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A Cross-Sectional Association Between Serum Aflatoxin and Micronutrient Status Among Children Aged 6–24 Months in Rural Tanzania

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ABSTRACT

Micronutrient deficiencies are a significant public health problem, particularly affecting children under five, caused by inadequate intake of micronutrient-rich foods or environmental factors like aflatoxin exposure. Three hundred sixty-nine children aged 6–24 months from Tanzania's Babati and Hanang districts participated in this study. Serum aflatoxin albumin adduct (AF-alb) levels were assessed as measures of aflatoxin exposure. Haemoglobin levels, serum ferritin, C-reactive protein, zinc and vitamins A, B9, and B12 were assessed to determine anaemia and deficiencies in iron, zinc and vitamins A, B9 and B12 based on WHO cut-off points. Seventy per cent of the children had detectable levels of AF-alb. The AF-alb geometric mean was 5.99 (95% CI: 5.99, 6.87) pg/mg. Of those with measured micronutrient markers, 37% were anaemic, and 33%, 75%, 4%, 4% and 73% were deficient in iron, zinc, vitamins A, B9 and B12, respectively. The child's age, gender and stunting were all significantly associated ($p < 0.05$) with anaemia and deficiencies in zinc, vitamin A and B12. Moreover, AF-alb was associated with iron deficiency even after adjusting for confounders; children with high AF-alb levels (> 6.07 pg/mg) were 1.40 times more likely to be iron deficient (AOR = 1.40, 95% CI: 1.11, 1.74). The high prevalence of micronutrient deficiencies and its association with aflatoxin exposure among young children highlights the urgent need for comprehensive intervention strategies, such as improving dietary diversity and enhancing food safety to reduce aflatoxin exposure. Importantly, longitudinal research is needed to understand the causal effect relationship between aflatoxin exposure and micronutrient deficiencies.

1 | Introduction

Aflatoxin is the toxin produced by fungi (*Aspergillus flavus* and *Aspergillus parasiticus*), which contaminate crops, mainly maize, nuts and oily seeds in the field and during storage under favourable temperature and humid conditions (WHO 2018). There are different types of aflatoxin, i.e., Aflatoxin B1, B2, G1 and G2, of which aflatoxin B1 (AFB1) ranks first as the toxin of most significant public health concern (Negash 2018; Kumar et al. 2017; Benkerroum 2020). Several studies in Sub-Saharan Africa have reported consumption of aflatoxin-contaminated cereal crops, such as maize and groundnuts and its association with high levels of aflatoxin B1 albumin adduct (AF-alb) in human serum (Gong et al. 2002, 2003, 2004; Chen et al. 2018).

Summary

- Micronutrient deficiencies and aflatoxin exposure remain high among under-five children in developing countries. However, few studies have assessed the association between these factors in East Africa, particularly in agro-pastoralist societies where maize and animal foods (highly susceptible to aflatoxin contamination), are staple foods.
- This study found that more than two-thirds of participants were detected with AF-alb levels and deficient in zinc and vitamin B12.
- Children with high levels of AF-alb adducts were 40% more likely to be iron deficient than those with low levels.
- Efforts should focus on improving micronutrient status and enhancing food safety by incorporating aflatoxin mitigation strategies in micronutrient interventions.

In East African countries, especially Tanzania, maize is considered a "staple food", and a lack of it signifies hunger/famine (Williams et al. 2010). In Tanzania, several studies have reported aflatoxin contamination of susceptible crops, including maize, groundnuts and oily seeds (Shirima et al. 2015), and their risk of human exposure has been ascertained (Wild and Gong 2010).

Dietary aflatoxin exposure has been reported to cause acute or chronic intoxications in humans, which is manifested in immune suppression, impaired growth in children and liver cancer (WHO 2018; Rasheed et al. 2021). Few studies have assessed the association between aflatoxin exposure and micronutrient status among children, yet aflatoxin exposure and micronutrient deficiencies are still at their peak in sub-Saharan Africa (Xu et al. 2018; Global Nutrition Report 2020). Studies across various parts of Africa have revealed alarmingly high levels of aflatoxin exposure assessed using a sensitive biomarker, i.e., AF-alb. In almost every study, the detection rate of the AF-alb was more than 60% in every population assessed (Gong et al. 2012; Castelino et al. 2014; Shirima et al. 2015; Watson et al. 2015; Chen et al. 2018; Alamu et al. 2020). In addition, the World Health Organization reported that, globally, more than two billion people were deficient in the essential micronutrients, i.e., vitamin A, iodine, iron and zinc, in the world, and the rates are even higher in Sub-Saharan African countries (World Health Organization and United Nations Children's Fund 2007).

Deficiencies in micronutrients, i.e., iron, zinc and vitamins A and E, can lead to several health problems in women of reproductive age and children under five (Kiani et al. 2022). Vitamin A deficiency can lead to night blindness and low immunity, hence increased susceptibility to infections (West 2003), while vitamin E deficiency can increase oxidative stress in the body (Traber 2014). Besides, iron deficiency can lead to fatigue, shortness of breath, impaired cognitive function and poor growth (Pasricha et al. 2021). Zinc deficiency can cause delayed wound healing, skin diseases and diarrhoea (Hussein et al. 2021). Micronutrient deficiencies can result from poor intake of micronutrient-rich foods (i.e., fruits and vegetables), gastrointestinal diseases (e.g., diarrhoea or worm infestation), which impair nutrient absorption and environmental factors, i.e., seasonality and climate change which reduces the availability of micronutrient-rich foods (Darnton-Hill 2018).

