Survey of Urinary Aflatoxin Levels Among Residents of Makueni County, Kenya: A Follow-Up Study

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ABSTRACT

Although fungi are known to be less pathogenic and mostly saprophytic in their nature as compared to other groups of microbes, those that produce aflatoxin have been associated with severe human disease. An example of such disease is Aflatoxicosis caused by soil-borne pathogenic fungi of the species *Aspergillus parasiticus* and *Aspergillus flavus*. They produce a mycotoxin substance that is carcinogenic to the human liver with severe outcomes. The objective of this study was to determine urinary aflatoxin levels among the residents of Makueni County, previously affected by Aflatoxicosis.

This was a cross-sectional study that involved the use of primary data collected from 106 participants. The method for data collection included a structured questionnaire and the collection of the urine samples for aflatoxin M1 analysis at Bora Biotech Laboratories LTD. The urinary levels of AFM1 were detected by use of an ELISA kit. Data was entered in SPSS and analysed through Chi-Square for the association.

The study participants, including both male and female, had an age of between 15 and 91 years and with an average age of 41±18. Out of the 106 study participants, n=68 (72%) were females and n=26 (28%) were males. Majority of the study participants were with a median age of 24 years old. AFM1 levels were detected in 99.1% % of all urine samples at a range of 25-2337 pg./ml. The mean and median concentration of AFM1 in urine was 637.6 ± 512.7and 525 pg./mL, respectively.

The results of this study provide information on the current situation of aflatoxin exposure. From what is evident from our study a lot needs to be done to mitigate on the long-term effect of this high exposure. Therefore, the study encourages the concerned ministry to have a broader focus on the extent of aflatoxin food contamination from this region plus other regions across the country.

Keywords: Urinary Aflatoxin Levels in Makueni County, Kenya

INTRODUCTION

Fungi are a group of microbes found everywhere in the environment and have been associated with human illness. Although fungi are known to be less pathogenic and mostly saprophytic in nature as compared to other groups of microbes, those that produce aflatoxin have been associated with severe human disease. An example of such disease is Aflatoxicosis caused by soil-borne pathogenic fungi of the species *Aspergillus parasiticus* and *Aspergillus flavus.* These two species are known to contaminate foodstuffs such as maize, rice, groundnuts, sorghum, wheat, millet and cassava among others. They produce a mycotoxin substance that is toxic to the human liver with severe outcomes. For those exposed to this carcinogenic Aflatoxin, the condition may range from acute to chronic state. The severity of the condition is related to the host factors, which include; age, diet, nutrition quality, the extent of the exposure, other underlying diseases condition and gender of the affected individual. Clinical manifestation in Aflatoxicosis include; severe jaundice, liver cirrhosis and imminent liver failure. Other organs affected by Aflatoxin B1 (AFB1) apart from the liver include; oesophagus causing oesophagial carcinoma which is the 6th most prevalent type of cancer globally

Historically Aflatoxin discovery was in Great Britain in the 1960s, after an outbreak of a disease in turkeys that was referred to as Turkey X disease by then. During this outbreak over 100.000 people lost their lives. After investigation, this mortality was discovered to be caused by a fungal metabolite called Aflatoxin common in mouldy cereal feeds. [1-3] This was followed by numerous outbreaks that were reported globally. Aflatoxins are poisonous molecules produced by certain kinds of fungi (mould) that are found naturally all over the world. They can contaminate food cereals and present a harmful effect on man and domesticated animals.

