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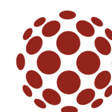

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The Additive Effect of Hepatitis B Virus and Aflatoxin B1 to Liver Disease Burden: A Case Study in Kitui, Makueni and Machakos Counties, Kenya

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Abstract
There are various causes of liver disease, including viruses, trauma, and toxins. Hepatitis B virus (HBV) is a major etiological agent for liver disease in lower eastern Kenya. This had compounded an already existing problem of aflatoxinB1 induced hepatoxicity associated with contaminated grain which had been reported over the years in parts of the region including Kitui, Makueni and Machakos counties. A study was carried out to evaluate the additive effects of hepatitis B virus (HBV) and dietary AFB1 in liver disease among the subjects. Liver disease bio markers HBSAg and AFB1 lysine albumen adducts were used in this study. The investigation was conducted as a case-control study where blood samples from appropriately selected subjects were collected and analyzed for exposure to dietary AFB1 and HBV. A non-probability purposive sampling method was used to choose and divide the study area into strata with 19 clusters. The sample size (n) for the human case-control study was determined as per the Schelsselman formula (1982), as 283 each for both cases and controls A computer software SPSS® version 18.0 was used to analyze the data statistically. For case subjects, 52.29% (n=148) of serum samples were positive for HBsAg with level range of 500 to 9800 Iu/mL and a mean of 3.204 x 103 Iu/mL {95%; CI= (2.76 to 3.65)x 103}, p≤ 0.05. For controls, 24% (n=68) of serum sample was positive for HBsAg with a level range of 50 to 990 Iu/mL and a mean of 347.57 Iu/mL (95%; CI= (278.35 to 416.80), p≤ 0.05. For AFB1 lysine albumin adducts, case subjects had 55.83% (n=158) of positive serum sample with a level range of 15.5 to 135.0 pg/mg and a mean of 42.93 pg/mg (95%; CI= (39.36 to 46.51) p≤ 0.05, while the controls with 31.0% (n=88) of positive serum sample had a lower AFB1 serum albumin adducts level range of 3.5 to 60.5 pg/mg with a mean of 14.30 pg/mg (95%; CI= (12.23 to 16.36), p≤ 0.05. Case subjects had higher means for both HBsAg and AFB1 lysine albumin adducts than controls, suggesting an additive effect on liver disease among the subjects. In control subject samples, lower HBsAg suggested either a carrier state or a recent exposure and recovery from HBV. In control serum samples, lower mean AFB1 lysine albumin adducts suggested a lower level of dietary aflatoxin B1 exposure among those subjects. The case and control cohorts, the higher total number of serum samples testing positive for HBsAg, 30.83% (n=175) and AFB1 lysine albumin adducts 36.13% (n=205) out of the total sample (N=566), implied that the causal factors for the liver disease

were endemic in the region. There was a higher dietary AFB1 exposure to residents than HBV exposure. It is concluded that AFB1 induced hepatoxicity was more prevalent than HBV infection among the study subjects.

Keywords: Hepatitis B Virus, Aflatoxin B1, Additive Effect to Liver Disease, Kitui, Makueni, Machakos

INTRODUCTION

Liver disease is known to have various causal factors, including viruses, toxins, autoimmune diseases, and even physical injuries. Infection with hepatitis B virus (HBV), leads to liver disease, which is grouped into two broad categories, namely: Acute hepatitis B and Chronic hepatitis B.

Acute hepatitis B manifest as an illness that begins with general ill-health, loss of appetite, nausea, vomiting, body aches, mild fever, and dark urine, and then progresses to the development of jaundice. It has been noted that itchy skin has been an indication of a possible symptom of an infection with hepatitis B virus sub types (Liaw *et al.,* 2010). The illness lasts for a few weeks and then gradually improves in most affected people. A few people may have more severe liver disease (fulminant hepatic failure), which may be fatal as a result. The infection may be entirely asymptomatic and may also go unrecognized (Liaw *et al.,* 2010). An estimated 30% of those infected do not show typical signs or symptoms (Terrault *et al.,* 2005; Liaw *et al.,* 2010). Generally, however, infection with hepatitis B virus leads to liver diseases including hepatocellular carcinoma, fulminant liver failure, liver cirrhosis and membranous glomerular nephritis (MGN), with the attendant symptoms (Gan *et al.,*2005). Between 85 to 95% of infected individuals develop permanent immunity to the disease, while 5 to 10% of adults and children older than 5years develop chronic infection and become HBV carriers, with the highest rate of infection occurring between the ages of 20 to 49 years. In children, 90% of those born to infected mothers acquire the disease, and only 5% of these newborn children develop full immunity to the disease (Bell and Nguyen, 2009).

