PAEDIATRICS

Aflatoxin exposure in utero causes growth faltering in Gambian infants

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Abstract

Background Growth faltering in West African children has previously been associated with dietary exposure to aflatoxins, particularly upon weaning. However, in animal studies in utero exposure to low levels of aflatoxin also results in growth faltering.

Objective This study investigated the effect of in utero aflatoxin exposure on infant growth in the first year of life in The Gambia.

Methods Height and weight were measured for 138 infants at birth and at regular monthly intervals for one year. Aflatoxin-albumin (AF-alb) adduct level was measured in maternal blood during pregnancy, in cord blood and in infants at age 16 weeks.

Results The geometric mean AF-alb levels were 40.4 pg/mg (range 4.8–260.8 pg/mg), 10.1 pg/mg (range 5.0–189.6 pg/mg) and 8.7 pg/mg (range 5.0–30.2 pg/mg) in maternal, cord and infant blood, respectively. AF-alb in maternal blood was a strong predictor of both weight (P = 0.012) and height (P = 0.044) gain, with lower gain in those with higher exposure. A reduction of maternal AF-alb from 110 pg/mg to 10 pg/mg would lead to a 0.8 kg increase in weight and 2 cm increase in height within the first year of life.

Conclusions This study shows a strong effect of maternal aflatoxin exposure during pregnancy on growth in the first year of life and thus extends earlier observations of an association between aflatoxin exposure during infancy and growth faltering. The findings imply value in targeting intervention strategies at early life exposures.

Keywords Aflatoxin, biomarker, The Gambia, growth faltering, in utero

Introduction

In sub-Saharan Africa, around 4.5 million children die each year under the age of 5 years and child death rates are about 30-fold higher than in industrialized nations.1 Growth faltering and under-nutrition has been suggested to underlie around 50% of this burden of mortality.1–3 In The Gambia, in common with much of the region, growth faltering in infancy and early childhood is common and does not appear to be completely explained by inadequate nutrition or infections such as diarrhoea, though the latter can cause acute
weight loss. The growth of Gambian infants appears comparable with that observed in more developed regions for the first two months. After which the onset of growth faltering coincides with the first introduction of foods other than breast milk, and the degree of growth faltering is associated with an intestinal enteropathy, characterized by an increased intestinal permeability. This ‘leakiness’ of the intestinal barrier can in part be explained by chronic inflammation and enteric infection.

Previously in young children in the same region of West Africa, we have reported an association between impaired growth, particularly stunting and exposure to aflatoxins. Aflatoxins are naturally occurring mycotoxins which commonly contaminate staple foods in the region, such as groundnuts and maize, and are known human carcinogens. However, they have broader toxicity and can affect a number of other organs, including the gastrointestinal tract. Aflatoxin metabolism by cytochrome P450 (CYP) 3A enzymes (CYP3A4, CYP3A5 and 3A7) generates a highly reactive aflatoxin-epoxide, which readily binds to cellular macromolecules including DNA and proteins such as albumin. Using aflatoxin-albumin adducts (AF-alb) as a biomarker of aflatoxin exposure, our studies revealed a consistent pattern of chronic exposure at high levels, with >90% of both adults and children having detectable adducts in their blood in West Africa. The liver exhibits the highest expression of CYP3A enzymes but it is notable that enterocytes within the human small intestine also express both CYP3A4 and CYP3A5 and thus could be a site of aflatoxin activation to its toxic metabolite. As a consequence frequent dietary exposure to high levels of aflatoxin may induce enterocyte damage and at least partially explain the ‘leakiness’ observed by Lunn and colleagues.

The above observations in human populations are consistent with evidence of growth faltering in animals treated with aflatoxin. However, growth faltering in animals is additionally observed following transplacental exposure. For example, in pigs exposed to aflatoxin during gestation, growth faltering was observed in the offspring at levels of contamination that occur in maize in sub-Saharan African countries. The potential for such effects in human populations is supported by the fact that CYP3A enzymes are expressed from intra-uterine life onwards and in utero exposure to aflatoxin results in the formation of AF-alb adducts in cord blood. This indicates that maternal aflatoxin exposure during pregnancy can translate to in utero toxicity for the child.

