The effects of aflatoxin exposure on Hepatitis B-vaccine induced immunity in Kenyan children

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Background: Globally, approximately three million children die each year from vaccine preventable infectious diseases mainly in developing countries. Despite the success of the expanded immunization program, not all infants and children around the world develop the same protective immune response to the same vaccine. A vaccine must induce a response over the basal immune response that may be driven by population-specific, environmental or socio-economic factors. Mycotoxins like aflatoxins are immune suppressants that are confirmed to interfere with both cell-mediated and acquired immunity. The mechanism of aflatoxin toxicity is through the binding of the bio-activated AFB_1-8, 9-epoxide to cellular macromolecules. *Methods*: We studied Hepatitis B surface antibodies [anti-HBs] levels to explore the immune modulation effects of dietary exposure to aflatoxins in children aged between one and fourteen years in Kenya. Hepatitis B vaccine was introduced for routine administration for Kenyan infants in November 2001. To assess the effects of aflatoxin on immunogenicity of childhood vaccines Aflatoxin B_1-lysine in blood serum samples were determined using High Performance Liquid Chromatography with Fluorescence detection while anti-HBs were measured using Bio-ELISA anti-HBs kit. *Results:* The mean ± SD of AFB_1-lysine adducts in our study population was 45.38 ± 87.03 pg/mg of albumin while the geometric mean was 20.40 pg/mg. The distribution of AFB_1-lysine adducts was skewed to the right. Only 98/205 (47.8%) of the study population tested positive for Hepatitis B surface antibodies. From regression analysis, we noted that for every unit rise in serum aflatoxin level, anti-HBs dropped by 0.91 mIU/ml (−0.9110038; 95% C.I −1.604948, −0.21706). *Conclusion:* Despite high coverage of routine immunization, less than half of the study population had developed immunity to HepB. Exposure to aflatoxin was high and weakly associated with low anti-HBs antibodies. These findings highlight a potentially significant role for environmental factors that may contribute to vaccine effectiveness warranting further research.

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Introduction

The World Health Organization [WHO] recommended "Expanded Program on Immunization" [EPI], has been one of the most cost-effective public health interventions in history.[1] This is exemplified by the eradication of smallpox, significantly lowering the prevalence of poliomyelitis and the dramatic reduction in morbidity and mortality from several other illnesses such as measles, rotavirus infection and tetanus.[2] Although a great deal has been achieved in diagnosis and treatment of many medical conditions, emerging and re-emerging infectious diseases remain a major threat to global health, causing severe morbidity and mortality worldwide.[2,3] To address these diseases, the World Health Organization put in place the Global Vaccine Action Plan 2011–2020 (WHO-GVAP) that was endorsed by 194 countries. This plan aims at strengthening current routine immunization to accelerate control of vaccine preventable diseases by introducing new and

improved vaccines and spurring development of the next generation of vaccines and technologies.[1]

Unfortunately, approximately three million children die each year of infectious diseases easily preventable with currently available vaccines globally.[4] Failure of vaccines to prevent infections and/or diseases may be due to suboptimal vaccine coverage, breakdown in cold chain of vaccine storage and delivery. In Kenya, diminished vaccine effectiveness is suspected to be partly due to the fact that the Kenyan population differs from the populations studied in the original vaccine clinical trials.[4] Furthermore, many infants do not receive recommended vaccines either on time or the required number of doses to provide optimal protection. Moreover, not all infants around the world develop the same protective immune response to the same vaccine. To tackle these issues requires identification of age and population-optimized vaccine schedules and formulations that can only be developed through research aimed at understanding the reasons why some children do not mount an efficacious immune response following vaccination.

An ideal vaccine must be able to induce a response over the basal immune response that may be largely driven by environmental and other population specific and socioeconomic factors. The importance of environmental factors modulating immunity is most readily recognized in early life, a period of rapidly changing environments.[5] Understanding the environmental engines that drive development and evolution of the immune system naturally and in response to childhood vaccines is not only necessary to address specific pediatric diseases but also to identify the strategies to change trajectories toward long-term, life-long protection from disease.[6]

There is emerging evidence of direct effects of mycotoxins to the immune system.

Malnutrition,[7] Soil-Transmitted Helminth infections,[8] early microbial exposure[9,10] and exposure to mycotoxins through the diet are few environmental factors that must be taken into consideration as they are likely to have an impact on the overall vaccine induced immunological response.[11]

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Epidemiological studies have shown that exposure to mycotoxins such as aflatoxins, fumonisins, deoxynivalenol, zearalenone and ochratoxin have significant negative health impacts on pediatric population.[12–15] Mycotoxins are naturally occurring fungal metabolites produced by filamentous fungi and commonly contaminate food supplies worldwide. Among the over 300 identified mycotoxins, aflatoxins are considered the most toxic as they are confirmed immune suppressants, and Group I carcinogens besides interfering with growth and development in children.[13,16] Moreover, aflatoxins are teratogenic, mutagenic and hepatotoxic.[16,17] The mechanism of aflatoxin toxicity is linked to the binding of the bio-activated AFB_1-8, 9-epoxide to cellular macromolecules.[18] Since the discovery of aflatoxins in the 1960s, exposure to high levels of aflatoxins in the diet have resulted in over 600 deaths in the Kenyan population.[19] The effects of acute exposure to high levels of aflatoxins came to the fore in Kenya following major outbreaks in 1981 and 2004.[20,21]

Deaths from consumption of aflatoxin contaminated grains has also been reported in India.[22] The United States Food and Drug Administration [USFDA] started monitoring aflatoxin levels in food supplies in 1969, concluding that they are unavoidable contaminant and recommended guidelines of 20 μg/kg for all foods and animal feeds.[23]

In addition to the known genotoxic and carcinogenic effects of mycotoxins, there is emerging evidence of direct effects of mycotoxins to the immune system.

