Pakistan Journal of Pharmaceutical Research
Faculty of Pharmacy, Bahauddin Zakariya University Multan, Pakistan
ISSN: 2410-6275 **Jan 2020** **DOI:** 10.22200/pjpr.202011-7

Research Article

Incidence of Aflatoxin M_1 in human breast milk in Karachi, Pakistan

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Received: Nov, 27, 2020
Revised: Dec, 27, 2020
Accepted: Apr, 04, 2021
Online: Apr 30, 2021

Abstract

Aflatoxins are strong toxic and cancer producing substance which can be excreted in lactating humans in the form of aflatoxin M_1 (AFM_1), exposed to food contaminated with mycotoxins. In the current study, breast milk samples of 62 lactating mothers collected from two well renowned hospitals at Karachi ,were evaluated for AFM_1 by High Performance Liquid Chromatography (HPLC). AFM_1 was detected in 10 out of 27 samples collected through Civil Hospital (CH) and 11 out of 35 from Jinnah Postgraduate Medical Centre (JPMC). In human breast milk sample collected from Civil Hospital, AFM_1 was detected in a range of $28pgmL^{-1}$ to $71pgmL^{-1}$, and in human breast sample from JPMC level of AFM_1 was ranging from $27pgmL^{-1}$to $970pgmL^{-1}$.The levels of AFM_1 in human breast milk detected in the period of studies was found to be frequent (Correlation coefficient (r^2= 0.99)), but were limited to a lower level. The levels were found to be lesser than that of FDA limit of $0.05mgkg^{-1}$. Development and improvement of the newborn child is quick and hence it is conceivable that exposure of AFM_1 through human breast milk contains a noteworthy health impacts. Current study helps to carry out further study to determine the possible sources of exposure of aflatoxin in the women in future.

Keywords: Aflatoxin M1, High Performance Liquid Chromatography (HPLC), Human Breast Milk. Toxicitv

Introduction

Human breast milk is believed to be the perfect nourishing food for the growth of newborn (Organization, 2003). However, along-with healthful and immunologically advantageous components, some carcinogenic substances such as Aflatoxin M (AFM_1) have also been observed in human milk (Navas *et al.*, 2005, Abdulrazzaq *et al.*, 2003). Aflatoxins M_1 has also been detected in cow and Buffalo milk and reported earlier (Raza, 2006). Several countries have regulated AFM_1 level in milk and milk products whereas the limit 0.05 $mgkg^{-1}$ set by European Union is considered to be the lowest in the world (Byrne, 2004). Various studies to identify the pre-natal and post-natal health effect on human by this carcinogenic substance have earlier been reported (El-Nezami *et al.*, 1995, Polychronaki *et al.*, 2006). Infants have a slower rate of metabolizing carcinogens than that in adults, causing long circulation period of the substance (Organization, 2003).

Recent reports have already indicated the presence of AFM_1 in dairy milk and human milk in different countries (Raza, 2006, Abdulrazzaq *et al.*, 2003, Polychronaki *et al.*, 2006, Hussain and Anwar, 2008, Meucci *et al.*, 2010, Polychronaki *et al.*, 2007, Turconi *et al.*, 2004). AFM_1 has been observed in the breast milk of lactating females of various parts of the world and it has become very important to understand that lactating mothers can be a potent source of AFM_1 exposure to the infant (Polychronaki *et al.*, 2006, El-Nezami *et al.*, 1995, Wild *et al.*, 1987, Zarba *et al.*, 1992). In the earlier studies it is observed that growth pattern of fetus to infant influence the risk to health in future life (Barker, 2004, Delisle, 2002, Firestone and Amler, 2003, Wild *et al.*, 1991, Wild and Kleinjans, 2003).

Keeping in view significant health hazards of aflatoxins, the aim and purpose of current study was to estimate the level of aflatoxin M_1 in human breast milk sample collected through two renowned hospitals of Karachi. The expected results of this study will

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highlight the occurrence of aflatoxin in human breast milk and danger of its presence for mother and child both. The data of the current study helps to carry out further study to indicate the possible sources of exposure of aflatoxin in the Pakistani women.

