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Effects of Aflatoxin Exposure on Child Growth in the City of Karachi, Pakistan

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Abstract

Aflatoxins excreted in lactating human, in the form of aflatoxin M_1 (AFM_1) are carcinogenic substances. These are metabolites of aflatoxin B produced by fungi, Aspergillus flavusand Aspergillu sparasiticus. The current study was based on the aflatoxin exposure to the infants through breastmilk feeding. Human breast milk samples of 62 mothers through two hospitals were included in the study for the evaluation of AFM_1. Standard HPLC method of analysis was used for analysis. A total of 21 samples out of 62 were found to contain AFM_1. In the present study, the mean concentration of AFM_1 in human breast milk (50 to 96pg/ L) has been found lower than the maximum acceptable limit ($0.05 mgkg^{-1}$) set by the European Union. The effects of aflatoxin contamination in breast milk on the growth of infants was assessed by maintaining anthropometric analysis of infants at 3, 6, 9 and 12months age. It has been observed that the aflatoxin contamination in the breast milk is significantly associated with infants impaired growth. The observed levels of aflatoxin in human breast milk was found to be associated significantly with weight, length, MUAC and head circumference deficit in the infants. The results confirming effect of chronic aflatoxin exposure to the growing infants and can be related with the impaired growth.

Keywords: Aflatoxin, infant growth, head circumference, length, weight, arm circumference

Introduction

For the growth of infant, human breast milk is considered to be the best (WHO, 2003). Human breast milk provides healthful components to improve the immune system of the infant. The human breast milk may contain harmful carcinogenic toxin i.e. AFM_1 (Abdulrazzaq, Osman, Yousif, & Al-Falahi, 2003; Andrews-Trevino et al., 2019; Navas, Sabino, & Rodriguez-Amaya, 2005; Turner et al., 2007). It has been observed that breast milk of nursing women was found to contain AFM_1 making them an effective source of aflatoxin exposure to infants (El-Nezami, Nicoletti, Neal, Donohue, & Ahokas, 1995; Polychronaki et al., 2006). It has been reported that buffalo and cow milk were also found contaminated with AFM_1 (R Raza, 2006). European Union set 0.05 mgkg-1 as the max acceptable limits of AFM_1 in milk (Rashid Raza, 2020). Various negative effects of AFM_1 on infant health has already been reported in literature (El-Nezami et al., 1995; Polychronaki et al., 2006). The effects include impaired growth of infants fed breast milk contaminated with AFM_1 (Andrews-Trevino et al., 2019; Hoffmann, Jones, & Leroy, 2018; Passarelli et al., 2020; Watson et al., 2018), that effect the growth pattern of infant creating lifetime negative health effects (Barker, 2004; Delisle, 2002).

The aim of current study was to determine the relation between aflatoxin exposure of infants fed with breast milk to impaired growth of child. The newborn children of the nursing mothers who found to contain aflatoxin in their breast milk (Rashid Raza, 2020) were included in the longitudinal study examining association between impaired linear growth in early childhood and aflatoxin exposure.

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Methodology

Data Collection

A permission letter was obtained from all volunteer nursing mothers before collecting the breast milk sample. Permission letter was obtained from both of the hospitals, while informed consent is obtained from the participants. Each lactating mother was agreed to provides her breast milk for the current study. Every volunteer mother has also provided information about her daily/weekly/monthly food intake i.e milk, fish, meat, poultry, legumes, dried fruits and vegetable oil etc. The demographic data was also recorded for every mother including education, monthly income, residential area etc.

Sample Collection

Milk samples of sixty-two nursing mothers were collected from Hospital A (27) and hospital B (35). The samples of volunteer mothers were collected by self-expression and by Chicco Mother Naturally Me Electric Breast Pump - Chi-00009199000000.

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 at 440nm emission and 360nm excitation. The 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 0.04-10 $ngmL^{-1}$ was prepared in 1 mL 2:3 vol/vol mixture of methanol and water and then it was filtered through PVDF membrane having pore size 0.45 μm. Since aflatoxins are possible carcinogen, care has always been practiced to avoid exposure and 10% sodium hypochlorite was used for decontamination.

Anthropometric Data Collection

Anthropometric data, such as weight (to the nearest 0.1 kg), length, head circumferenceand mid upper arm circumference (to the nearest 0.1 cm) of the infant wasrecorded at 3, 6, 9 and 12months age. Certeza Electronic Baby Scale BS-820 was used to measure child weight, during the survey visits. Length was measured using infant length measuring mat, all other measurements were taken by anthropometric tape.