Immune responses occur by macromolecular synthesis and cellular proliferation and this gives us an indication of how mycotoxins can cause immune-toxicity.[24] A major effect of aflatoxin exposure is the suppression of cell-mediated immunity (CMI).[24–26] Modulation by mycotoxins also increases the susceptibility to bacterial and parasitic infections that adversely affects acquired immunity, as evidenced following experimental challenge with infectious agents after vaccination.[24] Biologically reactive mycotoxins tend to inhibit protein synthesis or cell multiplication.[13] Ultimately, in order to bridge the gap of knowledge and understanding of vaccine immune

responses, it will be important to assess the possible role of environmental and nutritional factors that affect the general vaccine immune responses.

The interaction between Hepatitis B [HB] virus infection, and exposure to aflatoxins through the diet are confirmed to synergistically increase the risk of developing chronic liver diseases and hepatocellular carcinoma.[27–30] Hepatitis B vaccine was introduced for routine administration for Kenyan infants in November 2001. In this study, we selected anti-HBs antibody test to explore the immune modulation effects of dietary exposure to aflatoxins in children aged between one and fourteen years. Assessment of immune memory and measurement of Hepatitis B antibody, is key in assessing immune competence.[31] Hepatitis B vaccine was selected as the vaccine for exploration of the effects of aflatoxin on immune response due to the known association between hepatitis B infection and chronic aflatoxin exposure in the pathogenesis of chronic liver disease. The use of Hepatitis B vaccine has increased the usefulness of anti-HBs determination as a tool in monitoring seroconversion after immunization.

Generating vaccine-mediated immune protection is a complex challenge. Currently available vaccines have largely been developed empirically; their early protective efficacy is primarily conferred by the induction of antigen-specific antibodies. The quality of such antibody responses, specifically their avidity has been identified as a determining factor of efficacy. However, there is more to antibody-mediated protection than the peak vaccine-induced antibody titers. In addition, long-term protection requires the persistence of vaccine antibodies and/or the generation of immune memory cells capable of rapid and effective reactivation upon subsequent microbial exposure.[32,33] The determinants of immune memory induction, as well as the relative contribution of persisting antibodies and of immune memory to protection against specific diseases, are thus essential parameters of long-term vaccine efficacy.[31,34] The overall objective of this study was to estimate the prevalence and determine the clinical and immunological correlates of aflatoxin exposure among children aged between 1 and 14 years in Muuni sub-location, Makueni County in Kenya.

Methods

Setting of the study

Makueni County lies in the South-Eastern part of Kenya, approximately 180 km from the capital city Nairobi. Almost 80% of households rely on agriculture as the main source of income. The main subsistence crops include maize, sweet potatoes, cassava, peas, beans, sorghum and millet. Temperatures range from 18° to 33 °C which is prime for aflatoxin generating fungi to thrive. The study area, Muuni sub-location, is centrally located in Makueni as shown in Fig. 1. During the 2009 census, it was projected that the population of the area would reach 939,879 by 2014, an annual population growth rate of about 1.23%.[35] Makueni's population density is estimated at 110.4 people per km^2, against a country average of 65.3 people per km^2. It also has high fertility, which is currently 5.1 children per woman, compared to a national average of 4.6 children per woman.[36]

Study design and human participants

The study design is cross sectional. The Joint Ethics Committee of the University of Nairobi and Kenyatta National Hospital in Kenya approved the research protocol. Between July and December 2016, 930 households were randomly sampled in Muuni sub-location. 433 eligible households with children between the ages of 1−14 years were selected. Inclusion criteria were healthy children of both sexes, aged 1−14 years, availability of vaccination records, and the ability of parents or guardian to provide informed consent. When more than one child was eligible in a household, only one was selected randomly using Kish Grid Method.[37]

Recruitment of study subjects is summarized in Figure 2.

Subject recruitment and study procedure is shown in Figure 2 where TBC refers to total blood cell count, Anti HBS refers to measurement of hepatitis B surface antibodies, cytokines refers to a group of proteins secreted by cells of the immune system that act as chemical messengers. LFTs refers to liver function tests, AFB1 refers to measurement of aflatoxin B1 lysine adducts in serum, AFB2 refers to measurement of aflatoxin in grain, urinary fumonisins refer to measurement of sphingolipids in urinary cells. For each analysis, the number of samples analysed is shown beneath. For example, Anti-HBS N=205 indicates that we measured hepatitis B surface antibodies in 205 samples.

Makueni County performs poorly on most socio-economic indicators. The county scores a 0.48 on the Human Development Index (HDI)—a composite measure of development that combines indicators of life expectancy, educational attainment and income. This