A recent systematic review by Mshanga et al. (2025a) has highlighted that aflatoxin exposure can be associated with anaemia and vitamin A deficiency across various human studies. The review also explored the comprehensive biological mechanisms that could explain how aflatoxin might contribute to anaemia, deficiencies in zinc and vitamins A, C and E (see Figure S1). For instance, aflatoxin has been shown to contribute to anaemia/iron deficiency through three main pathways: by inducing haemolysis of red blood cells, suppressing erythropoiesis and inhibiting intestinal iron absorption (Smith et al. 2017; Figure S1). Furthermore, findings from animal studies suggest that aflatoxin can damage the intestinal lining and impair zinc absorption (Neathery et al. 1980). Similarly, Benkerroum (2020) reported that aflatoxin interferes with the intestinal absorption of vitamins A, C and E.

In Africa, only three studies from West Africa, Benin (Gong et al. 2004), Gambia (Turner et al. 2003) and Guinea (Watson et al. 2016) have explored the association between aflatoxin exposure and micronutrient deficiencies among children. Due to the lack of evidence on the association between the prevalence of different micronutrient deficiencies and aflatoxin exposure in East Africa, and specifically in Tanzania, this study explored the prevalence and association between aflatoxin exposure and micronutrient deficiencies among children aged 6–24 months in Babati and Hanang districts, among the major maize-producing areas in Tanzania (Tanzania Ministry of Agriculture, food Security and Cooperatives 2012; Tanzania Ministry of Agriculture 2016).

2 | Methods

2.1 | Study Design, Area and Population

A cross-sectional study of children aged 6–24 months was conducted from October to November 2022 (during the dry season) in the Babati and Hanang districts of the Manyara region, Tanzania. Hanang and Babati districts were purposively selected (out of six districts) as these are among the districts with high maize production in Tanzania (MRASC, 2008; AASS, 2017; Mutungi et al. 2012) and the only districts with unknown aflatoxin exposure and micronutrient status among under-five children. The study was conducted at Reproductive and Child Health Clinics (RCHs), including the mobile clinics, whereas all mother-child pairs of 6-24 months were informed about the study and invited to join. A total of four Health Centers (HCs) out of 10 were randomly selected, to have two HCs from each district (as shown in Figure 1) to represent other HCs located in the districts. Participants who were willing to participate were assessed for their eligibility, such as having a child aged between 6 and 24 months and who was not sick 3 days before and on the interview day.

2.2 | Sample Size

The sample size was calculated using the Kothari (2004) formula ($n = [(Z2 \times P(1 - P)/d2)]$) based on the percentage of

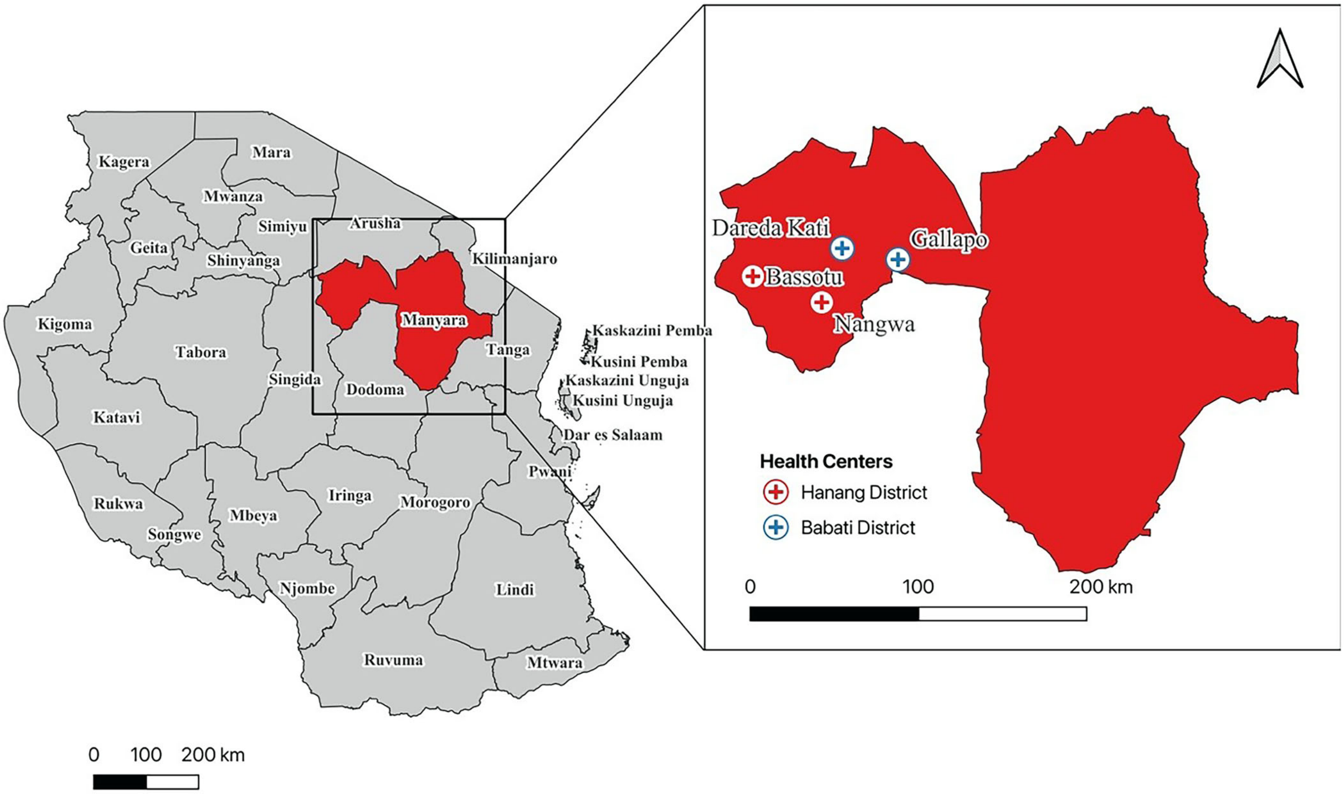

FIGURE 1 | Tanzania country map showing the Health Centers where data were collected (map drawn by QGIS).