Chronic hepatitis B infection could be asymptomatic or may be associated with a chronic inflammation of the liver (chronic hepatitis), leading to cirrhosis over a period of several years. This type of infection dramatically increases the incidence of hepato cellular carcinoma (liver cancer), more so in areas where dietary aflatoxin B1 toxicity is endemic.

Transmission of hepatitis B virus results from exposure to infectious blood or body fluids containing the virus. Possible forms of transmission include sexual contact and blood transfusions or transfusion with other human blood products (Buddeberg *et al.,* 2008; Fairley & Read, 2012). Re-use of contaminated needles and syringes and vertical transmission from mother to child (MTCT) during childbirth are other important means (CDC, 2012). However, at least 30% of reported hepatitis B infections among adults cannot be associated with an identifiable risk factor (Redd *et al.,* 2007).

The virus is divided into four major serotypes (adr, adw, ayr, ayw) based on antigenic epitopes presented on its envelope proteins, and into eight genotypes (A-H) according to overall nucleotide sequence variation of the genome. The genotypes have a distinct geographical distribution and are used in tracing the evolution and transmission of the virus. Differences between genotypes affect the disease severity, course and likelihood of complications, and response to treatment and possibly vaccination (Chan *et al.,* 2009).

Hepatitis B virus primarily interferes with the functions of the liver by replicating in liver cells, specifically the hepatocytes. A functional receptor for the virus is NTCP (Yan, *et al.,* 2012). There is, however, evidence that the receptor in the hepatitis B virus is a carboxypeptidase D (Glebe and Urban, 2007). The virions bind to the host cell via the preS domain of the viral surface antigen and are subsequently internalized by endocytosis. HBV-preS-specific receptors are expressed primarily in hepatocytes, however, viral DNA and proteins have also been detected in extrahepatic sites, suggesting that cellular receptors for HBV may also exist on extrahepatic cells (Coffin *et al*., 2011).

During HBV infection, the host immune response causes both hepatocellular damage and viral clearance. Although the innate immune response does not play a significant role in these processes, the adaptive immune

response, in particular, virus-specific cytotoxic T lymphocytes (CTLs), contributes to most of the liver injury associated with HBV infection. CTLs eliminate HBV infection by killing infected cells and producing antiviral cytokines, which are then used to purge HBV from viable hepatocytes (Iannacone *et al.,* 2007). Although liver damage is initiated and mediated by the CTLs, antigen-nonspecific inflammatory cells can worsen CTL-induced immunopathology, and platelets activated at the site of infection may facilitate the accumulation of CTLs in the liver (Sitia *et al.,* 2007).

According to Karayiannis *et al.,* (2009), hepatitis B surface antigen is most frequently used to screen for the presence of hepatitis B infection. It is the first detectable viral antigen to appear during infection. However, early in an infection, this antigen may not be present, and it may be undetectable later in the infection as it is being cleared by the host. Studies have shown that infectious virion contains an inner "core particle" enclosing viral genome. The icosahedral core particle is made of 180 or 240 copies of core protein, alternatively known as hepatitis B core antigen, or *HBcAg*. During this 'window' in which the host remains infected but is successfully clearing the virus, IgM antibodies to the hepatitis B core antigen (*anti-HBc IgM*) may be the only serological evidence of disease. Therefore most hepatitis B diagnostic kits contain HBsAg and total anti-HBc, including both IgM and IgG (Karayiannis *et al*., 2009). Individuals who remain HBsAg positive for at least six months are considered to be hepatitis B carriers. It is instrumental that, many carriers of the virus may have chronic hepatitis B, which would be reflected by elevated serum alanine aminotransferase (ALT) levels and inflammation of the liver, as has been revealed in many cases of liver biopsies (Lok and McMahon, 2007).