In the Gambia, groundnuts, a dietary staple, are frequently contaminated with aflatoxins and therefore human exposure occurs at high levels. In this study, we compared the growth pattern of Gambian infants in the first year of life with AF-alb adducts in maternal blood during pregnancy, in cord blood at birth and in the child’s blood collected at 16 weeks of age.

**Materials and methods**

**Recruitment**

This study permitted characterization of exposure to aflatoxin in early life in relation to growth and immunological outcomes in 138 singleton infants born in five villages in the West Kiang region over 14 consecutive months (ending June 2000). The study design and immunological data have been published. Detailed anthropometric data were collected within 72 h of birth by the principal investigator (ACC). Birth weight was measured to the nearest 20 g using a spring balance (Salter; CMS Weighing Equipment Ltd, London), which was standardized before each measurement. Infants were subsequently weighed to the nearest 10 g on Week one, Week four and then every fourth week of life for the first year using an electronic infant weighing scale (Seca model no. 834; Vogel & Halke, Hamburg, Germany). Crown-heel length was measured while the infant was in the supine position by using an infant length board (Kiddimeter; Raven Equipment, Great Dunmow, UK) over the same period. Gestational age was assessed by Dubowitz score. Ethical permission was granted by the joint Gambian Government and MRC Ethics Committee. Infants were enrolled ante-natally and studied from birth until one year of age. Other measurements included month of birth, birth size and maturity.

AF-alb adducts were determined in maternal blood samples twice during pregnancy, Collection 1 was on average 4.5 months (95% CI 4.1–4.9 months) prior to birth, and Collection 2 was on average 0.9 months (95% CI 0.7–1.1 months) prior to birth of the index child. The average maternal AF-alb was determined for use in statistical analysis. Additional samples were obtained from cord blood, and infant blood collected at Week 16. The levels of AF-alb adduct were determined by albumin extraction, digestion and ELISA as previously described. The detection limit was 5 pg AF-lysine equivalents per milligram of albumin (pg/mg). A value of 2.5 pg/mg was assigned to samples below the detection limit. Three positive and one negative control sample were analysed with each batch of samples as external controls. Samples were measured in quadruplicate on a given day to obtain a mean value and were analysed in this way on at least two occasions on separate days (coefficients of variation <25%) to obtain an overall mean value for statistical analysis.

**Statistics**

AF-alb adduct level was natural log transformed prior to statistical analysis. Simple comparisons of anthropometric values at birth and at age 52 weeks between genders were performed by t-test. Comparisons of AF-alb between seasons were performed by the Kruskal–Wallis test. Season was treated as a categorical variable with three seasons represented by December to March, April to July and August to November. The maternal, cord blood and Week 16 infant AF-alb levels were used to assess the effect of aflatoxin exposure on growth. Weight and height data were standardized for age separately for each gender to give weight-for-age Z-scores (WAZ) and height-for-age Z-scores (HAZ), using the parametric approach described in detail in Royston and Wright and reviewed in Wright and Royston. The generalized estimating equations (GEE) multiple regression approach was used to analyse the repeated measures of WAZ and HAZ, in relation to AF-alb, adjusted for covariates; covariates hypothesized to be associated with either AF-alb or growth were included in the model. This allowed for the correlation between observations.
Results

One hundred and thirty-eight infants born during the study were followed for 1 year. Table 1 shows descriptive maternal and infant data and infant anthropometry at birth and 52 weeks. The mean birth weight was 2853 g (95%CI 2800–2910) and height was 47.9 cm (95%CI 47.6–48.2). There was no significant difference between boys and girls in these parameters. The average weight and height gains were 5245 g and 16.5 cm over the first year, respectively. At 1 year, boys were significantly taller at 72.3 cm (95%CI 71.9–72.8) vs 70.9 cm (95%CI 70.5–71.4) than girls. Table 1 also shows the mean values of these characteristics by whether maternal AF-alb was above the median or not. The two groups were similar in most aspects except that at Week 52 infants with above median maternal AF-alb were significantly shorter (P=0.031).