Material and Method

Chemicals and Reagents

HPLC grade Methanol and acetonitrile (99.9%) were used for analysis. Standards of AflatoxinM_1 (analytical grade) were stored at 4^{o}C prior to use.

Data Collection

Before donating breast milk sample, every volunteer mother signed up a consent letter for providing her breast milk for analysis which includes; permission to take part in the research project, and to the use the volunteer's information. Volunteers filled up a set of printed questions, devised for the purposes of the statistical study of food intake including dried fruits and peanuts, fish, grain, meat, milk or milk products, legumes and vegetable oil. Employment status, total income, education, area of residence, and other data including age, health status and medication were also recorded. Mothers' and infants' height and mass (at the time of delivery and at the time of current study) were also obtained from the hospitals. In both of the hospitals patients appear from poor vicinity i.e. Lower Class (LC) and Lower Middle Class (LMC) of Karachi or various parts of interior Sindh. Most of the lactating women included in the study were born and residing in Karachi, Pakistan.

Sample Collection

A total of 62 milk samples were collected from two different hospitals (located at the central area of Karachi) 27 from Civil Hospital (CH) and 35 from Jinnah Post Graduate Medical Centre (JPMC). The samples were collected by self-expression of volunteer mothers approached through the nurses and paramedical staff working in the hospital. The samples were collected for a period of three months, i.e. three months after the start of nursing.

Sterile plastic containers were used to collect breast milk before feeding the infants. The samples were maintained at 4^{o}C before extraction. 10mL of each breast milk sample was heated to 37^{o}C with constant shaking and then it was centrifuged with a speed 3000rpmfor 15min at 5^{o}C. The samples were diluted with 20mL hot (80^{o}C) demineralized water. Before passage of diluted milk sample, it was rinsed with 10mL acetonitrile and then 10mL water. Washing of cartridge was carried out with 10mL water then 10mL ammonia:acetonitrile:water (1:10:89, v/v/v) and 10mL acetic acid:acetonitrile:water (1:10:89, v/v/v).

HPLC Analysis

Reverse-phase HPLC (model LC-10ADvp solvent delivery system; auto injection, Shimadzu, Japan) C_{18} Brownlee reverse phase column (220x4.6mm, particle size 5μm) with C_{18} guard column (Perkin Elmer) was used with fluorescence detection set at440nm emission and 360nmexcitation. The composition of mobile phase was water:acetonitrile: methanol (66:17:17, v/v/v). The oven temperature was maintained to 40^{o}C with a flow rate of 1mL/min and injection volume for standard and sample extracts was kept 30μL. The calibration solution of AFM_1 ranging from 0.04-10ngmL^{-1} was prepared in 1mL 2:3vol/vol mixture of methanol and water and then it was filtered through PVDF membrane having pore size 0.45μm. Since

aflatoxins are possible carcinogen, care has always been practiced to avoid exposure and 10% sodium hypochlorite was used for decontamination.

Statistical Analysis

Standard deviation was estimated by using one-way analysis of variance ANOVA. Calibration curves and linear regression curve showed r^2 values above 0.97 indicating good linearity.

Results and Discussion

Validation of Method and Detection limit

HPLC method for the quantitative determination of aflatoxins M_1 has been validated as described earlier{Landrigan, 2002 #108}. The limit of detection (LOD) of Aflatoxins was estimated as three times signal-to-noise ratio. LOD of aflatoxin M_1 for the human breast milk samples was obtained as 20pg/mL^{-1}. The calibration solutions of aflatoxins were prepared ranging from 0.025, 0.05, 0.1, 0.125 and 0.15ppb. Chromatogram of standard solution of aflatoxin M_1 is shown as Fig-1, and calibration curve constructed for aflatoxin M_1 is shown as Figure-2.