aflatoxin exposure ($p = 72\%$) among children aged below 36 months in Haydom ward of Mbulu district, one of the districts in the Manyara region (Chen et al. 2018). The confidence level was set at 95% ($Z = 1.96$), with an absolute precision of 5% ($d = 0.05$), and the statistical power was set at 80% to ensure adequate likelihood of detecting true associations. A total of 310 participants was proposed whereas 369 mother–child pairs (Figure S2), participated in the study, of which 149 were from Babati and 220 from Hanang districts. In cases where a mother had more than one child aged 6–24 months, one child was randomly selected. In some cases, the sample size for specific micronutrient analyses was reduced due to insufficient serum volume (as shown in Figure S1). This was partly due to biological variability, as some participants, particularly young children, had limited blood volume, which restricted the number of biomarkers that could be assessed.

2.3 | Blood Serum Collection

Experienced phlebotomists used butterfly needles to draw 3.5 mLs of blood from each child, which was then placed into red-top serum separator tubes. Two drops of blood were then placed in the microcuvette of the HemoCue Hb 201+ System (SN: 1143013516, manufactured by HemoCue AB Company, Ängelholm, Sweden) for Haemoglobin readings, which were recorded within seconds. The remaining blood sample was centrifuged, and the serum was aliquoted into vials. Each participant's serum was stored at −80°C in two separate vials for AF-alb and micronutrient analyses. Serums for AF-alb analysis were shipped on dry ice to the Biochemistry Laboratory of the University of Leeds, while serums for micronutrient analysis were shipped on dry ice to the Royal Wolverhampton NHS Trust Hospital in Birmingham, United Kingdom.

2.4 | Laboratory Assessment of Serum AF-Alb

A competitive Enzyme-Linked Immunosorbent Assay (ELISA) method, as described by Chapot and Wild (1991), was used to measure AF-alb concentrations in 250 μL serum samples. The method involved three steps: first, the albumin was extracted and digested, following which it was purified using Sep-Pak C18 cartridges, and lastly, the competitive ELISA was performed. For quality control, three positive and one negative control samples were analysed together with the study samples in one ELISA plate. All samples were analysed in triplicate, and the analysis was repeated on two separate days. To ensure precision, results were accepted only if the range between the highest and lowest values among the triplicates fell between 0.01 and 0.05. Additionally, 2-day results were accepted only if the coefficient of variation (CV) between repeats was less than 25%. Samples that did not meet either of these criteria were re-analysed. The limit of detection (LOD) for AF-alb was 3 pg/mg albumin; with any value below this termed as undetected and assigned a medium bound LOD of 1.5 pg/mg albumin value.

2.5 | Laboratory Assessment of Serum Micronutrients

Serum samples were analysed for various micronutrient statuses. Serum retinol was analysed to evaluate vitamin A status, serum active vitamin B12 was assayed for vitamin B12 status, and serum vitamin B9 was assessed for vitamin B9 status. Moreover, serum zinc (morning and non-fasting) was assessed for zinc status, while serum ferritin and C-reactive protein (CRP) were assessed for iron status and inflammation makers, respectively.

The Abbott Alinity I system, utilizing a chemiluminescent microparticle immunoassay with magnetic microparticles, was used to assess serum ferritin, vitamin B12 and vitamin B9 levels, following the methods described by Lee et al. (2023). For serum retinol, liquid–liquid extraction method was performed, and the analysis conducted using a high-performance liquid chromatography (HPLC) for vitamin A status, as detailed in Siluk et al. (2007). Serum zinc levels were measured after dilution in an acid diluent using the ICP-MS (Agilent 8900) in no-gas mode, according to the protocols by Meyer et al. (2018) and Laur et al. (2020). Finally, the inflammation marker, i.e., CRP, was assessed using the Abbott Alinity system through a latex immunoassay, as described by Komoriya et al. (2010). The Biomarkers Reflecting Inflammation and Nutritional Determinants of Anaemia (BRINDA) approach was used for adjusting serum ferritin and serum retinol with inflammation in the R software. However, serum zinc was not adjusted with BRINDA since we found no significant correlation between serum zinc and CRP. Thereafter, we applied the cut-off points developed by the World Health Organization (WHO) for each type of micronutrient among children under five, as shown in Table S1.

2.6 | Statistical Analysis

Descriptive statistics, such as frequency and percentage, were used to present the prevalence of each type of micronutrient deficiency among the study participants. Due to the skewed distribution of AF-alb, the AF-alb data were transformed using the natural logarithm. Subsequently, the geometric mean and its 95% confidence interval (CI) were calculated to present the average levels among participants. Independent sample t-tests or ANOVA were employed to assess the differences of AF-alb in micronutrient biomarkers, breast-feeding status and demographic variables. Since the current study aimed to test the hypothesis that aflatoxin exposure (measured by AF-alb) is associated with any micronutrient deficiencies (i.e., anaemia and deficiencies in Vitamin A, B9, B12, anaemia, zinc and iron), we considered any micronutrient deficiency as an outcome variable while the independent variable was aflatoxin exposure.

Logistic regression analysis was performed to assess the association between each type of micronutrient deficiency and AF-alb. Furthermore, adjusted logistic regression analysis was performed to assess the association between AF-alb and type of micronutrient, with the models adjusted for different confounders, i.e., child's age, gender, breastfeeding status, dietary intake (based on food frequency questionnaire [FFQ]), stunting, wasting, underweight and CRP. Confounders were selected based on previous literature (Turner et al. 2003; Gong et al. 2004; Watson et al. 2016), and a directed acyclic graph (DAG) was constructed using R software to illustrate the relationships among the exposure, outcome and confounders (see Figure S3). A *p*-value of less than 0.05 was considered statistically significant. All statistical analyses were performed using R Software v4.4.