Table 1 Descriptive maternal and infant data

<table>
<thead>
<tr>
<th></th>
<th>All subjects</th>
<th></th>
<th>Maternal AF-alb</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Mean</td>
<td>95% CI</td>
</tr>
<tr>
<td>Parity</td>
<td>125</td>
<td>4.2</td>
<td>3.7–4.7</td>
</tr>
<tr>
<td>Gestation/weeks</td>
<td>138</td>
<td>38.6</td>
<td>38.4–38.8</td>
</tr>
<tr>
<td>Maternal weight at delivery/kg</td>
<td>129</td>
<td>55.2</td>
<td>54.0–56.4</td>
</tr>
<tr>
<td>Placental weight/g</td>
<td>128</td>
<td>396</td>
<td>384–407</td>
</tr>
<tr>
<td>Infant birth weight/g</td>
<td>138</td>
<td>2855</td>
<td>2800–2910</td>
</tr>
<tr>
<td>Infant birth length/cm</td>
<td>137</td>
<td>47.9</td>
<td>47.6–48.2</td>
</tr>
<tr>
<td>Week 52 infant weight/g</td>
<td>132</td>
<td>8100ab</td>
<td>7927–8257</td>
</tr>
<tr>
<td>Week 52 infant height/cm</td>
<td>132</td>
<td>71.7b</td>
<td>71.3–72.1</td>
</tr>
</tbody>
</table>

*Mean boys weight 8409 g (95%CI 8191–8628), mean girls (mean 7734 g: 95%CI 7561–7907) were significantly different (P<0.001).

*Mean boys height 72.3 cm (95%CI 71.9–72.8), mean girls height 70.9 cm (95%CI 70.5–71.4) were significantly different (P<0.001).

*In univariate analysis at Week 52, infant length was significantly lower (P=0.031) where maternal AF-alb level was greater than the median level of exposure.

Aflatoxin exposure

The distribution of AF-alb is shown in Table 2. AF-alb adducts were present in all maternal samples collected during pregnancy. AF-alb was detected in nearly half of the cord blood samples, albeit at consistently lower levels than in maternal samples, and only 11% of infants at Week 16 had detectable AF-alb. Mean maternal AF-alb levels were significantly correlated with cord blood levels [Spearman’s correlation coefficient 0.383 (P<0.001)], but no correlation was observed between maternal and Week 16 AF-alb [0.151 (P=0.114)], or between cord blood and Week 16 AF-alb [0.099 (P=0.340)]. There was no difference in adduct level by gender of the infant (data not shown).

Maternal AF-alb level was significantly (P<0.001) higher in blood samples collected in December–March, geometric mean 70.8 pg/mg [Interquartile range (IQR) 43.8–106.7], than in April–July: 37.7 pg/mg (IQR 24.3–66.0) or in August–November: 26.6 pg/mg (IQR 16.1–46.5). Similar significant differences, though at different adduct levels, were observed for cord blood (P<0.001). There was no seasonal variation in AF-alb levels in samples collected from infants at 16 weeks of age (data not shown).

Aflatoxin and longitudinal WAZ

For 107 infants, there were no-missing values in average maternal AF-alb and the covariates concerned. They contributed a total of 1347 (average 14.5) weight-for-age measures to the GEE regression model. Table 3 shows the regression analysis results. After adjustment for covariates (gender, age, maternal weight, gestation time, season) a higher average maternal AF-alb was significantly related to lighter weight-for-age (−0.249 SD; P=0.012); WAZ dropped by a quarter of a standard deviation for every increase in one
unit in log average maternal AF-alb. Among the covariates, only placenta weight (per 100 g) was also significantly related to WAZ (0.260 SD; \( P = 0.032 \)). Model II further includes an interaction term for maternal AF-alb and infant age (in weeks). Infants with higher level of maternal exposure had a weight-for-age profile that fell over age (\(-0.004 SD; P = 0.001\)). Thus, for example, if a 6-month-old girl on the median WAZ had the corresponding maternal aflatoxin level of 110 pg/mg reduced to 10 pg/mg, the model predicts a weight increase of 0.60 SD or about 370 g. At age 12 months, the impact of the same difference in aflatoxin would be 0.87 SD or 800 g. In contrast, cord AF-alb had no effect on weight-for-age (\( P > 0.10; \) data not shown). Model III shows that AF-alb in cord blood was not associated with WAZ. Model IV shows the effect of Week 16 AF-alb adduct level on WAZ regression. This analysis excluded weights measured prior to Week 16. AF-alb at Week 16 was negatively related to weight-for-age, though this effect was not statistically significant (\(-0.355 SD; P = 0.062\)). Neither maternal nor cord blood AF-alb was significantly associated with lower birth weight (each \( P > 0.10; \) data not shown).

