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ORIGINAL ARTICLE

Aflatoxin B1 Level in Relation to Child's Feeding and Growth

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Abstract
Objective To study the influence of sociological factors, breast feeding and weaning on aflatoxin exposure in children as well as to determine the effect of aflatoxin exposure on child's growth.
Methods A questionnaire, administered to the mothers of forty-six children, obtained information on the child's age, sex, residence, feeding, weaning and general health status. Maternal parity, education and occupation were also collected. Height for age Z-score (HAZ) and weight for age Z-score (WAZ) of children were calculated at the time of recruitment. TLC analysis was performed for aflatoxin B1 level in studied children and their mothers.
Results Aflatoxin B1 was detected in 17 out of 46 (36.96%) of children's serum at a median concentration of 51.61 (30.565–62.795) ppm and in 17 out of 46 (36.96%) of mother's serum at a median concentration of 50 (35.59–84.93) ppm. Aflatoxin B1 level was neither affected by child's age, sex, residence whether rural or urban, maternal age, parity, education nor occupation. Aflatoxin B1 in breastfed patients was significantly lower than in non-breastfed ones (p=0.034). Weight for age Z-score (WAZ) showed no significant difference between aflatoxin B1 negative and positive cases (p=0.422) while height for age Z-score (HAZ) was significantly lower in aflatoxin B1 positive compared to negative cases (p=0.001). A significant positive correlation between aflatoxin B1 in the present cases and their mothers (r=0.881, p=0.0001) and a significant negative correlation between aflatoxin B1 in present cases and their height-z-score (HAZ) (r=−0.460, p=0.001) was detected.
Conclusions Breast feeding results in lower aflatoxin exposure. Also, a strong association between aflatoxin exposure and impaired child's growth exists.

Keywords Aflatoxin B1 · Breast feeding · Growth parameters · Weaning

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Introduction

Aflatoxins are a group of toxins produced in foods contaminated by the moulds Aspergillus flavus and Aspergillus parasiticus [1]. The toxins have been implicated as a causative agent in human hepatic and extrahepatic carcinogenesis [2, 3].

While a number of investigations have examined human exposure to aflatoxins from the diet, methodological constraints have inhibited extensive investigations to assess maternal to infant exposure, through breast milk, to a major carcinogenic metabolite of aflatoxin B1 (AFB1); aflatoxin M1 [1].

Aflatoxin M1 (AFM1) is the hydroxylated metabolite of AFB1 formed in liver by means of cytochrome P450-associated enzymes [4, 5]. It has been shown to be excreted in milk following exposure to AFB1 contaminated food and transferred to dairy products such as cheese which represents an important risk factor for consumers [6].

Several research workers reported that there is a linear relationship between the amount of AFM1 in milk and AFB1 in the food consumed by animals [7].

It has been reported that 0.3–6.2% of AFB1 in animal feed is transformed to AFM1 and excreted in milk [6]. However, this transmission rate varies from animal to animal, from day-to-day, and from one milking to the next [8]. Studies have clearly demonstrated that AFM1 causes toxic and carcinogenic effects, therefore this toxin is classified by the International Agency for Research on Cancer (1993) of WHO as a Group 2 human carcinogen [5].

Since milk and milk products are the most potent source of aflatoxin amongst food, humans are potentially exposed to these metabolites and it is generally assumed that neither storage nor processing provides a reduction of AFM1 content [9]. AFM1 is relatively stable in raw and processed milk products, and unaffected by pasteurization and ultra-high-temperature (UHT) treatment or processing into cheese [9, 10].

Earlier studies have demonstrated neonatal growth retardation in some animals exposed to aflatoxins prenatally. In addition, there is evidence of poor neonatal survival in piglets exposed to aflatoxins [11].

This study was conducted to establish the influence of sociological factors, breast feeding and weaning on aflatoxin exposure in children as well as to determine the effect of aflatoxin exposure on child's growth.

Material and Methods

Subjects

Forty six infants and children and their mothers, admitted in Mansoura University Children's Hospital during 18 months period, were recruited in the study. All subjects were free from any hepatic or renal diseases as well as from any chronic illness or malnutrition that may affect growth. A questionnaire, administered to the mothers of children recruited in the study, obtained information on the child, namely age, sex, residence, feeding, weaning status and general health status. The age range of infants and children included in the study was 1 month to four and a half year. They were 33 boys and 13 girls. Additional data concerning maternal parity, education and occupation were collected. Children's height and weight using a spring balance (SMIC Health Scale, RGZ-120,China) were measured as well as height for age *Z*-score (HAZ) and weight for age *Z*-score (WAZ) were calculated at the time of recruitment.