2.7 | Ethics Statement

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects/patients were approved by the Tanzania National Institute for Medical Research (NIMR/R.8a/Vol. IX/4077). Written and verbal informed consent was obtained from all subjects/patients. Verbal consent was witnessed and formally recorded.

3 | Results

3.1 | Socio-Demographic Characteristics of the Studied Children

The study included 369 children aged 6–24 months, with a mean age of 13 months (SD = 4.6), drawn from the Babati and Hanang districts. A larger proportion of participants (60%) were from the Hanang district (Table 1). Children from Hanang tended to be slightly older, with most aged between 13 and 24 months, and the majority were still breastfeeding while receiving complementary foods (Table 1). In terms of sex distribution, Babati had a higher percentage of female children (56%), while Hanang had a slightly higher proportion of male children (52%). Mothers in the Hanang district were more likely to have more young children, with a significantly higher proportion ($p < 0.001$) reporting two children under 5 years of age in the household, compared to mothers in Babati. This difference reflects the larger sample size from Hanang, which is partly due to the district's higher birth registration rates compared to Babati, as reported in the Manyara Census (2022).

3.2 | Aflatoxin Exposure Level Using AF-Alb and the Determinants of the Exposure

The overall geometric mean (95% CI) for AF-alb levels in the participants ($N = 369$) was 5.9 (5.2, 6.8) pg/mg. As shown in Figure 2a, there was higher geometric mean for AF-alb in the Hanang district 6.2 (6.1, 6.4) pg/mg as compared to the Babati district 5.6 (4.6, 6.9) pg/mg, though the difference was not statistically significant. Most study participants (70%) were detected with AF-alb adducts (detectable limit at 3 pg/mg), which was almost the same when presented by district; thus, Babati (68%) and Hanang (71%). Moreover, the geometric mean of AF-alb adduct was significantly high in participants with iron deficiency 8.50 (95% CI: 6.07, 11.89), $p < 0.001$ as compared to the ones who were not iron deficient 5.79 (95% CI: 4.78, 7.01), as shown in Figure 2b. However, we did not find any significant difference in the AF-alb levels by gender, age and breastfeeding status of children.

3.3 | Prevalence and Determinants of Micronutrient Deficiencies Among Children Aged 6–24 Months

Among the six micronutrients assessed, the most prevalent micronutrient deficiencies were zinc (75%) and vitamin B12 (73%) (see Figure 3), while vitamins A and B9 were the least micronutrient deficiencies. In addition, the current study found that more than one-third of participants were anaemic and iron

TABLE 1 | Socio-demographic characteristics of study participants by district.

Variable	Babati 149 (40%)	Hanang 220 (60%)	Total 369 (100%)	*p*-value
Gender				
Female	93 (56%)	74 (44%)	167 (44%)	< 0.001*
Male	56 (48%)	146 (52%)	202 (56%)	
Religion				
Christian	143 (96%)	210 (95%)	353 (96%)	1.12
Muslim	6 (4%)	10 (5%)	16 (4%)	
Age of the Child				
6–12 months	73 (44%)	94 (56%)	167 (45%)	0.23
13–24 months	76 (38%)	126 (62%)	202 (55%)	
Breastfeeding Status				
Breastfeeding + solid foods	124 (42%)	169 (58%)	293 (79%)	0.14
Stopped breastfeeding	25 (33%)	51 (67%)	76 (21%)	
Number of under-five children in a household				
1	99 (50%)	100 (50%)	199 (54%)	< 0.001*
2	42 (31%)	92 (69%)	134 (36%)	
> 3	8 (22%)	28 (78%)	36 (10%)	

Chi-square test.
*Significant at a *p*-value < 0.001.

