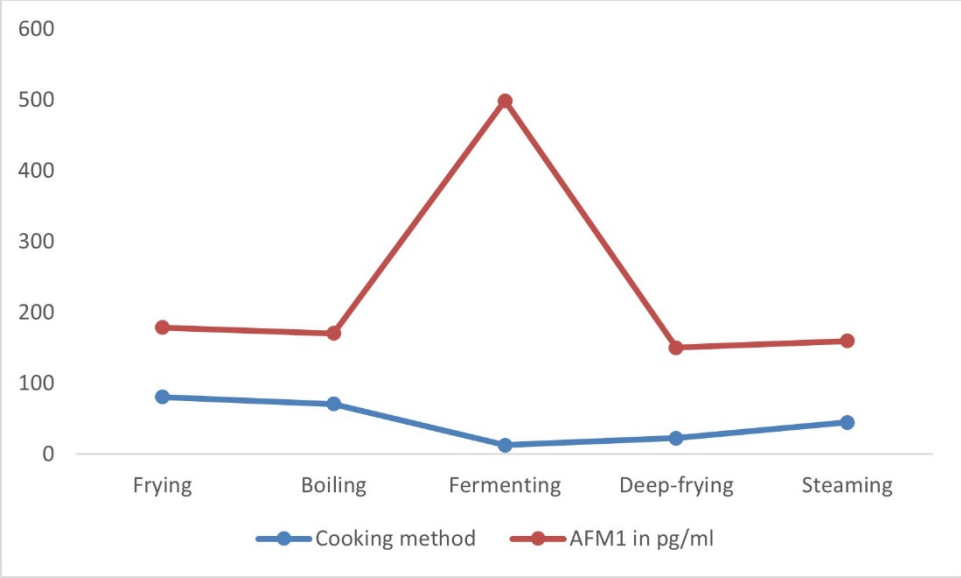

Figure 4: Line diagram describing the relation between cooking methods of food consumed by the lactating mother in percentage and median AFM1 concentration in mother's milk

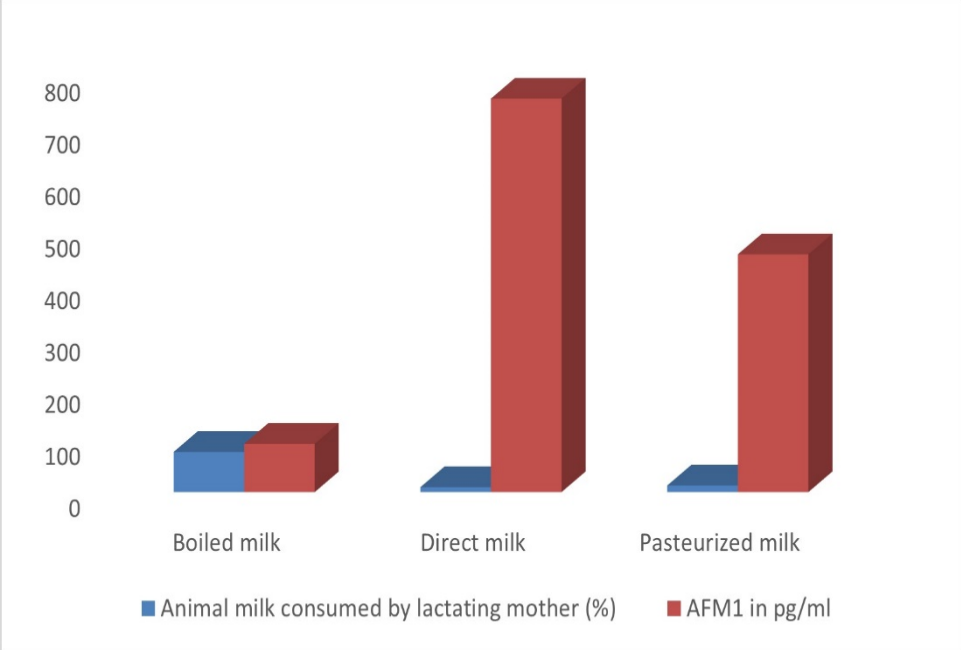

Figure 5: Bar diagram describing the relation between animal milk consumed by the lactating mother and median AFM1 concentration in mother's milk

Discussion

The study provided a sketch of AFM1 contamination of human breast milk in rural areas under the RHTC of JNUIMSRC in Rajasthan. The mean age of lactating mothers in our study was 24.5 years compared to a study done by Pourtalebi[6] S et al. where it was 28.99±5.2 years. Another research in Nepal[7] corresponding with our findings revealed that mean age of lactating mothers was 23.63 ± 4.74 years. According to European Guidelines[8] of infant formula, the concentration of aflatoxins has a safe upper limit of 25 ng/L or pg/ml (unit followed by the detection kit). The National Dairy Development Board[8], India, mentions that the level of AFM1 in animal milk should be lower than 0.5 ppb (500pg/ml). There are no recommendations till date about the safe limits of AFM1 in human milk. The AFM1 range in our study was 39 to 5100pg/ml. In a study conducted by Doğan[9] RA et al. in Turkey, AFM1 was detected in 83.3% of the samples whereas there was 100% detection of AFM1 in all our human milk samples. In another research from Nigeria by Adejumo[10] O et al. 82% human milk had detectable AFM1 and 16% exceeded the safe limits by European standards. Similar to our study, Salas[11] R et al also found 100% contamination of human milk samples in research conducted in Mexico; range from 5 to 66.23 ng/L, much lower than our range but only 13.01% higher than European standards. The high levels of concentration of AFM1 may be due to warm and humid conditions and the possibility of consuming contaminated foods.