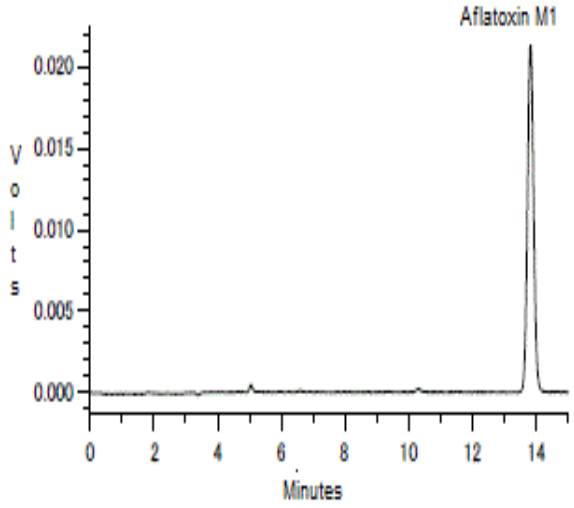

Figure 1: Chromatogram of Standard Solution of Aflatoxin M_1

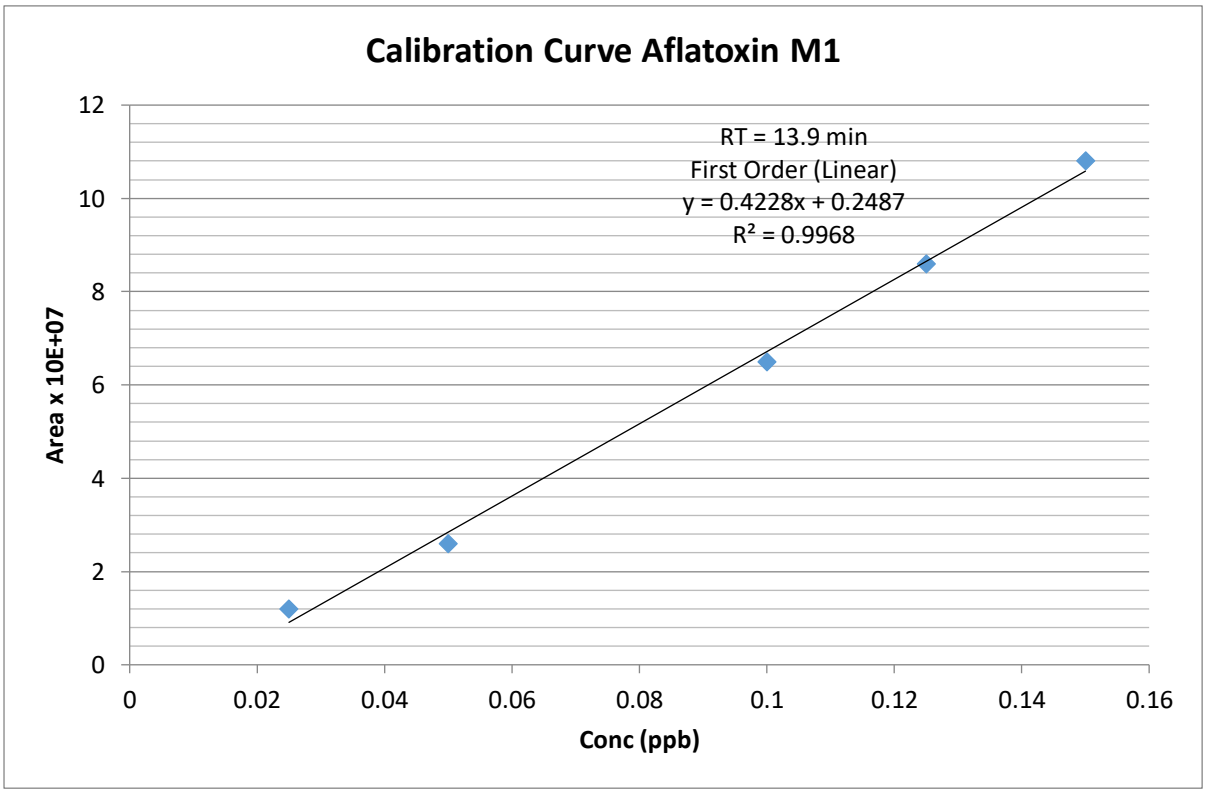

Figure 2: Calibration Curve Aflatoxin M_1

A linear result was obtained for AFM_1 concentration and their corresponding peak heights with correlation coefficient 0.99.