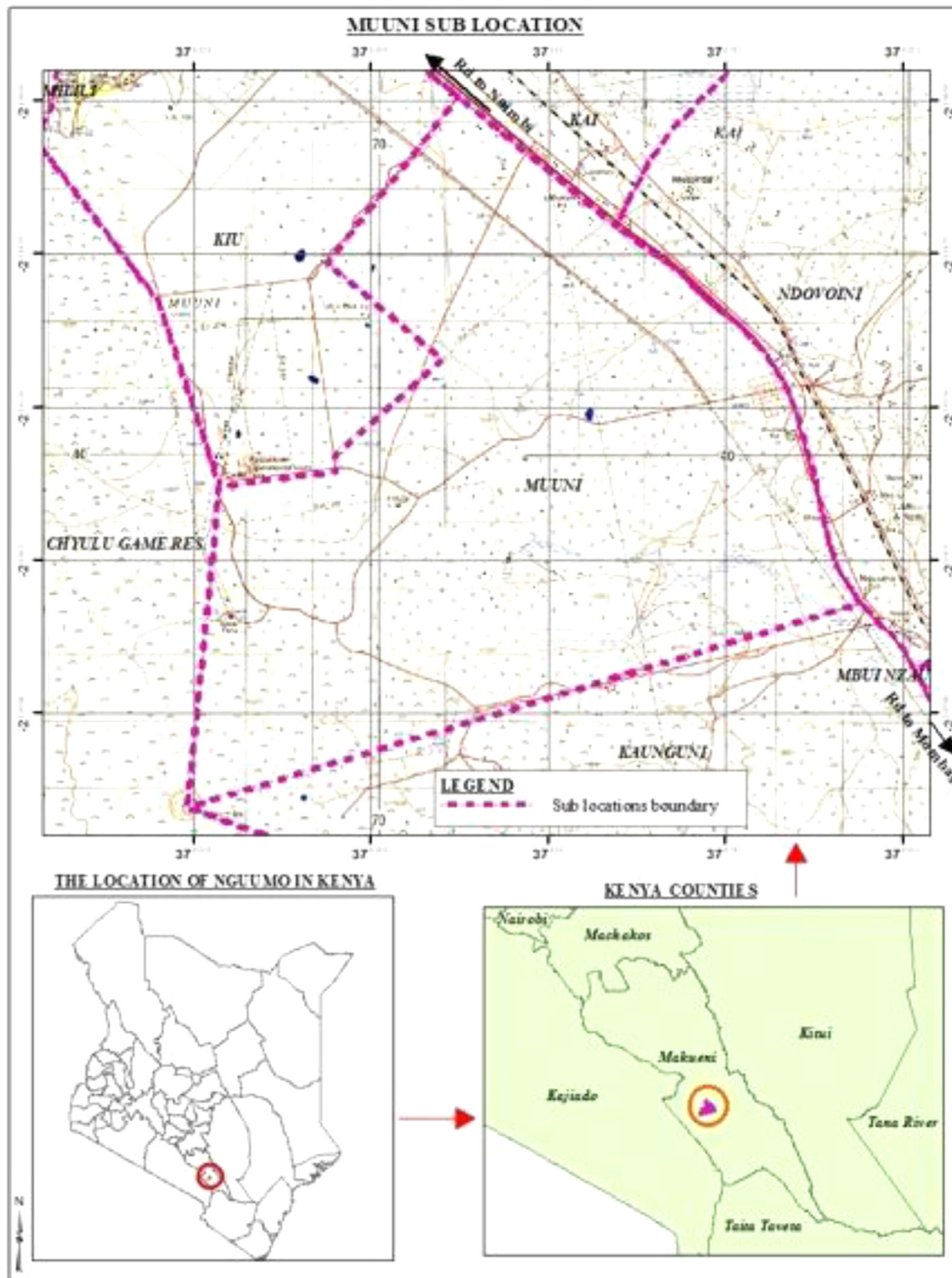


FIG 1. Map of Muuni sub-location. Bottom right panel: its position within Makueni county. Bottom left panel: Makueni County in Kenya.

is against a national average of 0.52.[36] Poverty is prevalent in the county and manifests itself in other socio-economic outcomes such as poor nutrition, health, and education, as well as a lack of access to basic services. Unemployment is a major challenge in the county, especially among youth. The majority of the population is employed in agricultural activities, with limited opportunities in commercial ventures and public service. The County ranks 9th out of 47 in poverty levels, with 60% of the residents living below poverty level.

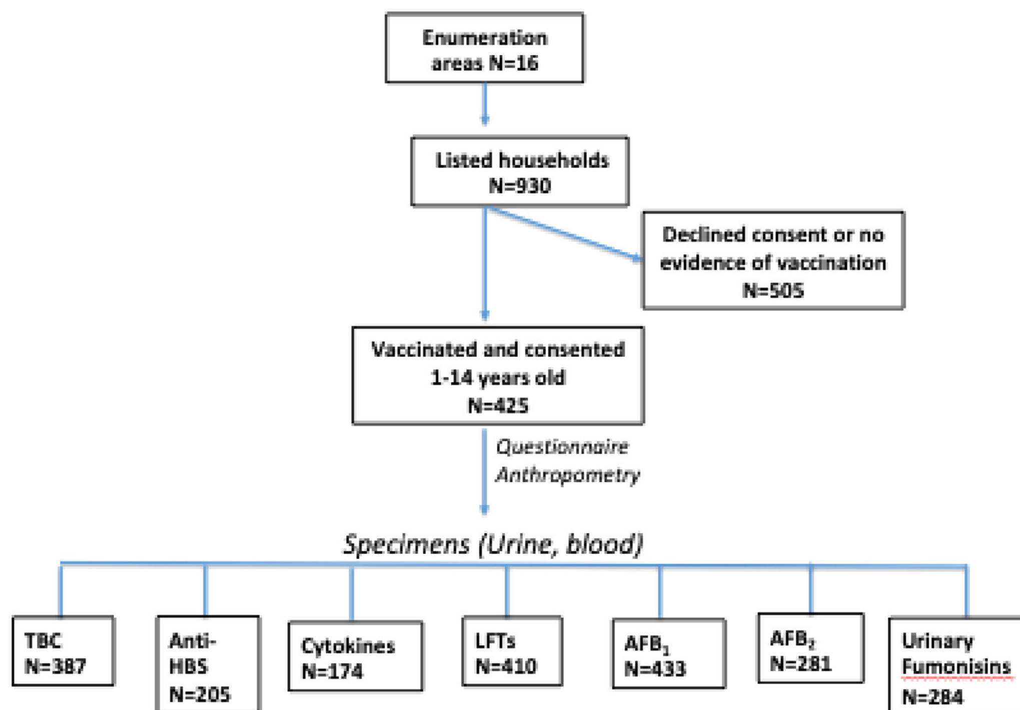


FIG 2. Subject recruitment and study procedures.

Children aged 1−14 years in Muuni sub-location were the focus of this study due to their particular vulnerability and susceptibility to the effects of aflatoxin exposure.