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 (Williams 1984).

Results and Discussion

Demographic Analysis

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. 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 (Lower Class) 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 (Lower Middle Class) 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).

Breast Milk Analysis

Table 1: AFM_1 Contents in Human Breast Milk Samples

Source	No. of Samples	No. of Positive for AFM_1 (%)	$AFM_1 pgmL^{-1}$ milk ± SEM	
			Samples*	Median ± SD
Hospital A	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	
Hospital B	35	11 (31.43%)	611 ± 24	96 ± 8.5
			197 ± 12	
			90 ± 10	
			116 ± 7	
			27 ± 3	
			29 ± 4	
			35 ± 2	
			970 ± 31	
			32 ± 5	
			64 ± 4	
			759 ± 17	

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. The maximum concentration of AFM_1 in human breast sample from hospital A 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 hospital B was found $970pgmL^{-1}$ and minimum level as $27pgmL^{-1}$. A total of 10 out of 27 breast milk samples collected from Hospital A and 11 out of 35 collected from hospital B found to contain AFM_1 (Fig-1). The results show large variation among all sample analyzed. According to the European Commission regulations, the

maximum level of AFM_1 for infant food commodities should not exceed 0.5mg/kg (Raza 2020). In the present study, the mean concentration of AFM_1 in human breast milk (50 & 96pg/L) was found lower than the maximum limit accepted by the European Union regulation.

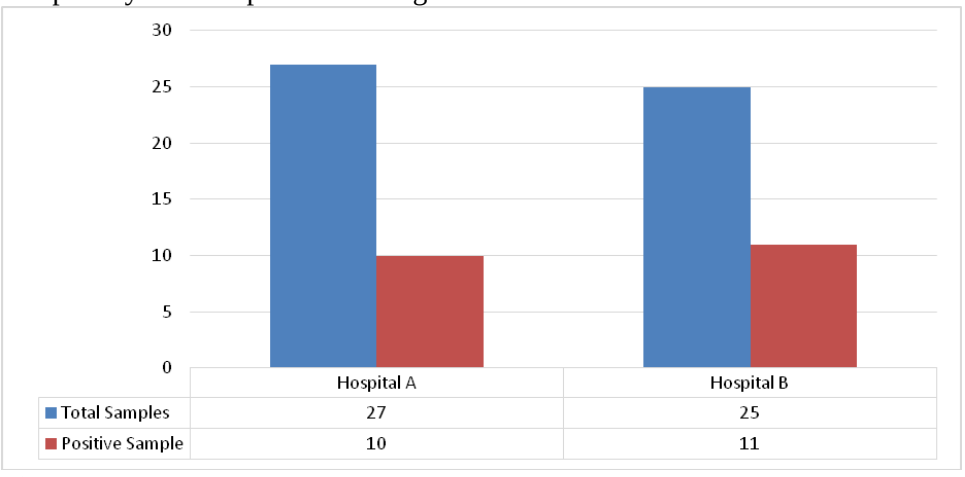

Figure 1: Frequency of Aflatoxin M1 Contamination in Human Breast Milk Samples

Anthropometric Analysis

Table 2: Anthropometry of Infants

Age (months)	AFM_1 Exposure (mg/kg)	Weight (kg)		Length (cm)		MUAC (cm)		Head Circumference (cm)	
		Estimated	Standard	Estimated	Standard	Estimated	Standard	Estimated	Standard
Birth	-----	2.83±0.46	3.3	48.2±2.15	50.0	------	------	32.0±1.46	34.5
3	0.11	5.52±0.83	6.3	59.0±2.51	61.5	12.48±0.94	13.5	37.9±1.83	40.5
6	0.15	6.89±1.00	7.9	65.6±2.57	67.5	13.20±0.93	14.2	41.2±2.00	43.3
9	0.2	7.47±1.04	8.9	69.3±2.67	72.0	13.43±0.93	14.5	43.1±1.94	45.0
12	0.2	7.94±1.06	9.7	72.3±2.72	76.0	13.74±0.96	14.7	44.2±1.72	46.1