**Aflatoxin and longitudinal HAZ**

There were also 107 infants with no missing values in average maternal AF-alb and the covariates concerned with HAZ assessment. They contributed a total of 1433 (average 13.4) HAZ values to the GEE regression model (Table 3). After adjustment for covariates (gender, age, placental weight, maternal weight, gestation time, season) a higher level of average maternal AF-alb was significantly related to a shorter height-for-age (\(-0.207 SD; P = 0.044\)). HAZ decreased by about one-fifth of a standard deviation for every increase in one unit of log average maternal AF-alb. As for WAZ, among the covariates, only placental weight (per 100 g) was positively related to the outcome (0.297 SD; \( P = 0.019 \)). Model II further includes an interaction term for maternal AF-alb and infant age (in weeks). Infants with higher level of maternal exposure had a height-for-age profile that fell with age (\(-0.008 SD; P < 0.001\)). Thus, for example, if a 6-month-old girl on the median HAZ had the corresponding maternal aflatoxin level of 110 pg/mg reduced to 10 pg/mg, the model predicts a height increase of 0.47 SD or about 0.8 cm; at age 12 months, the impact of the same difference in aflatoxin would be 1 SD or 2 cm. Consistent with the data for WAZ, cord AF-alb was not associated with HAZ (data not shown). Model III shows a lack of association between AF-alb in cord blood and HAZ. Model IV shows the effects of AF-alb at Week 16 on HAZ, excluding HAZ

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**Table 2** Maternal and infant AF-alb adducts

<table>
<thead>
<tr>
<th>Exposure</th>
<th>Mediana (pg/mg)</th>
<th>Geometric meanb (range) pg/mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal AF-alb</td>
<td>0.249 (0.042 to 0.055)</td>
<td>0.250 (0.150 to 0.520)</td>
</tr>
<tr>
<td>Cord Blood</td>
<td>0.197 (0.037 to 0.425)</td>
<td>0.207 (0.010 to 0.500)</td>
</tr>
<tr>
<td>Week 16 Infant</td>
<td>0.264 (0.049 to 0.545)</td>
<td>0.262 (0.052 to 0.548)</td>
</tr>
</tbody>
</table>

*aMedian of all data (samples below the detection limit of 5 pg/mg were assigned a value of 2.5 pg/mg).

*bGeometric mean of positive samples only.

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**Table 3** GEE multiple regression analysis (Units = SD)*

<table>
<thead>
<tr>
<th>Exposure</th>
<th>Weight-for-age</th>
<th>Height-for-age</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Model I</td>
<td>Model II</td>
</tr>
<tr>
<td>Maternal AF-alb</td>
<td>(-0.249)</td>
<td>(-0.250)</td>
</tr>
<tr>
<td>Maternal AF-alb×age</td>
<td>(-0.442 to -0.055)</td>
<td>(-0.444 to -0.056)</td>
</tr>
<tr>
<td>Cord blood AF-alb</td>
<td>(-0.024)</td>
<td>(-0.206 to 0.158)</td>
</tr>
<tr>
<td>Placental weight</td>
<td>(0.260)</td>
<td>(0.262)</td>
</tr>
</tbody>
</table>

*aadjusted for gender, age, placental weight, maternal weight, gestation duration and season.