Study procedures

The study objectives and purposes were explained to the community in local dialect before recruitment. Parents or guardians who agreed to study procedures provided written consent and completed a brief questionnaire on socio-economic indicators. Approximately 15 ml of urine, 5−8 ml of blood and 10 g of stool specimens were collected from children in the presence of parents or guardians who had provided informed consent. Anthropometric measures including, height, weight and mid-upper arm circumference were also taken. The questionnaire on socio-economic status data entry was done using CSEntry for Android application. Total blood count and stool samples were examined in the field shortly after collection. All other samples were placed in dry ice for transportation to the University of Nairobi KAVI-Institute of Clinical Research (KAVI-ICR) laboratories for analysis. The serum samples for aflatoxin levels and urine for urinary sphingolipid assessments were airfreighted in dry ice to Professor Jia-Sheng Wang's laboratory at the University of Georgia in Athens USA for analysis.

Laboratory analyses

Measurement of Hepatitis B surface antibodies

Hepatitis B surface antibodies (anti-HBs) were measured using Bio-Elisa anti-HBs kit according to the manufacturer's instructions. Bio-ELISA anti-HBs provides a direct immune-enzymatic method of the "sandwich" type in which the samples to be analyzed were incubated in wells of a microplate that were coated with highly purified HBsAg. Anti-HBs antibody assays were performed on 205 randomly selected samples.

Cytokines analysis

Cytokine analysis was accomplished by Bio-Plex Pro^{TM} Cytokine, Chemokine and Growth factor Assay kit. This assay provides a robust immunoassay test that enabled the quantification of cytokines in a single well in 3−4 h from a small volume of serum. One

hundred and seventy-four randomly selected serum samples were analyzed for Interleukin 2, 4, 6, 8,10, TNF-alpha, GM-CSF and IFN-gamma. This multiplex system uses xMAP technology on the MAGPIX^{TM} system.

Measurement of AFB_1-lysine adducts

Aflatoxin blood levels defined as Serum aflatoxin B_1 lysine adducts were analyzed using High-Performance Liquid Chromatography with Fluorescence Detection [HPLC−FD.] Detailed methodology is provided in a paper by Qian et al.[38]

Statistical analysis

STATA software was used for data analysis (StataCorp. 2017 Release 15.1). Continuous data with skewed distributions were log transformed prior to analyses. After data quality checks were done, descriptive analysis, univariate associations and multivariate logistic regression model with hepatitis B antibody levels as the outcome variable were done. Independent variables selected for inclusion in the final model were those that were significant at $p < 0.05$ in the univariate analyses.

We then assessed the association between aflatoxin blood levels with selected immune markers in serum samples collected from children aged between 1 and 14 years and that of aflatoxin levels on antibody response to Hepatitis B vaccine. Anti-HBs of 10 IU/L is the level of antibodies considered to give sero-protection.[34] In the analysis of this data, all study participants who had serum with Anti-HBs below 10 IU/L were considered to lack sero-protection against Hepatitis B infection. Due to logistical constraints, the rest of the serum samples were stowed away for analysis in future.

For purposes of analysis, aflatoxin blood levels herein referred to as AFB_1-lysine adducts level was dichotomized to define 'low' and 'high' based on the median of 19.98 pg/mg as cutoff due to the high variation. The median was chosen because the data was skewed to the right. This median level is selected to create a dichotomous variable of 'low' and 'high' aflatoxin level for purposes of exploring associations of aflatoxin blood levels and other factors of interest we may wish to study. To ascertain that selection bias did not influence the choice of who was tested, we compared age, gender, weight-for-age *Z*-score, BMI-for-age *Z*-score, height-for-age *Z*-score, Wealth Index and serum aflatoxin levels (AFB1) between the 2 groups.

Selection of variables for inclusion in logistic regression model

In order to select candidate variables for multivariate modeling, univariate logistic regression of the independent variable, aflatoxin blood levels, was carried out separately on individual variables. For each independent variable, the odds ratio, the 95% confidence interval and the *p*-value was reported. For variables with more than two groups, one-way analysis of variance statistical procedure (ANOVA) was carried out to compare the mean differences between the levels of anti-HBs. For each test, Bartlett's test for equal variances was carried to verify the validity of analysis of variance.

Results

We enrolled 433 children between the ages of 1−14 years. After data cleaning, 409 study participants had complete data available for analysis. The 24 samples were left out due to reasons that ranged from inadequacy of the sample volumes to run the other tests (n = 14), mislabeling (n = 7) and some samples were analyzed in duplicates (n = 3).

There were 204 males and 205 females with the mean age of participants was 8.07 ± 3.54 years. One hundred and five children (26%) of the children were under 5 years. The mean ± SD age of mothers or guardians who provided consent and were available when data collection took place was 37.32 ± 10.78. Children in this study were served on average about 3 meals per day 2.88 ± 0.62 with one meal constituted of maize based breakfast meal porridge. Majority of the children (357/433) or 82.4% of the parents in the study reported serving their children porridge. Characteristics of study participants and summary results (Table 1).

Due to logistical constraints, only 205 serum samples were selected for measurement of anti-HBs. Of the tested 205 children, only 98 (47.8%) tested positive for anti-HBs screening test. Of the 98 positive cases, four samples had anti HBs concentration of less than 10 IU/L and were therefore considered negative for purposes of further analysis. The range of anti-HBs level was 3-7833.8 IU/L while the mean ± SD is 543.7 ± 1375 IU/L.

TABLE 1. Characteristics of study participants and summary results.