Sampling

Five ml blood from each subject and his/her mother were collected. All samples were kept in refrigerator for TLC analysis for aflatoxin B1 according to Epply [12], modified by AbdelHamid [13]. The laboratory study was undertaken in the Laboratory of Animal Production Department, Faculty of Agriculture, Mansoura University.

Aflatoxins Assessment

Extraction 5 ml of the blood sample+25 ml (24 ml chloroform+1 ml 0.1 phosphoric acid) were mixed. This mixture was shaken in a separating funnel. Then the chloroform layer was received and transformed into a flask and dried under vacuum on Rota Vapor – M (Buchi – HB-140) at 60°C.

Qualitative Determination of Aflatoxin B1 by TLC Residues were dissolved in 20 ml chloroform for TLC spotting. Development of the plates was done in a closed developing tank with solution: Toluene : Acetic acid : Formic acid, 6 : 3 : 1 for about 40 min.

The plates were dried in air and examined under ultraviolet at wave length 366 nm by using: Min uvis, UDO-UV-Source for TLC, Desags (Heidelberg), with short wave length at 254 nm and long wave length at 366 nm.

The verification was done through R_f value and the fluorescence color, after comparison with external standard. The aflatoxin shows blue fluorescence. The fluorescent area which was shown under UV at 366 nm was demarked by a hard pencil.

Quantitative Determination of Aflatoxin B1 by TLC The fluorescent area which was confirmed as the tested aflatoxin was scratched using a stainless steel spoon. The aflatoxin was eluted from the silica gel using chloroform (5 ml). The chloroform elute was coloure-metrically tested using spectrophotometer (Ultrospec II, L.K. B, Biochrom) at 366 nm against a blank of chloroform and an external standard with known concentration of the tested aflatoxin.

For the calculation of aflatoxin concentration, the following equation was used:

$$\frac{\text{The O.D. of the unknown sample} \times \text{conc. of standard (1000 ng)}}{\text{The O.D. of the standard} \times \text{volume of the sample used (5 ml)}}$$

O.D. = Optical Density

Statistical Analysis

The collective data were computerized using SPSS for Windows Statistical Package (SPSS Inc., Chicago, IL, U.S. A.). Summary statistics of data were expressed as mean±

Table 1 Socio-demographic data of the studied aflatoxin positive and negative patients

Variables	Patients		*p*
	Aflatoxin+ve No. (%) 17 (36.96)	Aflatoxin -ve No. (%) 29 (63.04)	
Patient's age* (years)	0.858±0.437	1.234±1.024	0.158
Patient's sex			
Male	12 (26.1)	22 (47.8)	0.560
Female	5 (10.9)	7 (15.2)	
Patient's residence			
Rural	13 (28.3)	18 (39.1)	0.421
Urban	4 (8.7)	11 (23.9)	
Maternal age* (years)	26.812±0.9713	27.033±0.7783	0.985
Maternal parity			
Primi	5 (10.9)	8 (17.4)	0.930
2nd	7 (15.2)	14 (30.4)	
3rd	3 (6.5)	4 (8.7)	
4th	2 (4.4)	3 (6.5)	
Maternal education			
Illiterate	4 (8.7)	7 (15.2)	0.765
High school	10 (21.8)	18 (39)	
Institute	0 (0)	2 (4.4)	
College	3 (6.5)	2 (4.4)	
Occupation			
Housewife	16 (34.8)	26 (56.5)	0.667
Employee	1 (2.2)	3 (6.5)	

+ve=Positive; -ve=Negative

All data are presented as number (percentage)=No. (%) except * presented as mean±SD

$P<0.05$ is significant

SD, median and 25th–75th percentiles (interquartile range). The Kolmogrov-Smirnov test was performed to check normal distribution of data. Non-parametric data were assessed by the Chi-square test and the Mann Whitney *U* test for continuous variables. Independent samples *t*-test were used for parametric data. For correlation analysis, Spearman's correlation coefficients were calculated. A p value<0.05 was considered statistically significant.

Results

Aflatoxin B1 was detected in 17 out of 46 (36.96%) of the children's serum samples at a median concentration of 51.61 (30.565–62.795) ppm, it was also detected in 17 out of 46 (36.96%) of mother's serum samples at a median concentration of 50 (35.59–84.93) ppm. All mothers who showed positive Aflatoxin B1 serum concentration had children with positive Aflatoxin B1 serum concentration.