In Kenya, a first local case of Aflatoxicosis poisoning was reported in the late 1970s in the former eastern province. However, the worst aflatoxin outbreak happened in 2004 in Kitui and Makueni districts of the then eastern province of Kenya. [4, 5] Ever since, the eastern region remains to be an aflatoxin prone area.[6] By this time nobody knew what was happening until when samples collected from the affected patients to investigate for possible known causes of hepatitis returned negative results for known viral infections (Environ Health Perspect, 2005). Further evaluation, gave a similar clinical picture and resembles tthat seen in aflatoxin poisoning from symptomatic cases

reported in Machakos in 1981. [7] This was followed by maize sampling from the affected areas for analysis which confirmed the presence AFB1 with as high as 4400 μg / kg. This on average was far above the minimum required standard of 10 μg/kg of aflatoxin levels recommended for food meant for human consumption. [8-10] This outbreak was attributed to widespread aflatoxin contamination of maize grown locally characterized by poor drying, storage under damp conditions and exposure to humid conditions. [6]

Several preventive strategies are available with most of them focusing on proper pre and post-harvesting storage, adequate processing before consumption, of food cereals and many others. Aflatoxins contamination depends on various factors ranging from; how crops are planted, harvested, stored, and processed for human and animal consumption. However irrespective of having adequate measures to combat aflatoxins poisoning what we need to remember is that they are not destroyed by heating at the time of food preparation. The same is still applicable to manufactured products like peanut butter and other industrial processed products and their potency would still affect the consumer depending on the quantity of food available. [11-16] The focus of this study was therefore to determine the prevalence rates and compliance to aflatoxin preventive measures among the residents of Makueni County, previously affected by Aflatoxicosis.

METHODS

Study Area

This study was purposefully carried out in Makueni County, which had the highest number of fatalities following Aflatoxin poisoning in 2004. The County is made up of five Sub-Counties namely; Mbooni, Kaiti, Kilome, Kibwezi West and Kibwezi East. The County has a total population of 987,653 communities (Census, 2019). Its annual rainfall ranges between 800-1200 mm. Majority of the County is arid with temperature ranging between 20^0 C to 24^0 C. Farming in the area is largely for subsistence crops like maize, beans, peas, cassava sweet potatoes, millet, and sorghum. They also do fruit farming of watermelons, pawpaw, oranges, mangoes and lemons. Their main domesticated animals include cows, goats, sheep, and donkeys.

Study Design

This was a cross-sectional study and using primary information collected randomly from the patients attending Makueni County Referral Hospital through the collection of urine samples for laboratory analysis,

interviews and hospital records for those undergoing treatment or have completed treatment and information on any death.

Study Population, Inclusion and Exclusion Criteria
Sample Size
The sample size was 100 participants determined based on the incidence rate due to infected maize consumed at the time of the 2004 outbreak of aflatoxin in which Makueni County had the highest deaths. [17]

Sampling Procedure
Urine samples were collected by participants in clean containers at Makueni county referral hospital in the month of May 2020. The samples were frozen at –20°C until analysis.

Analyzing Urine for the Presence of Aflatoxin
The urine samples were collected in sterile urine containers and transported in a cool box to Bora Biotech Labs in Nairobi for aflatoxin M1detection. Before the samples were analyzed, they were allowed to thaw at room temperature. Then 5 ml of thawed urine from each participant was centrifuged at 4000 rpm for at least 10 min. Skatron assay tubes were labeled with the participant's sample identification number. Then 950 ml of distilled water were pipetted into skatron (SKATRON AS LIER, Norway. CAT. No 7071) tubes and 50 µl of standards or supernatant-urine was added into 950 µl of distilled water in the skatron tubes and mixed by priming pipetting at least five times. 200 µl of the assay buffer was added into the mixing well per plate and 100 µl of the diluted standards (ranging from 0 to 40 ppt) and urine samples were added into the wells.