Overall	*N* = 409[a]
Age	
< 5 years	105/409 (26%)
>5years	304/409 (74%)
Sex	
Males	204 (49%)
Females	205(51%)
[b]Children tested for anti-HBs antibodies	*n* = 205
• Positive [>10 mIU/ml]	98/205 [48%]
• Negative	107/205 [52%]
Anti-HBs levels Range	3–7833.8 mIU/L
Anti-HBs (mean (SD))	543.7 (1375) (mIU/ml
Age in Years (mean (SD))	8.07 (3.54) (yrs)
Height (mean (SD))	122.00 (24.73) (cms)
Weight (mean (SD))	23.71 (8.79) (kgs)
Growth patterns	
Stunted	67/420 (15.95%)
Not stunted	353/420 (84.05%)
Underweight	32/298 (10.74%)
Normal weight	266/298 (89.26%)
Wasted	5/105 (4.76%)
Not wasted	100/105 (95.24%)
Albumin g/dL (mean (SD))	4.88 (0.42) g/dL
Number of Meals (mean (SD))	2.88 (0.62)
Porridge (%)	
No	68 (15.7)
Yes	357 (82.4)
Non-specified	8 (1.8)
Aflatoxin blood levels	pg/mg of albumin
(Mean (SD))	44.94 (85.27) “
Geometric Mean	20.40 “
Median	19.98 “
Range	0.74–901.15 “
95% Confidence Interval	18.22–22.85 “
Grain aflatoxin levels μg/kg (mean (SD))	38.93 (184.69) ppb
Age of the mother (mean (SD))	37.32 (10.78) (yrs)
Dietary Diversity Score (mean (SD))	3.88 (2.05)

a433 children were enrolled in the study but only 409 samples had complete data for analysis.

b205 samples were used in Anti-HBs analysis due to logistical constraints.

All serum samples analyzed for aflatoxin blood levels had detectable levels. The limit of detection by the HPLC with fluorescence detection was 0.4 pg/mg of albumin. The lowest value of aflatoxin blood levels level recorded was 0.74 pg/mg of albumin and the highest was 901.15 pg/mg of albumin. The mean $\pm$ SD of AFB_1-lysine adducts in our study population is 44.94 $\pm$ 85.27 pg/mg of albumin. The geometric mean however was 20.40 pg/mg of albumin. The distribution of AFB_1-lysine adducts was skewed to the right, indicating that most of the children had levels below the median level of 20.4 pg/mg of albumin.

The mean height and weight were 122.00 $\pm$ 24.73 centimeters and 23.71 $\pm$ 8.79 kilograms respectively. Sixty seven out of 420 children (16%) in the study population were considered stunted, five of 105 less than (5%) wasted and 32/298 (11%) were underweight. Wasting ($p < 0.03$), dietary diversity score ($p < 0.02$) and total white cell counts ($p < 0.04$) were associated with decreased levels of anti-HBs in univariate models. We compared mean differences in anti-HBs in age bands of under 5 years, 5–8 years and 9–14 years using analysis of variance. Using the Bartlett's test for equal variances was carried to verify validity of analysis of variance, there were significant differences for age groups ($p < 0.04$) and dietary diversity score ($p < 0.005$).

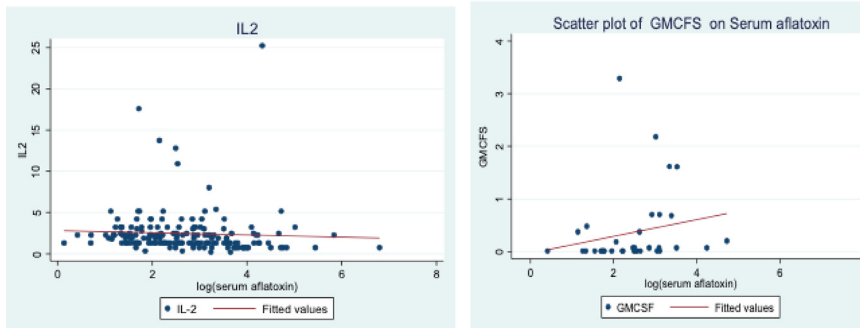
GM-CSF was only detectable in 36 (21%), IFN-gamma in 123 (71%), IL-4 in 146 (84%) and IL-10 in 156 (90%). For the other 4 cytokines (IL2, IL-6, IL-8 and TNF-alpha) were detectable in all the 173 samples that were analyzed. All the cytokines showed a negative correlation with respect to aflatoxin blood levels except IL-10 that showed positive correlation with 90% level of significance.

The relationship between the cytokine and aflatoxin blood levels were explored using scatter plots as shown in Fig. 3. Most of the cytokines showed negative correlation with aflatoxin except for IL-10, TNF-alpha and perhaps GM-CSF. Specifically, a decreasing trend for IL2 was observed as aflatoxin blood levels increases while GM-CSF increased with increasing aflatoxin levels. IFN-gamma and TNF-alpha show a decreasing trend as aflatoxin blood level increases. These results were not statistically significant [p 0.695]. Overall, the scatter plots revealed a mixed trends suggesting need for further detailed analysis.

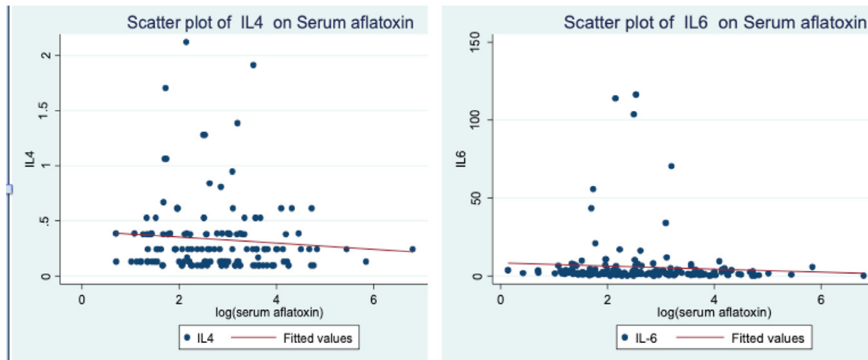
Cytokine profiles of IL-2. IL-6, IL-8 and TNF-α were compared against aflatoxin blood levels. The data suggest a tendency of depressed IL-4, IL-6 and IL-8 with up-regulation of TNF-alpha with high aflatoxin levels as shown in Fig. 4. None of the associations were significant when Mann Whitney test was run to compare medians.