Like is the case with HBV, various studies have linked exposure to aflatoxin B1 (AFB1) to chronic and acute hepato cellular injury leading to chronic liver diseases, including hepato cellular carcinoma. Aflatoxins could be defined as toxic secondary metabolites produced by fungal strains of the genus *Aspergillus,* mostly *A. flavus* and *A. parasiticus* that grow on a variety of substrates (Lawley, 2013). Aflatoxins are of particular concern due to their biochemical and biological effects on human and animal health (EFSA, 2013). Among the different aflatoxin compounds identified, aflatoxin B_1, B_2, G_1, and G_2 are the most important in terms of their toxic effects (Bao *et al*., 2010), with aflatoxin B_1 having shown the highest potency as a natural hepato-carcinogen and usually, is the major aflatoxin produced by the aflatoxigenic fungal strains.

Naturally, aflatoxin producing fungi occur in certain food products in the form of spores, and when conditions are favorable, the fungi produce aflatoxins in high amounts. These foods include maize, sorghum, pearl millet, rice, wheat, groundnuts, soybeans, sunflower seeds, cotton seedcake, chilies, coriander, turmeric, and ginger. Tree nuts, including almonds, pistachio, walnuts, and coconut, are also attacked. Other than aflatoxin M_1 found as a metabolite of aflatoxin B_1 in animal milk products, powdered milk can also be attacked directly by aflatoxin producing moulds (Lawley, 2013; EFSA, 2013).

The presence of these fungal toxins reduces the economic value of food products and devalues it as a commodity for human consumption (EFSA, 2013). Because of the hepatoxicity of these aflatoxins, the level of exposure is of particular public health concern. Both the Food and Agricultural Organization (FAO), and Agro-food and Veterinary Diagnostics Organization (AVD), estimates that mycotoxins contaminate 25% of agricultural crops worldwide (CAST, 1998; AVD, 2013).

Aflatoxin contamination of food stuff may occur during pre-harvest or post-harvest period, during storage, transportation, and processing (Li *et al.,* 2009; Rural21, 2013). Continued dietary exposure to aflatoxins is a major risk factor for hepatocellular carcinoma and general liver damage in populations, particularly in areas where hepatitis B virus (HBV) infection is endemic. Ingestion of higher doses of aflatoxins can result to acute aflatoxicosis, which manifests as hepatoxicity and in severe cases, fulminate liver failure (Golthardt *et al.,* 2009). Once ingested with food, aflatoxin B1 forms AFB_1 lysine albumin adducts which are carcinogenic and which are used as biomarkers for AFB1 induced hepatoxicity.

Objective of the study

The purpose of this study was, therefore, to evaluate the additive effects of the elevated levels of both HBsAg and AFB_1 lysine albumin adducts and determine whether the two etiological factors for liver disease are endemic in the region.

MATERIALS AND METHODS

Study design

The study was designed and conducted as a Case-Control study for human subjects which involved the collection of blood samples from both cases and controls after which a laboratory serum sample analysis for levels of HBsAg and AFB_1- lysine albumen adducts was performed. The presence or absence of HBsAg in human serum was to determine the actual exposure and non exposure to HBV while AFB_1 lysine albumin adducts would determine exposure to aflatoxin B1. For both case and controls, a structured questionnaire employing Likert scale was administered to help match the case and controls on the basis of age bracket, sex, alcohol consumption, and residency of the subjects.

Study Area

The study was conducted in Kitui, Makueni, and lower part of Machakos regions. The area was stratified into three (3) main strata and nineteen (19) sub-clusters based on health centers administrative units (districts and divisions) whose distribution was as below (Table 1).

Table 1: Distribution of the sub-clusters within the strata in the study area.

Kitui county	Makueni county	Machakos county
Mutomo	Makindu	Masaku
Kyuso	Wote	
Tei wa yesu	Sultan Hamud	
Mwingi	Mtito Adei	
Kitui	E mali	
Nuu	Kathozweni	
Kavisuni	Kibwezi	
Mtito ndooa		
Migwani		
Muthale		
Mathuki		

Study authorization

The study was granted approvals by Ethical research committee (ERC), of Kenya medical research institute (KEMRI), through the scientific steering committee (Protocol no.2988) and National committee on science, innovation, and technology (NACOSTI/P/15/5700/7331), after the vetting procedures for protocols of studies dealing with human subjects.

Target population

The target human populations for the case-control study comprised of all people who have been resident in Kitui, Makueni and lower Machakos all parts of lower eastern Kenya for at least a year for both males and females and who were between the age of 12 and 80 years.