The contents of the mixing well were shaken using a micro-shaker (DYNATECH) for 2 min. One hundred microliters of the mixture were transferred to the antibody-coated Reaction-Assay Plate (aflatoxin M1 assay for urine, Helica Biosystems Inc, 1527 W. Alton Santa Ana, California, USA). The samples were mixed by shaking for 1 min and incubated at 18-28°C in the dark for 1 h. The plate was washed three times using phosphate-buffered saline–Tween-20 (0.05%) using the well-wash (Thermo Scientific, Finland) machine with 3-min intervals between the washes. After drying, 100 µl of the conjugate was added into each well, mixed gently by tapping, and incubated at room temperature for 15 min in the dark. The plate was then washed and 100 µl of substrate reagent (tetramethylethidine) was added into each well, mixed gently by tapping, and incubated at room temperature for 15 min in the dark. The reactions were stopped by adding 100 µl per well of stop solution and the optical density (OD) read at 450 nm within 15 min of stopping the reaction. The level in each sample was determined using the program from the kit manufacturer, which allowed calculations of the levels based on the absorbance readings.

Ethical Consideration
Ethical approval was obtained from the Kenyatta University's Ethical review committee. Other authorizations sought before the commencement of the study include informed written consent from the research participants, approval from NACOSTI and authorization from the hospital administration.

Data Analysis
Collected data were entered in a computer excel spreadsheets and statistically analyzed using SPSS Version 20.0. Exploration of the data was by numerical summaries together with the use of graphics. Social demography characteristics of the participants were presented in contingency tables as mean ± standard deviation. The level of significance was accepted as $p < 0.05$. The strength of association between variables was determined using chi-square test.

RESULTS
Sociodemographic Characteristics of the Participants
Age distribution of studies of participants
The study participants, including both males and females, had an age range of between 15 and 91 years. On average the participants were between 41±18 of age. Majority of the study participants were with a median age of 24 years old. Half of the selected participants were aged between 15 and 35 years n= 50 (47%) (Figure 1). A total of 106 study participants underwent an initial examination that included a medical history taking. Of the 106 screened study participants, all were to be of sound health and were nonsmokers. For the analyses conducted in the current study, no study participants were excluded.

FIGURE 1: Age distribution of the research participants
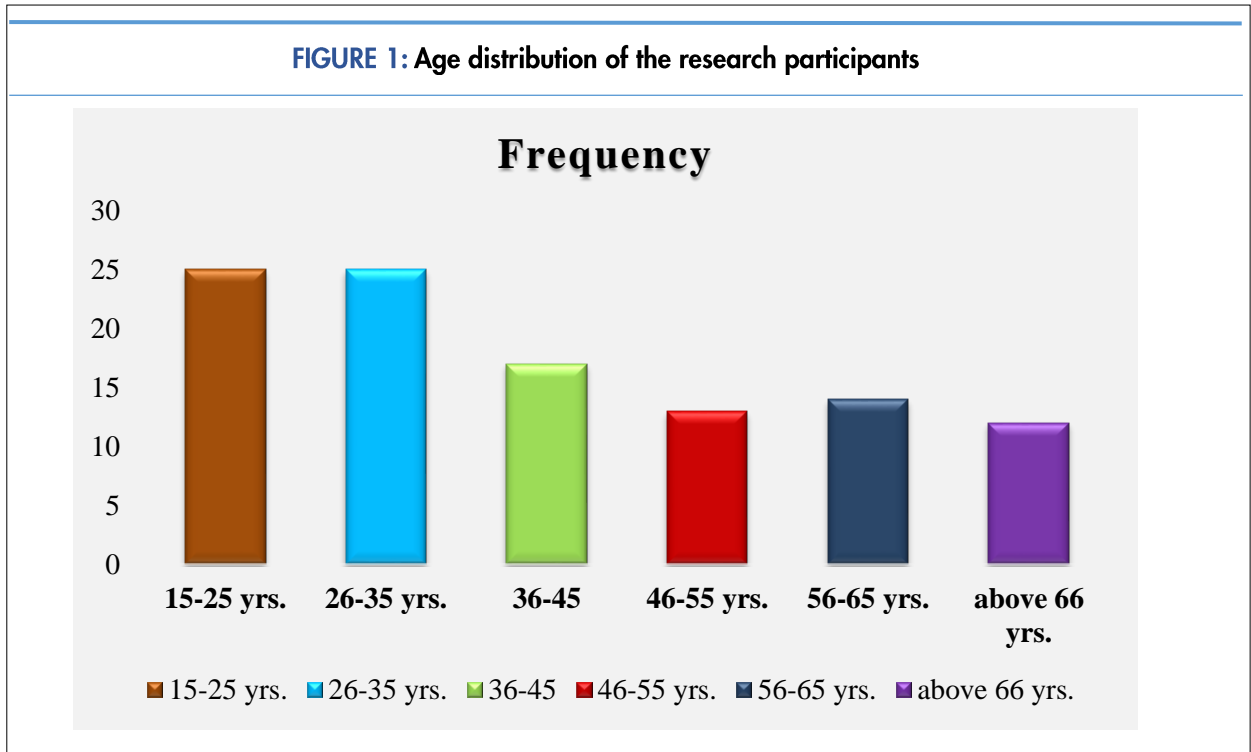
Gender distribution of studies of participants