*** \( P < 0.001 \); ** \( P = 0.002 \); * \( P < 0.05 \).
values measured prior to Week 16. AF-alb at Week 16 was significantly negatively related to HAZ ($-0.558$ SD; $P = 0.002$). HAZ decreased by more than half a standard deviation for every increase of one unit in log AF-alb at Week 16. There was no significant interaction between AF-alb at Week 16 and age on HAZ (data not shown). Neither maternal nor cord blood levels were significantly associated with lower birth length (data not shown).

**Discussion**

We have previously demonstrated that high levels of aflatoxin exposure in childhood are associated with growth faltering in infants and young children from West Africa. These observations are in agreement with growth impairment associated with aflatoxin exposure in animals. It is also known that trans-placental exposure to aflatoxin occurs in humans, as established by the presence of aflatoxins and AF-alb in cord blood samples. In the latter study in The Gambia, there was a positive correlation between the level of AF-alb in the mother’s blood at the time of parturition and that in the cord blood. In this study, we assessed maternal aflatoxin exposure during the second and third trimesters of pregnancy as an indicator of in utero exposure. As the AF-alb adduct provides a measure of exposure over a period of two to three months, these measurements provide exposure information over a significant proportion of the in utero period.

The generally high prevalence of exposure (100% of maternal samples and 48.5% of cord bloods with detectable AF-alb) is consistent with previous data in this region. Cord and maternal AF-alb levels are also similar to those reported earlier from The Gambia. In the present study, where both maternal and cord blood AF-alb were detected the difference between each paired sample ($n = 45$) was assessed. On average the cord blood had a 7.9-fold (95%CI 5.6–10.1) lower adduct level than the corresponding maternal samples, though it should be noted that maternal samples were not taken at the same time as the cord blood. The presence of AF-alb in cord blood is consistent with activation of AFB1 to the reactive 8,9-epoxide in fetal liver by CYP3A4 and confirms the potential for toxic and mutagenic events as a result of transplacental exposure.

The seasonal pattern of exposure in both maternal and cord bloods, with almost 3-fold higher median levels in December–March compared with April–July and August–November is related to harvest and storage activities as well as seasonal availability of the major source of aflatoxin in this region, namely groundnuts. This pattern is typical of previous measures in both Gambian adults and children.

At 16 weeks of age, there were relatively few infants (13/118: 11%) with detectable AF-alb and those positive were at low levels, probably due to the major contribution of breast milk to the child’s diet at this age. The AF-alb that we did see may represent residual adduct from in utero exposure, low levels of aflatoxins transferred into breast milk or be a result of the early introduction of weaning foods in some children. Breastfeeding is the exclusive form of nourishment during at least the first couple of months after birth, with the introduction of weaning foods likely to be associated with a rise in aflatoxin exposure. It is known, that where the lactating mother is exposed to aflatoxin, breast milk may be contaminated with aflatoxins, though at far lower levels than are found in solid foods. Although some of the aflatoxin metabolites in milk, such as AFM1, are less efficient at forming adducts than AFB1, they are still potent toxins and may form low levels of albumin adduct detectable by ELISA.

We assessed the effect of in utero aflatoxin exposure on growth during the first year of life by comparison of maternal AF-alb with WAZ and HAZ scores. High maternal AF-alb was strongly related to a lower level of weight-for-age in the Gambian infants. In contrast, AF-alb at Week 16 in the child’s blood sample was not significantly associated with WAZ ($P = 0.062$), though the estimated effect was consistent with the hypothesis of aflatoxin having a negative impact on growth. Maternal AF-alb was also significantly related to height-for-age in the infant, such that HAZ decreased by about one fifth of a standard deviation for every increase in one unit of log average maternal AF-alb. HAZ also decreased by more than half a standard deviation for every increase of one unit in log AF-alb at Week 16. These HAZ data on in utero and post-natal aflatoxin exposure are consistent with the strong association between AF-alb in infants and young children from Benin and stunting. It is possible that the association between aflatoxin biomarkers and growth in the present study is due to unmeasured confounding, for example by socio-economic factors, which are difficult to measure reliably in this setting and thus represent a limitation. Despite this, the strong dose-response relationship between AF-alb and growth in both this study and our previous work in Benin would argue against confounding. In addition, adjustment for socio-economic factors in Benin did not significantly affect the relationship between aflatoxin exposure and growth. Therefore the cumulative data indicate that aflatoxin exposure in utero and in early infancy has a significant impact on child growth in West Africa.