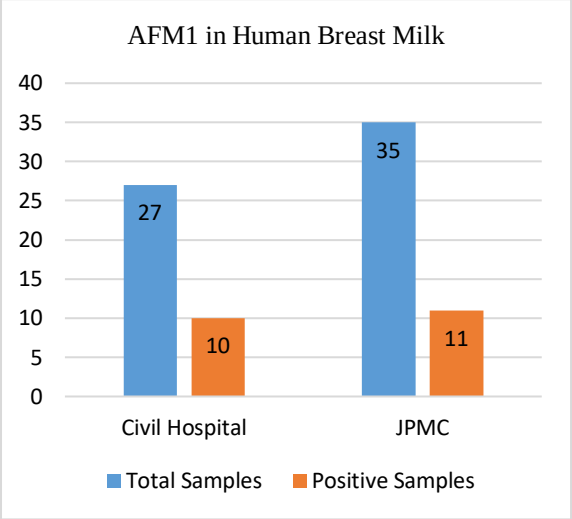

Figure 3: Aflatoxin M1 in Human Breast Milk Samples

The analytical results of the occurrence of AFM_1 in human breast milk samples of volunteer mothers from two different hospitals are summarized in Table-1. 10 samples out of 27 from civil Hospital (CH) and 11 out of 35 from JPMC observed to contain AFM_1 (Fig-1). The maximum concentration of AFM_1 in human breast sample from CH was found 71pgmL^{-1} and minimum level as 28pgmL^{-1}. On the other hand, the maximum level of AFM_1 in human breast sample from JPMC was found 970pgmL^{-1} and minimum level as 27pgmL^{-1}. The results show large variation among all sample analyzed. It is reflected from the questionnaire filled up by the volunteer mothers at the time of sample donation, that the variation is may be due to the group of food included grain products, milk or milk products, legumes, meat, fish, vegetable oil, dried fruits and peanuts used and different native place and demographic background of the volunteer mothers.

Table 1: Determination of AFM1 Contents in Human Breast Milk Samples

Source	No. of Samples	No. of Positive for AFM_1 (%)	AFM_1pgmL^{-1} milk ± SEM	
			Samples*	Median ± SD
Civil Hospital	27	10 (37.04%)	28 ± 4	**50 ± 11**
			71 ± 6	
			61 ± 4	
			37 ± 5	
			28 ± 4	
			64 ± 4	
			43 ± 5	
			50 ± 5	
			61 ± 6	
			49 ± 5	
Jinnah Post Graduate Medical Centre	35	11 (31.43%)	611 ± 24	**96 ± 85**
			197 ± 12	
			90 ± 10	
			116 ± 7	
			27 ± 3	
			29 ± 4	
			35 ± 2	
			970 ± 31	
			32 ± 5	
			64 ± 4	
			759 ± 17	

* Average of three essay ± SEM

Most of the lactating women included in the study were born and residing in Karachi, Pakistan.

The maternal data shows the average age of mothers that was 25 years (18-47) for current study. Most of the mothers (71%) already had one or more children, and mothers have miscarriage in past were 12.90%. A total of 76% of the volunteers female belong to LC and 92% were not employed anywhere, 8% of the volunteer were employed in the profession of teaching or nursing. The daily diet of the most of the volunteer mothers includes corn and wheat bread; Desi ghee, corn oil, legumes, and beans, but mothers belonging to LMC category found consuming less frequently than the mothers belonging to LC. 48% of the volunteer mothers were obese (>30). There were 25 girls and 37 boys in the study, with an average lactation period as 5 months (range 2 – 8).