Assessing determinants of low HepBs antibody levels

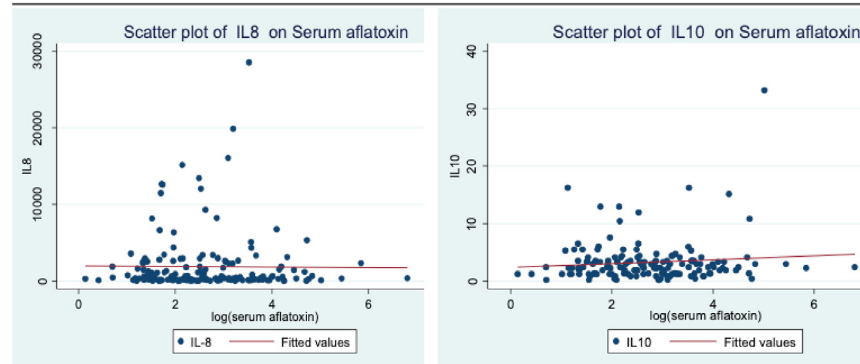
Variables included in the final fitted logistic regression model with 7 categories emerged. These were age (<5 and >/=5 years), gender, bmi-age-Z score and weight for height z-score which was defined as wasting and normal, aflatoxin blood levels (grouped high vs low), total white cell count, total protein and



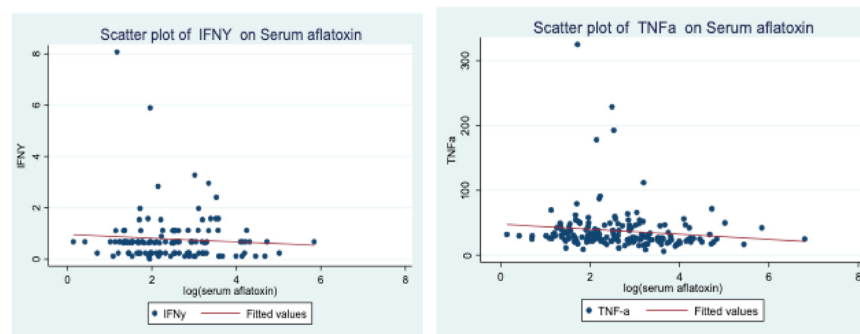
3a) Scatter plot of IL2 and GMCFS on Aflatoxin blood levels



3b) Scatter plot of IL4 and IL6 on Aflatoxin blood levels



3c) Scatter plot of IL8 and IL10 on Aflatoxin blood levels



3d) Scatter plot of IFNY and TNF-α on Aflatoxin blood levels

FIG 3. Scatterplots showing the relationship between aflatoxin blood levels and the different cytokines.

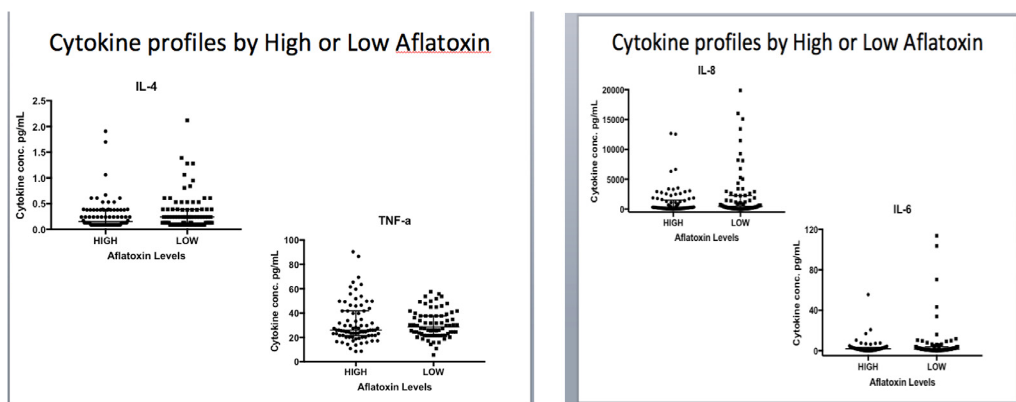


FIG 4. Cytokine profiles by high or low aflatoxin (IL4, TNF-a, IL8, IL4)

TABLE 2. Independent associations for low Hepatitis B antibody levels.

Characteristic	OR	95%CI	*P*
AFB1 high	**1.73**	**0.77–3.89**	**0.19**
Female	0.35	0.15–0.81	0.01
Age (Over 5)	3.56	1.34–9.43	0.01
Wealth index			
Middle	1.17	0.40–3.46	0.78
Poor	0.65	0.17–2.56	0.54
Total Protein	110.90	1.28–9603.96	0.04
Gamma GT	1.01	0.92–1.11	0.82
Total WBC	1.04	0.91–1.19	0.55
Hb	0.84	0.54–1.30	0.43
Wt for age Z score	1.35	0.37–4.95	0.65

wealth index-poor, middle class and wealthy-(Table 2). Serum AFB_1-lysine adducts was associated with increased odds of having low Hep B antibody levels after adjusting for age, sex, growth parameters, total protein, gamma GT, white blood cell count, hemoglobin levels and wealth index. The association was, however, not statistically significant (R 1.73; 95% CI 0.77–3.89; P = 0.19). Being a female child was associated with reduced odds of having low Hep B antibody levels and this association was statistically significant (OR 0.35; 95% CI 0.15–0.81; P = 0.01). Children aged above five years experienced a 3.5 fold increase in odds of having low Hep B antibody levels (OR 3.56; 95% CI 1.34–9.43; P = 0.01). In this analysis wealth index appeared not to be associated with Hep B antibody levels. Total protein level was weakly associated with low Hep B antibody levels (OR 110.9; 95% CI 1.28–9603.96; P = 0.04). Gamma GT, Total WBC count, hemoglobin and weight-for-age Z score were not associated with Hep B antibody levels.