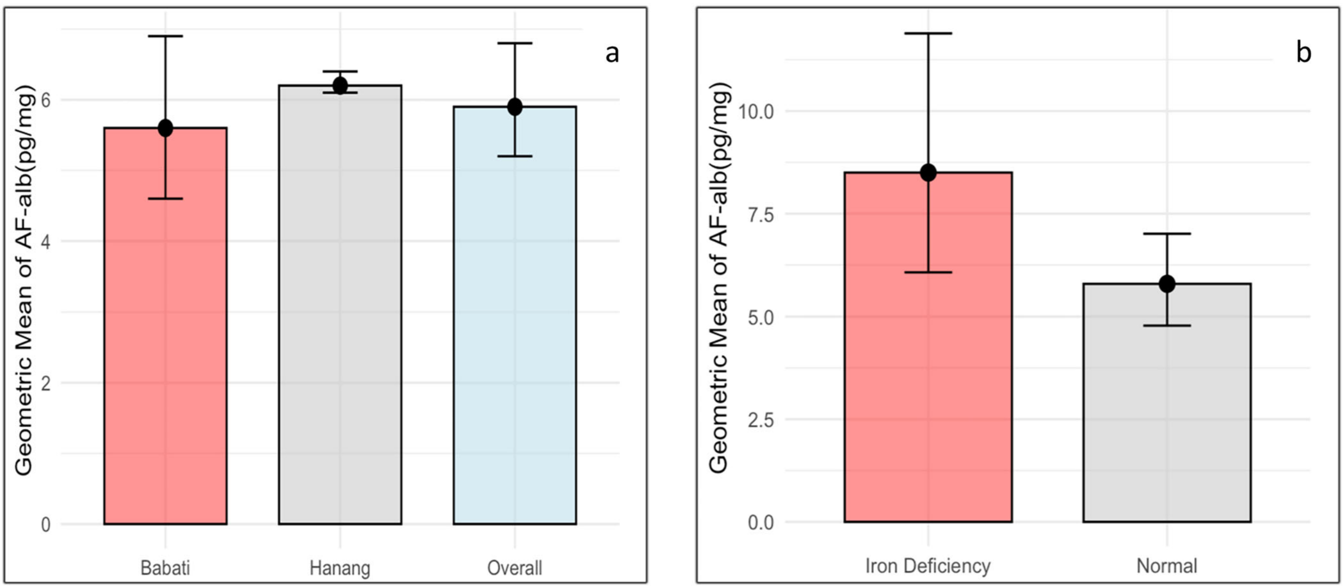

FIGURE 2 | Geometric mean of AF-alb adducts among participants by districts and iron status. (a) Geometric mean of AF-alb adducts among participants by district and overall. (b) Geometric mean of AF-alb adducts among participants with iron deficiency and without iron deficiency (independent sample *t*-test: *p*-value < 0.001).

deficient, as shown in Figure 3. In a separate manuscript (Mshanga et al. 2025a), focusing on iron markers, we reported that 13% of the same study participants had iron deficiency anaemia, while 28% had anaemia associated with vitamin B12 deficiency. On the other hand, the prevalence of having three or more micronutrient deficiencies (23%) was higher among 6- to 12-month-old children. The prevalence of having two micronutrient deficiencies (28%) was higher among children aged 13–24 months. Besides, only a small percentage of children had more than four micronutrient deficiencies as shown in Figure 4.

3.3.1 | The Association Between AF-Alb Adducts and Micronutrient Deficiencies Among Children Aged 6–24 Months

In the logistic regression model, our study found a significant positive association between AF-alb adducts and iron deficiency (OR = 1.20, 95% CI: 1.00, 1.44), $p = 0.04$, as shown in Table 2. However, we did not find any significant association between AF-alb adducts and other micronutrients, nor in multiple micronutrient deficiencies.

3.3.2 | Factors Associated With Micronutrients Deficiencies

In adjusted odds ratio (AOR), an increase in AF-alb adduct was consistently seen to be significantly associated with an increased likelihood of being iron deficient (AOR = 1.40, 95% CI: 1.11, 1.74) $p < 0.01$, even after adjusting for different confounders (see Table 3). Although AF-alb adduct was only seen to be associated with iron deficiency, other factors were seen to have a significant association with deficiencies in other micronutrients, as shown in Table 3. The odds of being anaemic was seen to increase as the child's age increase (AOR = 1.10, 95% CI: 1.01, 1.20) $p = 0.04$, while female children (AOR = 2.82, 95% CI: 1.49, 5.44) $p < 0.01$, were nearly three times more likely to be anaemic compared to their male counterparts. However, younger children were more likely to be zinc deficient compared to older ones (AOR = 0.90, 95% CI: 0.82, 0.99) $p = 0.04$. Moreover, stunted children (AOR = 2.54, 95% CI: 1.04, 6.42) $p = 0.04$, were two times more likely to have vitamin B12 deficiency compared to children who were not stunted. In addition, being a male child (AOR = 0.46, 95% CI: 0.23, 0.92) $p = 0.03$, significantly decreased the odds of being vitamin B12 deficient. However, factors such as breast-feeding status, dietary intake, underweight, wasting, place of residence and inflammation (CRP) were not significantly associated with any micronutrient deficiency.

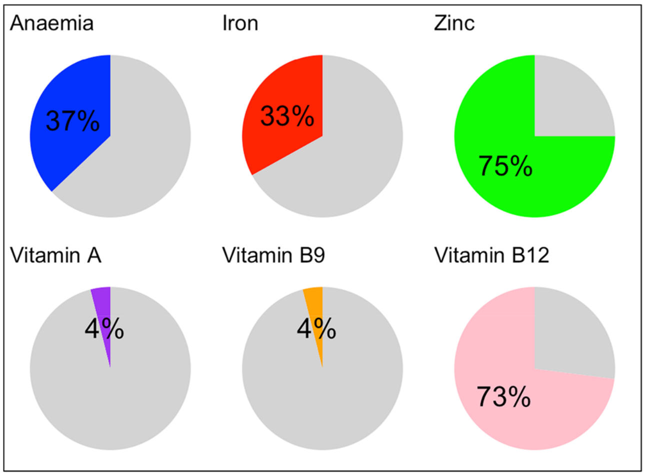

FIGURE 3 | Prevalence of micronutrient deficiencies among study participants.

4 | Discussion

This study aimed at exploring the prevalence of key micronutrient deficiencies and their associations with aflatoxin exposure among children aged 6–24 months, the first of its kind in Tanzania. The current study found that more than two-thirds of children were exposed to aflatoxin and deficient in zinc and vitamin B12, indicating the significance to public health issues. In addition, the study found more than one-third of children were anaemic and iron deficient, while vitamins A and B9 were the least deficient micronutrients. Most importantly, we found a positive association between AF-alb levels and iron deficiency among children aged 6–24.