Study population

The study population comprised of selected Case subjects from the Sub-district, District, and level 5 health centers that had been clinically diagnosed with liver disease. Controls consisted of those suffering from non-liver ailments within the same categories of hospitals and health centers under investigation and who were matched in terms of sex, age, residency, and non-use of alcohol

Case subjects identification

Human subject Cases were identified from health facilities within the study area on condition that they had the clinical manifestation of liver disease. Hospital admission registers at various county or district hospitals in the counties were also used to confirm admissions due to liver disease.

Control subjects identification

Controls were drawn from the samples of the population where the Cases were drawn from. Appropriate numbers of Controls equal to the number of Cases were picked from the same hospital where the cases were admitted but from the non GIT sickness sections, including surgical, orthopedic, and trauma wards to avoid confounding and or Berkson bias (Westreich *et al*., 2012; Pearce *et al.,* 2014)

Controls were matched to Cases as much as possible, including using personal characteristics, including age, sex, food preference, and residency. Matching was, however, limited to three factors, including age, sex, and locality (residency) of the participants.

This was a case-control study which employed non-probability stratified purposive sampling method on the basis of clinical diagnosis of liver disease among cases and non-disease in control subjects

Participant characteristics

a) Inclusion criteria

The human subjects were included into the study on condition that:

i) They were between ages of 12 to 80 years for all sexes.
ii) They had been residents of the study area for at least one year.
iii) They gave an informed consent to participate in the study in case of those above 18 years (a prescribed standard consent format was used for this procedure).
iv) They gave an ascent to participate in the study in case of those who were below 18 years, and an informed consent of the guardian or parent was also to be provided.
v) They had been clinically diagnosed to have liver disease.
vi) They did not belong to the vulnerable groups, including prisoners, blind persons, pregnant women, mentally impaired, or persons lacking consent capacity.

b) Exclusion criteria

The human subject was excluded from this study if:-

i) Below 12 yrs and above 80 yrs
ii) He or she had not been a resident of the study area for more than one year
iii) He or she had not given an informed consent to participate in the study in case of those above18 years.
iv) He or she had not given an ascent to participate in the study in case of those who are below 18 years and irrespective of whether an informed consent of the guardian or parent had been provided.
v) He or she was a consumer of alcohol in any form and did not meet all or some of the inclusion criteria above.
vi) They had not been clinically diagnosed to have liver disease
vii) He or she belonged to the vulnerable groups, including prisoners, blind persons, pregnant women, mentally impaired persons, or any person lacking consent capacity due to various reasons.

Subjects sampling procedure

Non-probability stratified purposive sampling method was used to pick human subjects on the basis of clinical diagnosis of liver disease for Cases and non-liver disease for Controls. Recruitment of human participants to the study was done at health facilities within the study area, and questionnaires were used to link individuals in strata to serum samples by use of code numbers for anonymity.

Sample size for the case-control design

The sample size (n) for the case-control study was determined by use of the Schlesselman (1982), formula, thus:-

$$n = \frac{\left(Z_{\alpha}\sqrt{2\bar{p}\bar{q}} + Z_{\beta}\sqrt{p_1 q_1 + p_0 q_0}\right)^2}{(p_1 + p_0)^2}$$

Where P_0 = exposure rate of Controls (general population)

P_1 = exposure rate of Cases

Z_{α} = 1.96 for 95% confidence level; Z_{β} = 0.84. Since it can be shown that

$$p_1 = \frac{P_0 R}{[1 + P_0(R-1)]}$$

Then $q_1 = 1 - p_1$; $q_0 = 1 - p_0$,
While $\bar{p} = \frac{p_1 + p_0}{2}$; and $\bar{q} = 1 - \bar{p}$.

Given that the aflatoxin prevalence rate in the study area was 9.3% (Lauren *et al.,* 2004; CDC, 2004), then the general population (controls), exposure rate was estimated to be 10%. Hence

$p_0 = 0.1$; $p_1 = 0.1818$; $q_0 = 0.9$; $\bar{p} = 0.1409$; $q_1 = 0.8182$; $\bar{q} = 0.8591$; $Z\alpha = 1.96$;
$Z\beta = 0.84$; then (n) = 283.