Discussion

This study showed an association between high aflatoxin blood levels and low hepatitis B antibodies. Only 48% (98/205) of the study participants tested positive for HBs screening tests. Four of the samples did not achieve the protective threshold of 10 mIU/ml, the level considered to give protection.[39] This means that only 46% of the studied children can be considered to have sero-protection antibodies against hepatitis B infection as estimated by antibody levels.

Even though using antibodies as a measure of vaccine sero-protection is practical, it has some limitations. In a study by Liao et al, 46% of children vaccinated 5–7 years previously had titers below 10 mIU/ml.[40] When these children were given a booster dose, 90% showed evidence of an anamnestic response. The long incubation period of the disease has been reported to allow the anamnestic response to be highly protective.[41] In that study, Plotkin went further to suggest that $CD4^+$ responses are better correlates of protection than antibody titers.

Passively acquired maternal antibodies and hepatitis B infection are other two reasons that may affect levels of anti-HBs. None of the children in our study were less than a year old. The asymptomatic children were drawn from the community at the time of testing. Vaccine antibody responses elicited before 12 months of age has been shown to have rapid decline.[42,43] Van Damme et al reported low to undetectable levels of Hepatitis B antibodies in 15–50% of fully vaccinated patients 5–15 years post vaccination.[44] Our study selected children who had been vaccinated within the past one to thirteen years since the introduction of the hepatitis B vaccine in 2001.

That 54% of the children did not have protective antibodies in an area of high aflatoxin exposure, is a matter of public health concern given the compounding carcinogenic effects of hepatitis B infections and chronic low dose exposure to aflatoxin. Vaccine delivery-related issues like site and dose of vaccine, the cold chain integrity, timelines of vaccinations, were not considered in this study.

That 54% of the children did not have protective antibodies in an area of high aflatoxin exposure, is a matter of public health concern

Of the 433 children studied, AFB$_1$ was detected in all, ranging from 0.74 pg/mg albumin to 901.15 pg/mg albumin with a median of 19.62 pg/mg with a skew to the right. The geometric mean is 20.40 pg/mg. These results are comparable to Turner et al who reported a geometric mean adduct level of 22.3 pg/mg in children from the Gambia.[26] Aflatoxin blood levels are higher in children compared to adult populations. For example, Leroy et al reported median levels of 7.47 pg/mg albumin,[45] while Yard and colleagues reported a median level of 1.78 pg/mg albumin.[46] The overall higher level of AFB1-lysine in children reveals a greater sensitivity to aflatoxins possibly due to lower body mass, higher metabolic rate, and underdeveloped organ functions and detoxification mechanisms. Another study conducted in Nepal revealed median levels of 3.62 pg/mg of albumin.[47] This study reported that aflatoxin exposure during the first 36 months of life was not associated with impaired growth in children from Nepal. Nonetheless, it is worth noting that Nepal is geographically located between 1000 and 2000 m above sea level, with a generally cooler climate while aflatoxins thrive in hot and humid environments.

A trend of higher level of aflatoxin blood levels in the females compared with the male children was observed after adjusting across wealth index, however, this was not statistically significant.

The females in this study also had lower anti-Hbs antibody levels compared to the males (OR 0.35; 95% CI 0.15–0.81; P = 0.01). There is no clear indication whether this may be a biological association.

The females in this study also had lower anti-Hbs antibody levels compared to the males (OR 0.35; 95% CI 0.15–0.81; P = 0.01). There is no clear indication whether this may be a biological association.

It is known that there are sex differences in immune response that are not necessarily related to differences in pubertal hormone levels.[48–50] Nevertheless, this does not mean that this is a spurious occurrence; there may be a difference between the metabolism of aflatoxins in males compared to females. Moreover, it has been established that the reaction between males and females is different for vaccines way before the onset of hormonal differences.[41] Some genes that encode for immunological proteins are found in the X chromosome. This may lead to higher expression in females compared to males. This observation is likely plausible for aflatoxin exposure as well. Future studies may consider exploring if indeed there exists sex differences as regards aflatoxin exposure in pre-pubertal children.

There was a significant association between the level of serum aflatoxin and the socio-economic class. Wealth index predicted the likelihood of a child having high serum aflatoxin. Those in the middle socio-economic class had an 18.5% lower likelihood of having high aflatoxin compared to those in the lowest class. The children in the rich social class had 57.2% less likelihood of having high serum aflatoxin compared to those in the poor class. These results are in agreement with previous studies that have shown a significant association between poverty and aflatoxin exposure.[45]

In addition, this study showed a strong association between dietary diversity score and wealth score index. Whereas these findings may seem obvious, they point to the centrality of poverty in aflatoxin exposure and direct us to an important starting point, certainly at a policy level, in aflatoxin mitigation measures.

Malnutrition occurs when there is one or more of the following situations; wasting (<-2SD WHZ), underweight (<-2SD WAZ) or stunting (<-2SD HAZ). Aflatoxin impairs the intestinal barrier function resulting in mal-absorption and micronutrient deficiency.[13,51] Systemic immune activation following increased permeability of the enterocytes and inhibition of protein synthesis are other postulated mechanisms that lead to malnutrition.[52,53] Malnutrition as an independent factor is the biggest risk factor for global burden of disease as previously indicated

and by extension, one could infer that malnutrition has significant contribution to immunomodulation. Aflatoxin modulates infectious diseases as shown by a number of studies.[25,54,55]

In the presence of high prevalence of serum aflatoxin in vulnerable populations, infectious diseases are likely to continue contributing to our excess morbidity and mortality if we fail to intervene on upstream factors like ensuring safe food supplies from aflatoxin contamination.

In the presence of high prevalence of serum aflatoxin in vulnerable populations, infectious diseases are likely to continue contributing to our excess morbidity and mortality if we fail to intervene on upstream factors like ensuring safe food supplies from aflatoxin contamination.