The high prevalence of zinc deficiency observed in our study was similar to findings from a randomized control trial in

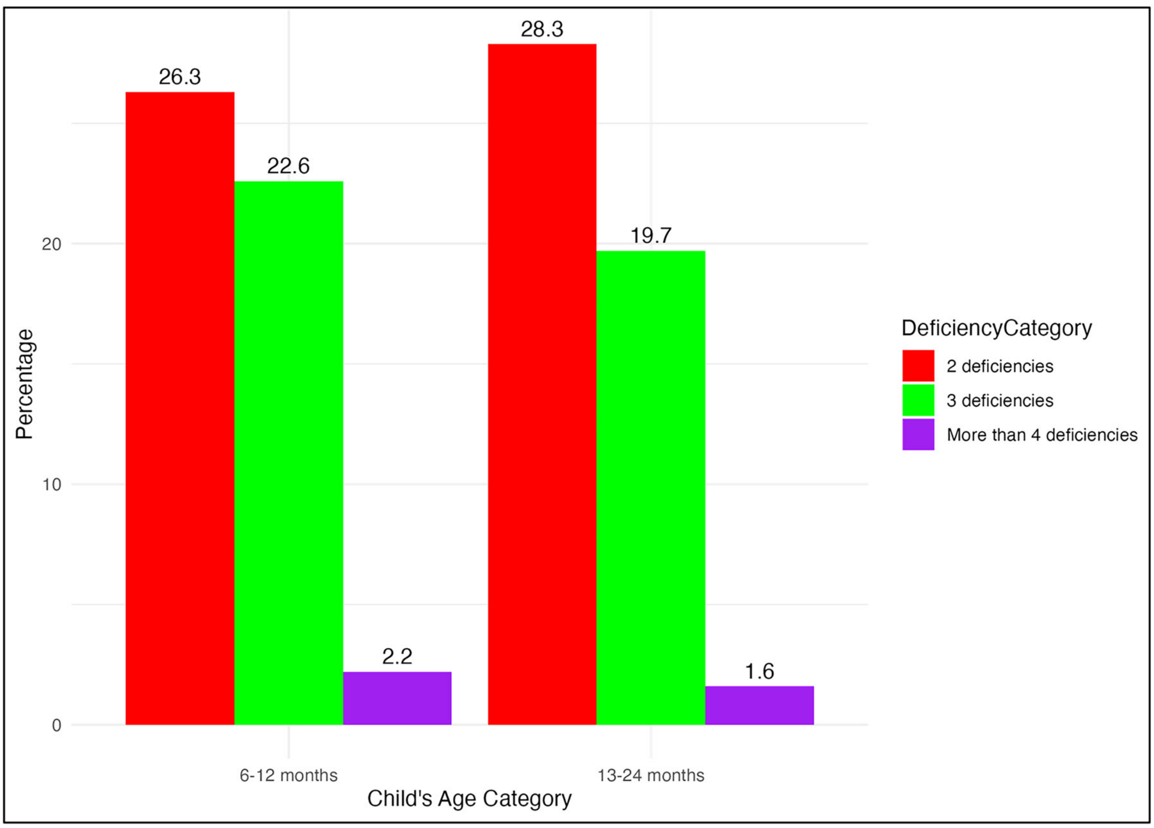

FIGURE 4 | Percentage of multiple micronutrients deficiencies by age group.

TABLE 2 | Association between aflatoxin exposure and micronutrient deficiencies among 6–24 months children.

Micronutrient deficiency	OR	95% CI	*p*-value
Anaemia	0.95	0.80, 1.14	0.487
Iron deficiency	1.22	1.01, 1.46	**0.04***
Zinc deficiency	1.02	0.83, 1.25	0.86
Vitamin A deficiency	0.95	0.76, 1.19	0.68
Vitamin B9 deficiency	1.83	0.15, 1.91	0.21
Vitamin B12 deficiency	1.01	0.82, 1.27	0.86

OR: Odds Ratio.
*Statistically significant if $p < 0.05$.

TABLE 3 | Adjusted odds ratio for factors associated with micronutrient deficiencies among children aged 6–24 months.

	Iron deficiency		Anaemia		Zinc deficiency		Vitamin B12 deficiency	
Variables	AOR (95% CI)	PV	AOR (95% CI)	PV	AOR (95% CI)	PV	AOR (95% CI)	PV
AF-alb adducts	1.40 (1.11, 1.74)	**0.01***	0.80 (0.62, 1.01)	0.07	1.10 (0.82,1.40)	0.66	1.02 (0.79, 1.33)	0.87
Child's age	0.90 (0.90, 1.07)	0.71	1.10 (1.01, 1.20)	**0.04***	0.90 (0.82,0.99)	**0.04***	0.95 (0.86, 1.04)	0.25
Gender (default Male)								
Female	1.54 (0.82, 2.90)	0.17	2.82 (1.49, 5.44)	**0.01***	0.71 (0.34,1.48)	0.37	0.46 (0.23, 0.92)	**0.03***
Breastfeeding status (default yes [continue breastfeeding])								
No (stopped)	1.73 (0.84–3.57)	0.13	0.42 (0.17–1.05)	0.05	0.57 (0.26–1.19)	0.15	0.59 (0.26–1.38)	0.21
Stunting (default non-stunting)								
Stunting	0.96 (0.42, 2.18)	0.92	1.15 (0.51, 2.57)	0.73	1.46 (0.59, 3.69)	0.41	2.54 (1.04, 6.42)	**0.04***

AOR, adjusted odds ratio; PV, *p*-value.
Adjusted for age in months, gender, breastfeeding status, dietary intake, stunting, wasting, underweight and CRP.
*Statistically significant if $p < 0.05$.

Tanzania, which reported a prevalence of zinc deficiency at baseline (67%) among under-five children in Handeni district (Veenemans et al. 2011). In addition, the high prevalence of zinc deficiency was also reported in other sub-Saharan African countries, i.e., Kenya (82%) and Malawi (60%) in under-five children (Gupta et al. 2020). For the case of vitamin B12, the high prevalence observed in our study was similarly reported among children aged 1–12 years (65%) in India (Umasanker et al. 2020). The Tanzania food composition table highlights foods of animals original, such as fish, beef, chicken liver as foods with high amount of vitamin B12 ranging from 3 to 7.3 mg per 100 g and zinc ranging from 4 to 5 mg per 100 g (Lukmanji et al. 2008). Therefore, the reason for the high prevalence of deficiencies in vitamin B12 and zinc might be due to high intake of plant-based complementary foods (which are low in zinc and vitamin B12) and poor dietary diversification, as reported in our previous observation in the same study participants (Mshanga et al. 2025b).