Table 2 Feeding and growth measurements of the studied aflatoxin positive and negative patients

Variables	Patients		*p*
	Aflatoxin+ve	Aflatoxin -ve	
Feeding			
Breast-fed	3 (6.5)	14(30.4)	
Non-breastfed:			0.034
Artificial milk	11 (23.9)	10 (21.7)	
Cow's milk	2 (4.3)	0 (0)	
Fully weaned	1 (2.2)	5 (10.9)	
WAZ	−0.535±1.944	0.983±1.546	0.422
HAZ	−0.95 (−1.89–0.33)	−0.19 (−0.605–0.665)	0.001

Feeding is presented as number (percentage)=No. (%)

WAZ is presented as mean±SD, while HAZ is presented as median (IQR)

$P<0.05$ is significant

Socio-demographic data of the studied patients having negative or positive serum levels of aflatoxin B1 are shown in Table 1. Aflatoxin B1 positive and negative patients showed no significant difference regarding their age, sex, residence, maternal age, parity, education nor occupation.

Patients were classified as either breastfed (partially or wholly breastfed) (17/46), receiving artificial milk (partially or wholly artificially fed) (21/46), receiving cow's milk (2/46) or fully weaned (6/46). Aflatoxin B1 concentration in breastfed patients was significantly lower than in non-breastfed (artificially-fed, cow's milk fed or fully weaned) ones (p=0.034). As regards growth parameters, weight-z-score (WAZ) was lower in aflatoxin B1+ve patients compared to the -ve group (mean±SD −0.535±1.944 versus 0.983±1.546 respectively). However, this didn't reach a statistically significant difference (p 0.422), while height-z-score (HAZ) was significantly lower in aflatoxin B1 positive compared to aflatoxin B1 negative cases (p=0.001).These results are shown in Table 2 and Fig. 1.

A significant positive correlation between aflatoxin B1 concentration in the present cases and their mothers (r=0.881, p=0.0001) is shown in Fig. 2; a significant negative correlation between aflatoxin B1 concentration in the present cases and their height-z-score (HAZ) (r=−0.460, p=0.001) is shown in Fig. 3 while correlation between aflatoxin B1 concentration in the present cases and weight-z-score (WAZ) was non-significant (r=−0.199, p=0.185).

Discussion

The present study demonstrated that the level of Aflatoxin B1 was neither affected by child's age, sex, residence whether rural or urban, maternal age, maternal parity, maternal education nor maternal occupation. Socioeconomic status might have been expected to correlate with poor food quality and higher aflatoxin level but no strong effect was

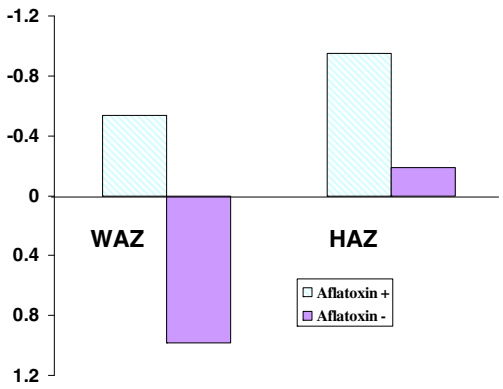

Fig. 1 Growth measurements of the studied aflatoxin positive and negative patients

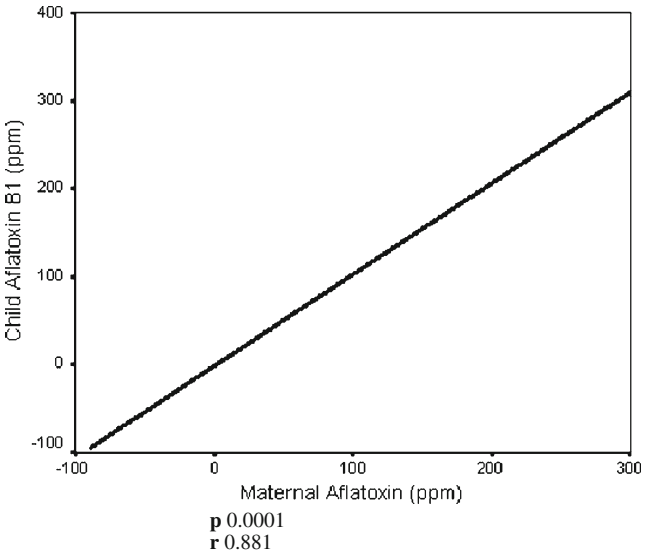

Fig. 2 Correlation between maternal and child's aflatoxin B1 level

observed. Gong et al [14] found no statistically significant difference in aflatoxin-albumin level between male and female children. Similarly, they found that household socioeconomic status showed no statistically significant correlation with aflatoxin-albumin level. They also demonstrated that aflatoxin-albumin levels were lowest in children aged <1 year and increased with age up to 2–3 years, at which point levels reached a plateau. However, this trend wasn't significant when adjusted for weaning and socioeconomic status. Furthermore, Sadeghi et al [15] didn't show significant correlation between aflatoxin M1 and postnatal age, gender, the number of children nor the number of family members.