In Gambian children, there is therefore evidence that aflatoxin can cross the maternal placental barrier, the fetus has the requisite CYP3A capability for aflatoxin bioactivation and AF-alb adducts are observed in cord blood (indicative of in utero formation of the highly reactive aflatoxin-epoxide). This evidence is consistent with in utero aflatoxin toxicity having a direct effect on subsequent early child growth. In an experimental study, the offspring of Wistar rats treated with AFB1 (125–1000 μg/kg bw daily) on Days 6–15 of gestation had lower birth weights and lengths and histopathological lesions were also indicative of a direct toxic effect of exposure.

To date the mechanism of growth faltering in relation to maternal aflatoxin exposure during pregnancy is unknown. It is possible that it is a consequence of inhibition of protein synthesis, caused by aflatoxin-induced disruption to RNA synthesis. However, the fact that we observed no effect on weight and length at birth diminishes the likelihood of this explanation. Alternatively, it has been suggested experimentally that an intestinal malabsorption may occur in the offspring following maternal aflatoxin exposure. For example, piglets from aflatoxin-exposed sows had defects related to zinc intestinal adsorption. There was a >50% reduction in plasma zinc in piglets from AF-exposed sows compared with non-exposed sows, despite maternal zinc levels being unaffected. Zinc deficiency has effects on both growth faltering and
immune competence. In our earlier study, the Beninois children were older and growth faltering was assessed only in relation to infant biomarker level.

The current study in The Gambia therefore extends the above observations and suggests that aflatoxin is having a direct toxic effect in utero, manifest as growth faltering in early infancy. It is notable that whilst maternal AF-alb, used as a surrogate of in utero exposure, was associated with growth faltering, the specific measure of cord blood AF-alb was not. This may reflect the fact that AF-alb levels are lower in cord blood, with more non-detectable samples and a consequent loss of ability to resolve inter-individual differences in exposure. Alternatively, given that AF-alb represents exposure over the previous two to three months, the availability of two maternal measures means a greater period of in utero exposure is represented than by the cord blood sample, which will represent exposure in the weeks just prior to birth. Thus, maternal AF-alb may provide a more accurate and relevant measure of in utero exposure than cord blood. The association between aflatoxin exposure and growth faltering will be further influenced by the introduction of weaning foods; these will contribute both to post-natal aflatoxin exposure and the introduction of other factors likely to influence growth e.g. infectious agents. These additional factors may be more prevalent in households with high maternal AF-alb, if the latter is an indicator of generally poorer quality food (e.g. unhygienic, less nutritional value) in a household. This association could consequently partially explain the interaction of maternal AF-alb and age of the child on growth faltering, described in model II.

Growth faltering and under-nutrition has been suggested to underlie around 50% of the burden of infant and early childhood (<5-years-old) mortality in developing countries. The present study and our earlier observations have linked aflatoxin exposure and growth faltering in childhood. It is therefore reasonable to suggest that aflatoxin may in part contribute to childhood morbidity and mortality in this region. Further studies are required to more fully understand these potential health risks, though it is clearly important to focus efforts on economically feasible and sustainable intervention strategies to reduce exposure.

Acknowledgements

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Conflict of interest: None declared.

**KEY MESSAGES**

- Growth faltering in West African children is common and causes a significant health burden in terms of early life morbidity and mortality.
- We have previously demonstrated that chronic aflatoxin exposure at high levels during infancy was associated with growth faltering.
- This study in The Gambia demonstrated that maternal exposure to aflatoxin during pregnancy was (i) frequent and at high levels; (ii) had a deleterious effect on the subsequent growth of the infant.
- A reduction of maternal AF-alb from 110 pg/mg to 10 pg/mg would lead to a 0.8-kg increase in weight and 2-cm increase in height within the first year of life.
- The findings emphasize the need for effective intervention strategies targeted at early life exposures.

**References**

AFLATOXIN AND GROWTH FALTERING IN GAMBIAN INFANTS