Based on the three categories of health facilities, mainly sub-districts, district and level 5 (provincial facility), the sample size (n) of 283 cases was divided into a ratio of 1:2:3 so that there was a minimum of 47, 94 and 142 cases to be enrolled from sub-districts, district and level 5 facilities respectively. The number of case sample (n), per hospital, was worked on depending on mean totals from each hospital register. Thus if admission from all causes in a particular health facility was (t), and the total mean admission for all health facilities in that category was T, then sample (n) per hospital was determined as (t/T x 47), (t/T x 94), and (t/T x 142) respectively for sub-district, district and level 5 health facility. Since the hospital admissions differed, the case sample (n) from one facility to another also differed. Similarly, since Controls were matched for each Case, they similarly differed (Table 2).

This implied that the minimum total number of subjects, both case and controls in the study was to be 283×2 = 566. Since this sample was representative, the study enrolled those admitted to health centers since following outpatients was not practical in this case.

Table 2: Case and control serum sample per health center

Health center	Case (n)	Control (n)	Category
Mutomo	17	17	District
Tei wa yesu	4	4	Sub district
Kitui	21	21	District
Mtito ndooa	7	7	Sub district
Mwingi	10	10	District
Kyuso	2	2	District
Migwani	10	10	Sub district
Kavisuni	3	3	Sub district
Nuu	6	6	Sub district
Muthale	15	15	District
Mathuki	5	5	Sub district
Kibwezi	3	3	District
Wote	45	45	Level 5
Sultan H	12	12	Sub district
Masaku	97	97	Level 5
Kathozweni	12	12	District

Makindu	7	7	District
Emali	4	4	District
Mtito Adei	3	3	District
Total	283	283	

Procedure for collection of blood samples.

The invasive procedure for collection of blood samples was undertaken by a qualified lab technologist (phlebotomist) as per the WHO, (2010) guidelines.

Blood samples were drawn after consent was obtained, then frozen and transported for analysis.

Analysis and determination of AFB_1 albumin serum adduct levels

Preparation of samples and AFB_1-albumin standards.

The direct competitive ELISA Kit (Glory® Science Co., Ltd. USA), was used for the total determination of AFB_1-albumin adducts in the human serum samples for both case and controls in the study. The kit manufacturer (Glory Science Co. Ltd USA), had put the kit detection lower limit to 0.3ug/L (0.3ug/mL) with extracts from feed fish, shrimps urine or serum samples.

Each sample serum extract in a $10cm^3$ test tube was diluted using methanol (1:10) solution, then centrifuged for 3 minutes to get the liquid supernant (serum) for the test.

Six aflatoxin-albumin adduct standards, vials each of 1ml, and the concentration 0 ng/ml, 0.1ng/ml, 0.3ng/ml, 0.9ng/ml, 2.7ng/ml, and 8.1ng/ml was arranged in a test tube rack and labelled; S_1, S_2, S_3, S_4 and S_5(Glory Science Co,. Ltd. USA).

Preparation of AFB_1- albumin adduct-enzyme conjugates.

The ELISA kit (Glory® Science Co., Ltd USA), had an already prepared AFB_1-albumin adduct-enzyme conjugate which was used for tests, in both micro-titre and standard wells.

Preparation of TMB-enzyme substrate

The ELISA kit was supplied with an already made enzyme colour marker with TMB-substrate, but for accuracy purposes, the solution was prepared by mixing a portion of (1:1), citric acid buffered solution (pH 3.8), containing 325ul of 30% hydrogen peroxide per litre of solution and one portion of a solution of 50.4 mg tetra methyl benzidine (TMB) in an acetone-methanol (1: 9) solution.

Analysis/Method:

Fifty (50) ul, of the standard AFB_1-albumin serum adduct solution, was pipetted in duplicate to the pre-coated aflatoxin albumin adduct antibody removable micro-titer plates in the order S_0, S_1, S_2, S_3, S_4 and S_5 representing standard dilutions of 0 ng/ml, 0.3ng/ml, 0.9ng/ml, 2.7ng/ml and 8.1ng/ml. Similarly, 50 ul, of sample serum was pipetted into adjacent pre-coated wells. Aliquots of 50 ul, of AFB_1-albumin adducts enzyme conjugate (Glory® Science Co. Ltd USA), was added to all the wells of both the standards and the sample, covered with an aluminum foil and incubated at room temperature (28ºC) for two (2) hours.