When grouping the children according to type of feeding, whether breastfed (partially or wholly) or non

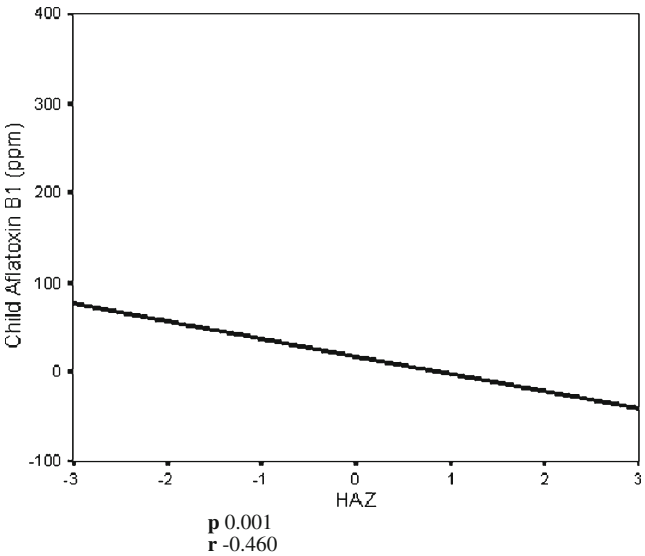

Fig. 3 Correlation between child's aflatoxin B1 level and HAZ

breastfed (artificially fed, cow's milk fed or fully weaned), it was found that aflatoxin B1 level was significantly higher in non breastfed than in breastfed children. Thus it can be hypothesized that the increase in aflatoxin level in the non-breast fed children might be due to the possible effect of breast milk on intestinal absorption of aflatoxin or on its metabolism to reactive metabolites once ingested. However, this hypothesis isn't supported by experimental data so far to authors' knowledge. Gong et al [14], suggested weaning status, rather than age, to be the major determinant of aflatoxin exposure level in young children. The lower aflatoxin-albumin levels associated with breast feeding almost certainly reflect lower levels of aflatoxin in breast milk than weaning and family foods and even lower levels of exposure might be expected in wholly breastfed infants. The authors' observation was also similar to the finding by Gong et al [16] who found that the change from partial breast-feeding to complete weaning correlated with the largest increase in aflatoxin-albumen level, possibly reflecting the increasing proportion of total food consumption coming from the weaning and family foods as the child gets older.

A striking association between aflatoxin and impaired growth in children has been previously reported by Gong et al. [17] who showed a very strong association between aflatoxin albumin (AF-alb) level and stunting (HAZ scores) and being underweight (WAZ scores) in a group of children (9 months to 5 years of age) from Benin and Togo, who may be more sensitive to the growth-inhibitory effects of aflatoxin. Turner et al [18] have previously demonstrated a weak association between AF-alb adduct level and wasting (WHZ scores) but not for stunting (HAZ-score) or for being underweight (WAZ-score) in infants and young children from West Africa. These observations are in agreement with growth impairement associated with aflatoxin exposure in animals [19]. In the present study, HAZ but not WAZ score was significantly lower in aflatoxin positive than in aflatoxin negative children. HAZ score showed a significant negative correlation with aflatoxin B1 level. This was in accordance to the results of Gong et al [16] who showed a significant inverse correlation between serum AF-alb adducts and HAZ score but not WAZ. To date the mechanism of growth faltering is unknown. It is possible that it is a consequence of inhibition of protein synthesis, caused by aflatoxin-induced disruption to RNA synthesis. Alternatively, it has been suggested experimentally that an intestinal malabsorption may occur following aflatoxin exposure [20]. The significant association between aflatoxin B1 level and HAZ score but not WAZ score can be explained by the possibility that the degree of sensitivity of the present cases to the growth inhibitory effects of aflatoxin might have played a role. Furthermore, the dose of aflatoxin in the present studied cases might had an effect on HAZ score but didn't reach to the level that leads to a significant effect on WAZ score. This is in accordance with the results of Marin et al., [21] who observed a dose-related decrease in weight gain in weaning piglets exposed to 140 ppb and 280 ppb aflatoxin.

Regarding the relation between aflatoxin B1 level in studied children and their mothers, a significant positive correlation was demonstrated between them which might be explained by exposure to maternal aflatoxin during fetal life as supported by the findings of Abdulrazzaq et al [22] who found a strong correlation (p <0.0001) between mothers' and cord blood levels of aflatoxin. Another possibility is that these children share the same type of foods with their mothers which might be the source of aflatoxin for both of them.

Conclusions

This study suggests that breast feeding results in lower aflatoxin exposure and that there is a strong association between aflatoxin exposure and impaired child's growth.

Conflict of Interest None.

Role of Funding Source None.

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