Figure 2 gives a representation of the gender distribution of study participants. Out of 106 study participants, n=76 (72%) were females and n=30 (28%) were males.

FIGURE 2: Gender distribution of the study participants
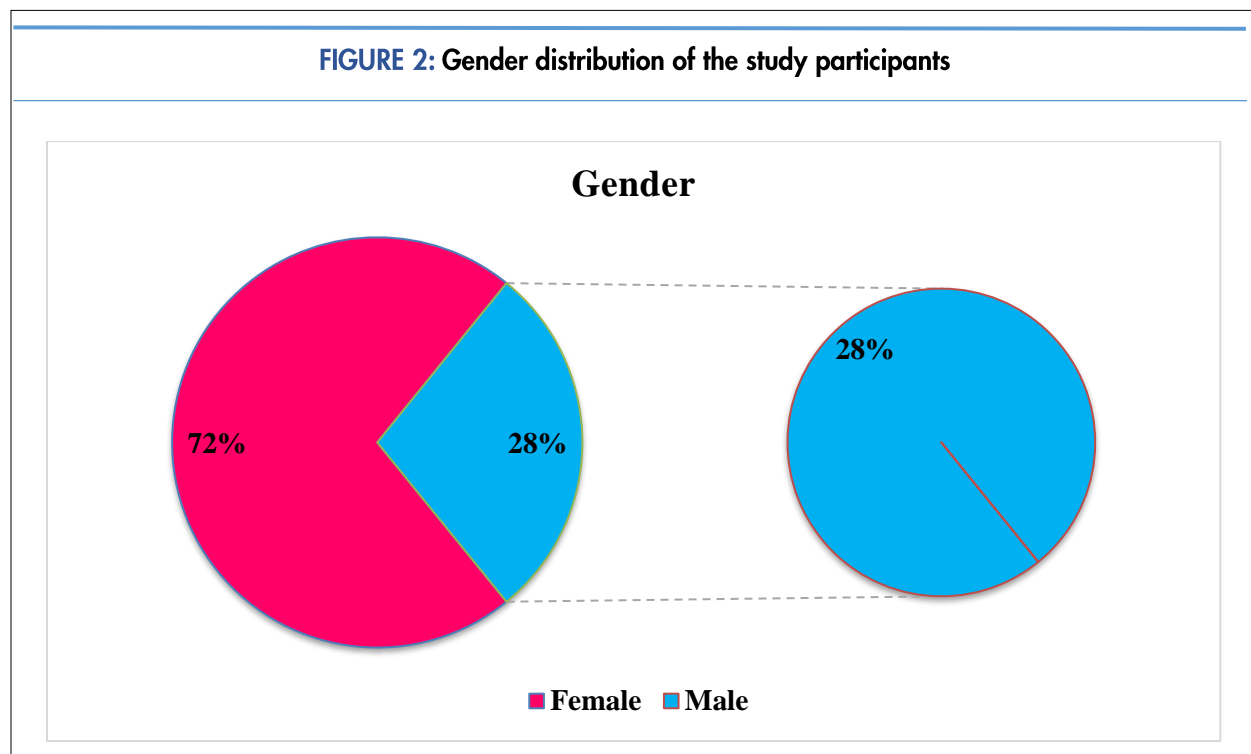
The current prevalence rates of Aflatoxicosis