Data of anthropometric analysis of infants has been recorded as Table-2. The average exposure of infant to AFM_1 showed that in the beginning months of life (0-3months) infants are exposed to an average of 0.11mg/kg AFM_1 daily from breast feeding in the sample analyzed in the current study. The daily amount of AFM_1 is calculated according to the standard set for the amount of breast feeding to infant (Organization 2009). As the amount of milk fed to the infants increased the amount of aflatoxin ingested by the infants is also increased to 0.15 mg/kg (per day) at 6months and 0.2mg/kg (per day) at 9-12months of age. The exposure of aflatoxin to the infants has affected the growth pattern of infant which appeared in the anthropometric analysis. At the time of birth children were 0.4kg lighter than the standard weight of infant (Organization 2006); and the shortage was increased to 1.0kg at 6 months and 1.7kg at 12months of age. The length of infants was found to be 1.7cm shorter than the standard length of infant (Organization 2006), which moves to 1.9cm at 6moths and 3.7kg at 12moths of age. Head circumference and MUAC were also determined during the study and head circumference was found to be 2.5cm lesser than the standard set by WHO (Organization 2006). The difference in head circumference was decreased to

2.1cm at 6months and 1.9cm at 12moths age. No regular pattern of increase or decrease had been observed for MUAC at the age of 3, 6 and 12months.

Discussion

The effects of aflatoxin contamination in breast milk on the growth of infants was assessed by maintaining anthropometric analysis of infants at 3, 6, 9 and 12months age. Differences observed in the anthropometric analysis of infants to the set standards (Organization 2006) is may be due to the effects of aflatoxin contamination in breast milk of nursing mothers. However, due to the low biotransformation rate of infants, attempts should be directed toward finding ways to reduce the contamination of foods with aflatoxins. Therefore, in order to reduce the presence of aflatoxins in breast milk and infant exposure, people, especially mothers, should be educated about the ways of conveyance of aflatoxin into foods, and associated hazards following unsuitable food storage and ingestion of contaminated foods. There is a need for monitoring the AFM_1 level in human breast milk samples over the breastfeeding period.

References

Abdulrazzaq, YM, *et al.* (2003). "Aflatoxin M1 in breast-milk of UAE women." Annals of tropical paediatrics **23**(3): 173-179.

Andrews-Trevino, JY, *et al.* (2019). "Relatively low maternal aflatoxin exposure is associated with small-for-gestational-age but not with other birth outcomes in a prospective birth cohort study of Nepalese infants." The Journal of nutrition **149**(10): 1818-1825.

Barker, DJ (2004). "The developmental origins of chronic adult disease." Acta paediatrica **93**: 26-33.

Delisle, H (2002). "Foetal programming of nutrition-related chronic diseases." Cahiers d'études et de recherches francophones/Santé **12**(1): 56-63.

El-Nezami, H, *et al.* (1995). "Aflatoxin M1 in human breast milk samples from Victoria, Australia and Thailand." Food and Chemical Toxicology **33**(3): 173-179.

Hoffmann, V, *et al.* (2018). "The impact of reducing dietary aflatoxin exposure on child linear growth: a cluster randomised controlled trial in Kenya." BMJ Global Health **3**(6): e000983.

Navas, S, *et al.* (2005). "Aflatoxin M1 and ochratoxin A in a human milk bank in the city of Sao Paulo, Brazil." Food additives and contaminants **22**(5): 457-462.

Organization, WH (2006). WHO child growth standards: length/height-for-age, weight-for-age, weight-for-length, weight-for-height and body mass index-for-age: methods and development, World Health Organization.

Organization, WH (2009). Infant and young child feeding: model chapter for textbooks for medical students and allied health professionals, World Health Organization.

Passarelli, S, *et al.* (2020). "Aflatoxin exposure in utero and birth and growth outcomes in Tanzania." Maternal & child nutrition **16**(2): e12917.

Polychronaki, N, *et al.* (2006). "Determinants of aflatoxin M1 in breast milk in a selected group of Egyptian mothers." Food Additives and Contaminants **23**(7): 700-708.

Raza, R (2006). "Occurrence of aflatoxin M-1 in the milk marketed in the city of Karachi, Pakistan." Journal of the Chemical Society of Pakistan **28**(2): 155-157.

Raza, R (2020). "Occurrence of Aflatoxin M1 in Human Milk Investigated in Karachi, Pakistan." Pakistan Journal of Pharmacy Research, **6**((1),): 1-7

Turner, PC, *et al.* (2007). "Aflatoxin exposure in utero causes growth faltering in Gambian infants." International journal of epidemiology **36**(5): 1119-1125.

Watson, S, *et al.* (2018). "Impaired growth in rural Gambian infants exposed to aflatoxin: a prospective cohort study." BMC Public Health **18**(1): 1-9.

Who, G (2003). "Global strategy for infant and young child feeding."

Williams, S (1984). Official methods of analysis, Association of Official Analytical Chemists