There was AFM1 present in all the samples of breast milk above the recommended level among the women who consumed cereals (100%) daily followed by curd 87.09%, Cheese/Paneer 77.42% and peanut 38.71%. A similar study in Iran[12] showed the presence of AFM1 as 65.8% in rice, yogurt 66.7% and peanut 72.7%.

There was a positive association between alfatoxin level and type of milk consumption in the present study. It was more in the women who consumed the milk directly followed by pasteurized milk. The levels and incidence of AFM1 in milk in developing countries like India are higher and more frequent than in developed countries. This difference could have several causes, including lack of regulations on aflatoxins in dairy animals feed and milk products, unawareness of the problem amongst dairy farmers, inadequate laboratory and analytical facilities, improper farm management practice, and lack of safe feed storage facilities[8].

We have included all known food items which are highly likely to get contaminated with aflatoxin B1. The food items were culturally and geographically commonly consumed by the families in the field practice area. Since all mothers had high concentration in breast milk, we did not grade the food items that are specific in raising the AFM1 concentration. We have taken the most important kitchen micro-environment, storage and procurement of food stuffs and described graphically the median AFM1 concentration in association with the parameters concerned.

In our study the families who procured food from the public distribution system had the maximum AFM1 concentration in breast milk of lactating mother compared to mandi and grocery stores procurement. There was high median concentration of AFM1in food turnover of 1-3 months (553 pg/ml), and highest in less than 1 month (2237.5 pg/ml) turnover. This finding can suggest poor refrigeration or methods of storing food in gummy bags or uncovered bins in a damp environment. As described by Wagacha[13] et al, the fungi do not thrive under low-oxygen conditions and contamination can be minimized by use of air-tight, moisture proof bags. Infestation by insects, also cause an increase in moisture content and temperature, thereby creating favourable conditions for fungal

growth. According to Pretari[14] et al, sun drying of grains and hermetic bags for storage reduced Aflatoxins during post-harvest period among households of farmers in Kenya.

According to FSSAI[15] guidelines to prevent aflatoxin contamination among consumers, storage of food must be damp free, away from walls to increase its shelf-life. Also consume food well before expiry date. Grain turnover should be frequent, stockpiling in bins should be avoided. In our study dampness and grain turnover of 1-3 months showed higher amount of median AFM1 concentrations, also less than 1 month turnover can be associated with poor storage and procurement from public distribution system where the quality of storage might be poor. However, very high levels of contamination of human milk (500 pg/ml and more) did not correspond with PDS procurement as only 2 households out of high AFM1 mothers confirmed procurement from PDS. High median AFM1 in human milk was also corresponding with consumption of stale food.

A study by Hussain[16] et al, found that the aflatoxins are resistant to autoclaving, thermal inactivation and pasteurization and other food processing procedures. The present study also showed that even after frying, steaming or boiling the food stuff (AFB1) consumed by the mothers, the concentration of AFM1 was still high in the breast milk concluding that these toxins are heat resistant. As a striking finding, consumption of fermented food corresponded to high median AFM1 concentration in human milk.

Conclusion

All lactating mothers were showing AFM1 concentration higher than safe limit (25 pg/ml) in their milk, according to European standards for infant milk substitutes taken as cut-off. The Standard Deviation depicted large variations among human milk samples. Most mothers consumed boiled animal milk every day, all fed on cereals daily. The women who consumed stale food, stored grains in damp kitchen, fed on direct or pasteurized animal milk, procured grains from Public Distribution System, with frequent kitchen turnover corresponded to very high median concentrations of AFM1. Fermentation of food corresponded to maximum median AFM1 concentration among cooking methods.

Limitations

Foremost, this was a descriptive study. Follow-up of mothers to re-test the AFM1 secretion should have been done to check the persistence of such high levels of AFM1 in human milk throughout the lactation period. This was not feasible due to time lag between the procurement of testing kit and its expiry. Secondly, we did not apply tests of significance as all samples were found to be positive for aflatoxins. Correlation between a qualitative entity and quantitative AFM1 concentrations was not possible. Thirdly, we did not follow-up the infants for possible hepatic manifestations (AST/ALT ratio) or growth faltering along with measurement of Aflatoxin M1 in their urine to develop a causal association due to financial constraints but could be planned.

Recommendations

The general FSSAI[15] recommendations hold good for lactating mothers also. These are as follows:

- Consume well dried, clean and sorted food products free from mouldy growth.
- Discard mouldy, damped, shrivelled and discoloured foods.
- Promote dietary diversification with a view to reducing exposure to aflatoxin contamination.
- Avoid old and damaged bags used for storage as they may be infested with pests.

- Close containers tightly immediately after use and avoid unnecessary stockpiling.
- Examine food well before you buy it. Look at the stem areas on fresh produce, and avoid bruised produce.
- Avoid consumption of broken nuts as they are most likely to contain aflatoxins as compared to whole nuts. Its is also advised not to consume bitter/mouldy nuts
- If bread and top layer of pickles in a container show signs of mould growth, immediately discard the complete product.
- Buy foods which are properly packed and labelled bearing FSSAI Logo. Check "Best before date", "Use by date" and "Expiry date" of products before consumption.

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