The prevalence of anaemia observed in our study was low as compared to the existing data for the Manyara region (46%) and in Tanzania (59%) among under-five children as reported by the Tanzania Demographic Health Survey and Malaria Indicator Survey Report of 2022 (Tanzania Demographic and Health Survey and Malaria Indicator Survey TDHS-MIS. 2022). The reason for the observed low prevalence may be explained by smaller population studies as only two out of six districts in the Manyara region were assessed. On the other hand, the current study reported a higher prevalence of iron deficiency as compared to another study that was done among under-five children in the Kilimanjaro region, Tanzanian (Kessy et al. 2019). This might be due to the difference in the methods used to estimate iron deficiency. In this study, we adjusted for ferritin levels that might arise due to inflammation, while in Kessy et al.'s study, this adjustment was not done. Given that inflammation tends to increase ferritin levels even in situations with iron depletion (Kernan and Carcillo 2017), this might have resulted in having the lower prevalence in the study by Kessy et al. (2019) compared to the current study. Although our study was conducted in a pastoral society, it was expected to have a low prevalence of anaemia and iron deficiency due to the intake of iron-rich foods such as meat. However, our previous study (Mshanga et al. 2025b), observed a high consumption of complementary foods made of maize and high intake of cow's milk in our current study participants. This might explain the high prevalence of anaemia and iron deficiency since maize food is low in iron (Piskin et al. 2022) and cow's milk has high calcium, a nutrient that inhibits iron absorption (Lönnerdal 2010). In addition, maize is rich in phytates, a compound that inhibits iron and zinc absorption (Al Hasan et al. 2016), which could also contribute to the high prevalence of iron and zinc deficiencies observed in the current study.

Compared with the reported AF-alb level in previous studies in Tanzanian children (Shirima et al. 2013, 2015), children in this study had similar level of aflatoxin exposure; thus, in the previous study, the AF-alb detection was 67% with a geometric mean of 4.7 (95% CI: 3.9, 5.6) pg/mg. The reason for the slightly higher levels of aflatoxin exposure in our study is likely due to the customary use of maize-based complementary foods (maize is among the foods that are highly susceptible to aflatoxin contamination (Xu et al. 2018)) in the Manyara region. This is further supported by previous studies (Hanselman et al. 2018; Kamala et al. 2016), with the latter one reporting an outbreak of aflatoxin among children who consumed maize-based complementary food in the Manyara region. In this study, aflatoxin exposure did not significantly vary by child's age, gender, or location. Possibly this trend is due to the observed uniformity in consumption of maize-based complementary food among children, the narrow age range and the similarities in agriculture, food storage and handling practices in Hanang and Babati districts. The important finding of this study is that aflatoxin exposure was associated with iron deficiency. Although the mechanism behind the interaction between aflatoxin exposure and iron deficiency is little known, this is an important finding because it suggests that aflatoxin exposure can predispose children to iron deficiency. A study performed in poultry (Lanza et al. 1980) found that aflatoxin exposure can stimulate the production of hepcidin hormone, which can prevent the absorption of iron in the intestine and iron uptake from macrophages, resulting in iron deficiency. Due to limited evidence in humans and the potential health implications of this co-occurrence to children, we call for more studies with longitudinal design to assess this observed relationship between aflatoxin exposure and iron deficiency.

Although other micronutrients did not show a significant association with AF-alb adducts, other variables showed a significant association in various micronutrient deficiencies. For instance, we found that the odds of being anaemic are two times higher for a female child compared to a male child. However, this observation is inconsistent with findings from other studies in Kenya and Ethiopia, which reported higher odds of being anaemic among male than female children (Ngesa and Mwambi 2014; Mohammed et al. 2019). In Tanzanian pastoralist communities, such as the Maasai, iron-rich animal products like meat are often viewed as symbols of wealth and pride. These foods are primarily given to male children as a sign of respect and recognition of their future leadership roles within the community (Ndiku et al. 2011; Frumence et al. 2023). This cultural practice may significantly contribute to the lower prevalence of anaemia observed among male children. In addition, we found that the likelihood of being anaemic increases with age. A similar trend was reported by the Ethiopia Demographic Health Surveys (Mohammed et al. 2019). The possible explanation for this trend is that more iron is needed to support growth as the child ages (Chaparro 2008). Conversely, Hailu (2023) highlighted the increased odds of being anaemic in younger children from 11 East African countries.

The current study found that the odds of being zinc deficient decreased as the child's age increases. This is in line with a meta-analysis study that found a significant negative association between zinc deficiency and a child's age in studies with infants below 12 months old (Cai-Jin et al. 2021). Some of the subjects, in our study, were aged from 6 to 12 months, transitioning from exclusive breastfeeding to complementary feeding. The reason for the significant negative association between zinc deficiency and age might be due to the customary intake of plant-based complementary foods in many Tanzanian communities. Plan-based foods are low in zinc, hence increasing the risk for zinc deficiency (Kulwa et al. 2015; Vitta et al. 2016; Kinabo et al. 2017; Mollay et al. 2021). In addition, the dietary survey component of the current study revealed that most participants reported an increased consumption of complementary food made from maize (Mshanga et al. 2025b). However, we did not find a significant association between maize intake and zinc deficiency.

We found that stunted children were two times more likely to be vitamin B12 deficient than non-stunted children. Similar findings were reported in Brazil (Salvatte et al. 2023) and Nepal (Ng'eno et al. 2017). These studies found a significant positive association between stunting and vitamin B12 deficiency in children aged 6–59 months. Vitamin B12 is among the micronutrients supporting the synthesis of DNA and proteins crucial for growth and development (Shane and Stokstad 1985), hence, lack of it can result in poor growth/stunting in children. In addition, we observed an increased odds of having vitamin B12 deficiency in male children compared to their female counterparts. However, we could not find any published reports on the relationship between vitamin B12 and gender; therefore, we call for more research to assess this link.