Sixteen per cent of children in our study population were stunted compared to the national average of 26% reported in the Kenya Demographic Health Survey of 2014.[56] However, the national average assessed a younger age range of 18−23 months, while our study included children aged 12 months to pre-pubertal age of 14 years. This may suggest a decline in stunting rate as the children get older. Stunting indicates the effects of chronic food shortage and has serious implications especially for the first one thousand days. It is the most prevalent form of malnutrition in the world particularly in Sub-Saharan Africa.[57] It is worth noting that the 2014 KDHS anthropometric measurements were determined using data that were already 5 years old. Moreover, only 25% of our study population fell between the ages of 18−23 months. Any comparisons of malnutrition rate in the two groups may not be appropriate due to different age groups of the study population. There is also a possibility that food supplies may have improved among Kenyan households in 2016, when we conducted our fieldwork and data collection. Alternatively, the stunting rate of 16% found in this study is lower than the 26% of the national rate may suggest that Makueni County has had some useful interventions in the last 5 years.

Wasting generally suggests recent illness especially diarrhoea or rapid deterioration in food supplies. Wasting rates in this study was 5% against a national rate of 4% 2014 KDHS, with a higher prevalence among under one-year-olds in the national study. Our study excluded children under one year old therefore the wasting rates are incomparable. It should be noted that underweight reflects both effects of acute and chronic malnutrition status. The rate of underweight in our study was 11% similar to the 2014 KDHS national rate that showed that, Makueni County had registered a slightly lower underweight rate of 10.2%.

From the literature, we know that the levels of cytokines are usually low-to-undetectable in the absence of an appropriate trigger. Most of the cytokines showed negative correlation with aflatoxin except for IL-10, TNF-alpha and GM-CSF. In our analysis, aflatoxin levels were not significantly associated with the levels of any of the eight cytokines tested. All the cytokines showed a negative correlation with respect to serum aflatoxin. Dose-response relationships with varying levels of aflatoxin in peripheral blood mononuclear cells (PBMCs) would greatly aid our understanding of cytokine signature with regards to aflatoxin exposure.

The relationship between cytokine and aflatoxin blood levels was explored using scatter plots. A decreasing trend for IL2 was observed as serum aflatoxin increases while GM-CSF increased with increasing aflatoxin levels. IFN-gamma and TNF-alpha show a decreasing trend as serum aflatoxin increases. These results were not statistically significant. Overall, the scatter plots revealed a mixed trend that should prompt for further detailed analysis. The baseline cytokine data in this study was more difficult to interpret especially because the levels were very low or below limit of detection not to mention use of dichotomized levels of aflatoxin to define ‘low’ and ‘high’ based on the median in a very wide range of aflatoxin levels. A study using peripheral blood mononuclear cells (PBMCs) that are stimulated with varying levels of aflatoxin would greatly augment the study of relationships between cytokine responses with varying levels of serum aflatoxin levels. Taken together, the results we have from this study form a valuable baseline for future studies.

This study set out to estimate possible effects of aflatoxin exposure on immunity by measuring the levels of hepatitis B antibodies. While recognizing that not all measured antibodies are functional, quantifying hepatitis B antibodies is both practical and inexpensive compared to cell -mediated based tests which are best at giving conclusive estimations. Laboratory

equipment necessary for this immunoassay has a higher possibility of being found in an easily accessible reference laboratory. The measures of IgG subclass and antibody avidity are known to give a better indication of quality of the humoral response again barring the costs and complexity of the procedure. ‘In-house’ functional assays such as the neutralization assays used in assessing polio and measles antibody responses can provide additional information. These tests are however both laborious and difficult to standardize rendering them less useful to the clinician in a County hospital.

Vaccine-preventable conditions continue to cause unacceptably high mortality rates compared to countries with similar programs in the Northern hemisphere.

Three doses of Hepatitis B vaccines should achieve a protection rate of >90%.[58] Anti−HBs concentrations have been reported to rapidly decrease in the first year and more slowly thereafter but immune memory persists longer.[59] Expanded Programs on Immunizations have been in place for a long time in countries that contribute the highest child mortalities. That notwithstanding,

vaccine-preventable conditions continue to cause unacceptably high mortality rates compared to countries with similar programs in the Northern hemisphere.

This calls for urgent well-designed, adequately powered prospective studies to determine the effects of mycotoxins on robust clinical and immunological outcomes like IgG subclass, antibody avidity and cellular immune responses. This need is particularly crucial in the context of discussions on Sustainable Development Goals, Universal Health Coverage and the role for high impact interventions such as vaccines in achieving defined targets. The researchers of these studies must synthesize and present the results of these studies specifically for the political leadership who are pivotal in ensuring policy formulation and operationalization.

The outcomes of similar research may spur the scientific community interested in vaccines to re-evaluate the current vaccine scheduling to ensure it is informed by contextual data. Basic science research is increasingly experiencing difficulties in obtaining funding compared to operational research. One could make a case that the immunization programs take a considerable part of the health budget in low-income countries that stand to benefit most from tailor-made vaccination schedules. Many of the schedules included in the Expanded Program on Immunization were developed empirically with little understanding of the cellular immune responses in the early days. Malnutrition, poor sanitation and personal hygiene, overcrowding, gut microbiota, sex, genetic factors, contaminated food and water are non-vaccine factors that impact on effectiveness of vaccinations in developing economies.

Despite high coverage of routine immunization, less than half of the study population had developed immunity to Hepatitis B. Exposure to aflatoxin was high and weakly associated with low anti-HBs antibodies. This finding should spur further work across the region with high burden of vaccine-preventable diseases. The explanation of the inter-relationships of the various environmental factors would enrich our knowledge pool, further spurring the multi-disciplinary effort that is necessary and pivotal in our quest to address the resultant morbidity and mortality patterns in children. Taken together, this will be in line with Sustainable Development Goal policies that aim at ending preventable deaths of newborns and children under 5 years of age.

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