The plate was then emptied and washed with saline tween solution (8.55gm sodium chloride dissolved in 1000 ul distilled water, plus 0.25ml of poly oxy ethylene sorbitan monohydrate), and dried by tapping with a blotting paper.

An enzyme-substrate (Glory® Science Co. Ltd USA), which consisted of Horse radish peroxidise and tri methyl benzidine, was added and the plates incubated in the dark for 10 minutes, after which the enzyme reaction was stopped by adding 100 ul of 2M sulphuric acid simultaneously into all micro-titre wells. The colour had changed from blue to yellowish.

The intensity of colour in all wells was determined by measuring absorbance at 450nm, using an ELISA reader (Uniskan II® Lab systems, Finland). The absorbance value data for standards and serum samples were entered into computer software (R-ridasoftwin® version 1.60, R-bio pharm., Germany), which used percentage absorbance against known standard aflatoxin adducts concentrations to draw a standard curve. The software automatically generated AFB_1-albumin adducts levels in ng/mL, which was converted to pg/mg of albumin (Gathumbi *et al.,* 2001).

(b Determination of HBsAg level in case and control serum samples

The quantification of HBsAg was done by automated analyzers available commercially, namely Architect QT® (Abbot Laboratories). This investigation used Architect QT® (Abbot Laboratories), to quantify HBsAg in blood samples since it was more easily available, and it was also the oldest kit in use among many other Immuno assays analyzers. The assay was capable of processing up to 800 HBsAg tests per hour

Architect QT is a Chemiluminescence Microparticle Immuno assay (CHIA), in which 1ml serum and anti-HBsAg-coated paramagnetic micro particles were combined. After washing, acrinium- labeled anti-HBs-conjugate was added; and after another washing step, pre-trigger and trigger solution were added.

The subsequent chemiluminiscent reaction was measured in relative light units (RLU), which are converted to HBsAg units, using a previously graduated Architect HBsAg calibration curve (Deguchi *et al.,* 2004). The range of the assay for this test was between 0.05 IU/mL to 250 IU/mL of HBsAg in undiluted sera. Manual dilution could be done up to a ratio of 1: 999, but in this study, an On-board auto dilution was done up to a ratio of 1: 500, to offer a wider range of quantification (O' Neil *et al*., 2012). Auto dilutions demonstrated better precision values within and between runs. The performance of this automated analyzer is comparable to the new generation chemiluminiscent assay for hepatitis B surface antigens (Chen *et al.,* 2006)

A sample of blood each for case and control was run through and analyzed for HBsAg in IU/mL units, and data entered in the data collection tool for data analysis.

Data analysis

Laboratory data on HBs Ag and AFB_1 lysine albumin adducts level, was analyzed for means, medians, ranges, standard deviation (Sd) and confidence intervals of the means (CI) by use of a computer software SPSS version 18.0. The confidence interval (CI) statistical manipulations was cross worked with Casio® fx-82EX (Casio®, Japan), statistical tool at 95% confidence level ($0 < p \leq 0.05$).

Analysis of means was done to determine any synergistic (ʒ) or additive effect on liver disease due to a combined sero presence of HBsAg and AFB_1 lysine albumin adducts in subjects compared with those determined to be HBsAg sero positive only, in the study.

RESULTS

In case cohort sample, (N=283), 32% (n=91) of subject serum samples were positive for HBsAg, while 36% (n=101) was positive for aflatoxin B1 lysine albumin adducts (AFB_1 lysine albumin adducts). In the same cohort, 20% (n=57) of the serum sample had evidence of mixed infection with serum samples positive for both HBsAg and AFB_1 lysine albumin adducts. Case subjects had 12% (n=34) of the serum samples testing negative for both HBsAg and AFB_1 lysine albumin adducts and therefore was of unknown status. Table 3 shows the case subject serum samples which tested positive and negative for HBsAg and AFB_1 lysine albumin adducts as biomarkers of liver disease in the study.

Table 3: Case subject serum samples positive or negative for biomarkers of liver disease

Disease factor	sample(n)	ratio	percentage %
HBsAg	91 positive	0.32	32.00
AFB_1 lysine albumin adducts	101 positive	0.36	36.00
AFB_1 lysine adducts + HBsAg	57 positive	0.20	20.00
All above	34 negative	0.12	12.00
Totals	283	1.00	

The chart shows percentage of serum samples positive for the biomarkers of liver disease with 12% (n=34), of total sample negative for both etiologic factors for liver disease.