The study had an aim of establishing whether there was a reduction in the prevalence rate in Makueni County and this after some intensive prevention and control strategies after the initial outbreak of 2004. To achieve this aim urine aflatoxin levels were used as a parameter to measure the exposure rates of resident of Makueni county. At the time of the study samples with AFM1 concentration below 0.0 pg/ml were categorized as negative samples and those with detectable level above 1 pg/ml of AFM1 through the extrapolation from the standard curve were categorized as positive samples. Of 106 urine samples analyzed during the study period, 105 had AFM1 urine concentration levels of above 1 pg/ml which were categorized as a positive finding. Only one sample had an assay value reading of 0.0 pg/ml and this was categorized as a negative value. From those with positive AFM1, 39 had Aflatoxins value below 300 pg/mL. The rest had a value greater than 300 pg/ml, where the majority fall. The positive 105 samples had urinary AFM1 concentration ranging from 25 to 2375 pg/mL, with an average of 637.6±512.8 pg/mL. The urinary levels of AFM1 were detected by enzyme-linked immunosorbent assay. AFM1 was detected in 99.1 % of all urine samples at a range of 25 to 2375 pg/mL (Table 1). This gives a typical picture of what happens when aflatoxins are ingested in contaminated food and animal products. In the liver, the ingested aflatoxin (AFB1) is converted to aflatoxin M1 (AFM1), a metabolite that we have used in our study as a biomarker of AFB1 exposure, as it is excreted in the urine, and therefore suitable for our study.

TABLE 1. The urinary concentration of Aflatoxin in residents of Makueni County

Characteristics	The concentration of AFs in pg/ml									
	Below 100	101-300	301-500	501-700	701-900	901-1100	1101-1300	1301-1500	1501-1700	Above 1701
Age groups										
15-25	7 (28.0%)	2 (8.0%)	1 (4.0%)	3 (12.0%)	3 (12.0%)	4 (16.0%)	1 (4.0%)	2 (8.0%)	0 (0.0%)	2 (8.0%)
26-35	2 (8.0%)	9 (36.0%)	1 (4.0%)	10 (40.0%)	0 (0.0%)	0 (0.0%)	1 (4.0%)	2 (8.0%)	0 (0.0%)	0 (0.0%)
36-45	2 (11.8%)	2 (11.8%)	3 (17.6%)	2 (11.8%)	4 (23.5%)	1 (5.9%)	1 (5.9%)	1 (5.9%)	0 (0.0%)	1 (5.9%)
46-55	4 (30.8%)	1 (7.7%)	1 (7.7%)	1 (7.7%)	0 (0.0%)	3 (23.1%)	1 (7.7%)	1 (7.7%)	0 (0.0%)	1 (7.7%)
56-65	2 (14.3%)	2 (14.3%)	1 (7.1%)	0 (0.0%)	3 (21.4%)	2 (14.3%)	1 (7.1%)	2 (14.3%)	1 (7.1%)	0 (0.0%)
above 66	2 (16.7%)	4 (33.3%)	2 (16.7%)	1 (8.3%)	2 (16.7%)	1 (8.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Gender										
Male	7 (23.3%)	3 (10.0%)	3 (10.0%)	6 (20.0%)	3 (10.0%)	3 (10.0%)	1 (3.3%)	2 (6.7%)	0 (0.0%)	2 (6.7%)
Female	12 (15.8%)	17 (22.4%)	6 (7.9%)	11 (14.5%)	9 (11.8%)	8 (10.5%)	4 (5.3%)	6 (7.9%)	1 (1.3%)	2 (2.6%)