Tanzania has implemented policies that require the fortification of wheat and maize flour with essential micronutrients, including iron, zinc and vitamins B9 and B12 (Tanzania Food and Drug Authority 2011). Additionally, the country has focused on the biofortification of maize and sweet potatoes with provitamin A, as well as beans with iron (MOA, 2020). However, in our study, few participants reported consuming fortified flours or foods (Mshanga et al. 2025b). This is likely because food fortification is mainly conducted by large-scale producers, and fortified products are primarily available in commercial markets (Kiwango et al. 2021). In contrast, most of our study participants were subsistence farmers who consumed maize, maize flour and beans from their own harvests, which are typically unfortified (MRASC, 2008; AASS, 2017). Only a few participants reported occasionally giving their children foods made from wheat flour, but this practice was infrequent (Mshanga et al. 2025b).

Previous studies have demonstrated that maize biofortified with provitamin A can help reduce both aflatoxin contamination and vitamin A deficiency (Suwarno et al. 2019; Mboup et al. 2024). Therefore, we recommend further research to assess the effectiveness of provitamin A maize in decreasing aflatoxin contamination and vitamin A deficiency in Tanzania. Such research is crucial for generating evidence to inform food-based strategies aimed at improving vitamin A status and reducing dietary aflatoxin exposure among vulnerable populations that heavily depend on maize.

Since this study did not evaluate the impact of any specific intervention to reduce anaemia/iron deficiency, we recommend a multifaceted approach to address the issue. One promising strategy is the use of multiple micronutrient powders (MNPs), which are widely available in local health clinics and have proven effective in reducing micronutrient deficiencies, particularly anaemia among children under five in Tanzanian pastoral communities

(Kejo et al. 2018). Additionally, strengthening maternal nutrition education, promoting dietary diversity and encouraging the inclusion of vitamin C-rich foods (such as oranges) in meals can help enhance iron absorption (Dewey 2007). Food processing techniques, such as germination and soaking of cereals used in complementary feeding (reduce phytates which inhibit iron absorption), have also shown a potential to enhance iron bioavailability and reduce iron deficiencies (Dewey 2007).

This study aimed to investigate the relationship between aflatoxin exposure and micronutrient deficiency. The use of biomarkers in assessing micronutrient and aflatoxin exposure status in young children has been one of the strengths of this study, as it presents a more accurate exposure assessment of children compared to studies that used dietary intake or aflatoxin exposure estimations based on food intake and contamination. Additionally, the current study presents the prevalence of multiple micronutrient deficiencies among children in Tanzania. There were limited reports on multiple micronutrient deficiencies in Tanzania; the commonly reported micronutrient deficiency in Tanzania is anaemia. Although all samples were collected in the morning to minimize diurnal variation and we used the recommended cutoff point for non-fasting, morning samples (< 9.9 μmol/L), the lack of fasting may still have introduced minor variability in serum zinc concentrations. Another limitation of this study is its cross-sectional design, which may have missed seasonal variations in micronutrient status and aflatoxin exposure. Since the current study was conducted during the dry season, the observed deficiencies and exposure levels may be higher than those found in studies conducted during other seasons (e.g., rain season). Though the prevalence of malaria infection among children in the Manyara region is less than 1% (Tanzania Demographic and Health Survey and Malaria Indicator Survey TDHS-MIS. 2022), the current study did not directly assess infections such as malaria and worm infections, which are known confounders of micronutrient deficiencies. To minimize potential confounding, we assessed whether children had received key health services, i.e., deworming tablets to reduce worm infections and multiple immunizations, within the 3 months preceding the interview. In addition, we cross-checked with local health officers in the study areas and mothers to confirm on the occurrence of malaria cases, however, none of the doctors/mothers reported any malaria cases during the data collection period and 3 months prior data collection.

5 | Conclusion

The current study revealed that children with high levels of AF-alb adducts were 40% more likely to be iron deficient than those with low AF-alb levels. Furthermore, the study identified factors such as gender, child's age and stunting to be associated with anaemia and deficiencies in zinc and vitamin B12. These findings are primarily intended to be applicable to the Hanang and Babati districts or to other aflatoxin-affected areas with similar socioeconomic and environmental conditions. However, generalizability to other regions, the broader national context, or different age groups may be limited.

Since this cross-sectional study provides a snapshot of the health conditions at a single point in time but does not establish causality, further research should focus on longitudinal studies to further investigate the causal effect relationships and long-term impacts of aflatoxin exposure and micronutrient deficiencies on health outcomes across different populations, which will allow for the development of comprehensive strategies for improving public health in Tanzania.

Moreover, while the Tanzania National Multisectoral Nutrition Action Plan recommends the routine provision of MNPs, vitamin A supplementation, deworming tablets and the use of iodized salt for children under five during clinic visits, the current study found limited uptake of these interventions. Participants reported receiving vitamin A drops and deworming tablets approximately every 6 months and reported using iodized salt. However, none reported the use of MNPs, highlighting a critical gap in the implementation or community-level awareness of this key intervention.

Author Contributions

Naelijwa Mshanga: study curation, data collection, laboratory analysis, statistical analysis, prepared the manuscript drafts. **Suchaya Sonto:** laboratory analysis. **Neema Kassim, Haikael D. Martin, Sally Moore, Monica Pirani, Carolyn I. Auma, Martin Kimanya:** reviewed all the manuscripts drafts. **Yun Yun Gong:** supervised all the laboratory and statistical analyses and reviewed all the manuscript drafts.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.