Study and monitoring of food intake alongwith food analysis will help to determine aflatoxin exposure to human. Observing presence of aflatoxins in biological fluid could be a more coordinate and solid pointer of exposure. Human breast milk is a better biological fluid used to monitor aflatoxin exposure as it is easily collected and provide direct assessment of the exposure of aflatoxins to newborn can be investigated. A variety of reasons makes children more susceptible group of population for exposure to natural toxicants. Newborn babies have lower capacity to detoxify, are rapidly growing; and can absorb higher amount of food, and therefore, early child hood exposure to aflatoxin may be basic determinants of future health (Weaver *et al.*, 1998).

The Food and Drug Administration (USA) set a tolerance limit of 0.5 µg/kg of AFM_1 in milk. (Organization, 2003). Human breast milk is believed to be the perfect nourishing food for the growth of newborn. Occurrence of variety of contaminants in human breast milk is responsible in producing prominent health issues for newborn (Turconi *et al.*, 2004, Landrigan *et al.*, 2002, Pronczuk *et al.*, 2004, Solomon and Weiss, 2002, Organization and Cancer, 1993). Study and monitoring of breast milk intake alongwith analysis will help to determine aflatoxin exposure patterns which are excreted through mother's breast milk.

In the current study, investigation of aflatoxin in human breast milk of various cohorts of women in Karachi has shown unexpected levels of AFM_1 indicating substantial exposure of mothers to aflatoxin in their daily intake. In this study, 33.4% of human milk sample of volunteer mothers were observed positive for AFM_1 and the level of AFM_1in the 21 positive samples out of 62samples analysed was in a range of 27-970pgmL^{-1}, which is very much lower than that reported earlier. 92% of human breast milk sample were observed to be contaminated with AFM_1 (5-3400pgmL^{-1}) in United Arab Emeritus with an average level of 560pgmL^{-1} (Abdulrazzaq *et al.*, 2003). AFM_1 reported in 11 out of 73 (15%) breast milk samples as 71pgmL^{-1} with in a wide range of range 28-1031pgmL^{-1} in the sample analysed in Victoria, Australia; and 5 out of 11 (45%) of human breast sample in Thailad were reported to contain 664pgmL^{-1} with a range of 39-1736pgmL^{-1}AFM_1 (El-Nezami *et al.*, 1995). The human breast milk samples that were analysed and reported to contain AFM_1 in a range of 2100-9200pgmL^{-1} (Zarba *et al.*, 1992). The current study is

comparable to the study which indicates 138 out of 388 (34%) samples contaminated with $AFM_1$with a median level of the positive sample of 160pgmL^{-1} (Polychronaki *et al.*, 2006).

In the current study a moderate level of contamination of aflatoxin is observed that can be compared to some of the high exposure area. Be that as it may, a threshold tolerable value for carcinogenic substances such as aflatoxins, should be exist (Organization and Cancer, 1993). The tolerance limit of 0.5 µg/kg of AFM_1 in milk is set by Food and Drug Administration (USA). Human breast milk is considered to be the perfect nourishing food for the growth of newborn (Organization, 2003). It the toxicological significance of aflatoxin contamination in human breast milk be taken in consideration the child exposure to the AFM_1 can be reduced.

Conclusion

Aflatoxin exposure to the residents of Karachi has been estimated earlier in buffalo milk (Raza, 2006) and human breast milk in current study. The current study provides important evidence of contamination of aflatoxin in human breast milk, but at a very limited number of samples and in the period of study. In view of the results of current study, a detailed study is required to be carried out to reduce the hazard of aflatoxin to newborn baby through human breast milk in Karachi. It is also recommended to analyze individual exposure to aflatoxin in lactating women residing in various provinces of Pakistan. A longitudinal study to indicate the possible sources of aflatoxin exposure in the Pakistani diet may also be conducted.

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