DISCUSSION

Aflatoxins metabolites are common in groundnuts, cereals, and spices and herbs, which are widely used as the main food commodities for human and animal consumptions globally. [17-27] All food supplies are plant-related products, animal products such as meat and dairy products are in danger of being contaminated with aflatoxin. Animal products get tainted when such animals are on plant diet that is tainted with aflatoxin. The level of contamination in both cereal and animal products varies from country to country or even at a local level. For example, the Kenya National Bureau of Standards, a state organ with the mandate to ensure the safety of consumer products in the country found that 19 out of 53 samples of dairy products were tainted with AFM1, ranging from 3.5 to 100.5 ng/L. [28-34] The Kenyan population is among some of the African people who consume cereals products daily as this forms one of their staple food supply. [35]

The current prevalence rates of Aflatoxicosis

In our study, the prevalence rate was at 99.1% from the 106 urine samples analyzed. This prevalence of urinary AFM1 was relatively higher than the 79% and 83% reported by Kang'ethe et al 2017 [36, 37] in their previous studies in children below the age of five years in Makueni and Nandi counties, respectively. The high prevalence found in the present study could be attributed to continuous exposure to aflatoxins tainted food even at the time of adulthood. These results were also similar to the 86% prevalence reported by Polychronaki et al. 2008 among children in Guinea [38] The prevalence rate was also higher than those reported in other countries such as 30 % in Egypt [38], Sanchez et al. 2019 reported a 41.7% in Colombia children [39], Ali et al also found a 40 % prevalence rate among a rural population in Bangladesh [40] and Ayelign et al 2017 reported a prevalence rate of 17 % from Ethiopian children. [41] The presence of AFM1 in urine as seen in this study gives a picture of continuous exposure to aflatoxin-tainted food commodities in the entire lives of individuals from this County. This high number can be explained by the high level of aflatoxins contamination and continuous ingestion of AFB1 in food, which is eventually degraded to AFM1 by the liver and easily excreted by kidneys. Aflatoxins metabolized by the liver results into end products such as AFB1-lysine adduct [42-44], AFB1-N7-guanine adduct [45], and urinary AFM1 commonly found in urine. Aflatoxin contamination of animal products has been reported in various studies across the world. [46-57] The first Aflatoxicosis case related to aflatoxin contamination was reported in turkeys and ducklings in the 1960s, where millions died as a result of consuming AFB1-tainted feeds. [58-60]

All these cases could be related to the higher consumption of aflatoxin-tainted staple food, an aspect that contributed to the largest outbreak of human Aflatoxicosis and food contamination in Makueni and subsequent attacks as reported by various studies from different regions of the country.[4, 7, 10, 36, 48, 61-85] The outbreaks reported as high as 8000 µg/kg of aflatoxin contamination in maize in which 125 of people who consumed it never survived the poisoning. Most mycotoxins are stable at the time of food processing [17, 86-90] so it can even show up in food products such as peanut butter and many processed products. However, certain food preparation techniques can minimize the level of toxicity. [91]

CONCLUSION

The results from this study provided the current situation on aflatoxin exposure to the people of Makueni and at the same time offered feedback that served to monitor what is happening and if the initiated preventive strategies for Aflatoxicosis are working. From what is evident from our study, the findings suggest higher aflatoxin concentration among the resident of Makueni County, where there is an urgent need to mitigate the long-term effect of this high exposure. Therefore, the study is recommending to the concerned ministry to have a broader focus on the extent of aflatoxin food contamination from this region plus other regions across the country. This will be so important to protect vulnerable communities and the general population of the country as a whole from this carcinogenic exposure. Some of the recommendations we propose include; regular training on good agricultural practices, routine food sampling for aflatoxin levels, the introduction of a modern method to control aflatoxin food contamination and more research to be done to understand the impact of this high exposure to the health of the vulnerable population.

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Conflict of Interest

The authors declare no conflict of interest related to this study

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