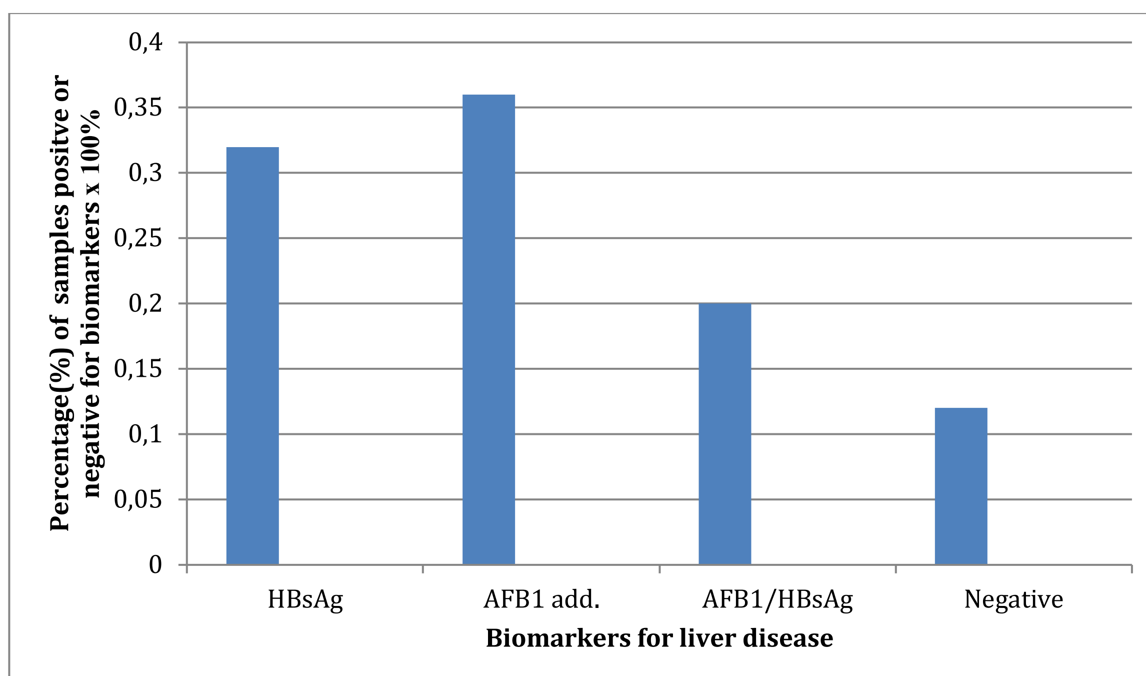

Figure 1: A comparative bar chart on case samples analyzed for liver disease biomarkers

In case cohort, the ratio of serum samples testing positive for AFB_1 lysine albumin adducts was higher at 36%, than that of serum samples testing positive for HBsAg at 32%. The serum samples testing negative for both etiologic factors for liver disease had a lower ratio at12%.

The control subjects (N=283), were clinically disease-free, however, 15% (n=42) of serum samples were positive for HBsAg, while 22% (n=62) of the sample was positive for AFB_1 lysine albumin adducts. In the same cohort, 9% (n=26) of the serum sample was positive for both HBsAg, and AFB_1 lysine albumin adducts an indication of recent exposure to disease factors or recovery from liver disease by the same factors. Control cohort had 54% (n=153) of the subject serum sample testing negative for the etiologic factors for liver disease (Table 4 and figure 2).

Table 4: Control subject serum samples positive or negative for biomarkers of liver disease

Disease factor	sample (n)	ratio	percentage (%)
HBsAg	42 positive	0.1484	14.84
AFB_1 lysine albumin adducts	62 positive	0.22	22.00
AFB_1 lysine albumin adducts + HBsAg	26 positive	0.09	9.00
All above	153 negative	0.54	54.00
Total	283	1.00	

Figure 2 is a control comparative bar chart showing percentage (%) of subject serum sample which tested positive and negative for liver disease biomarkers (HBsAg and AFB_1 lysine albumin adducts), but at much lower levels comparatively than case subject serum samples.

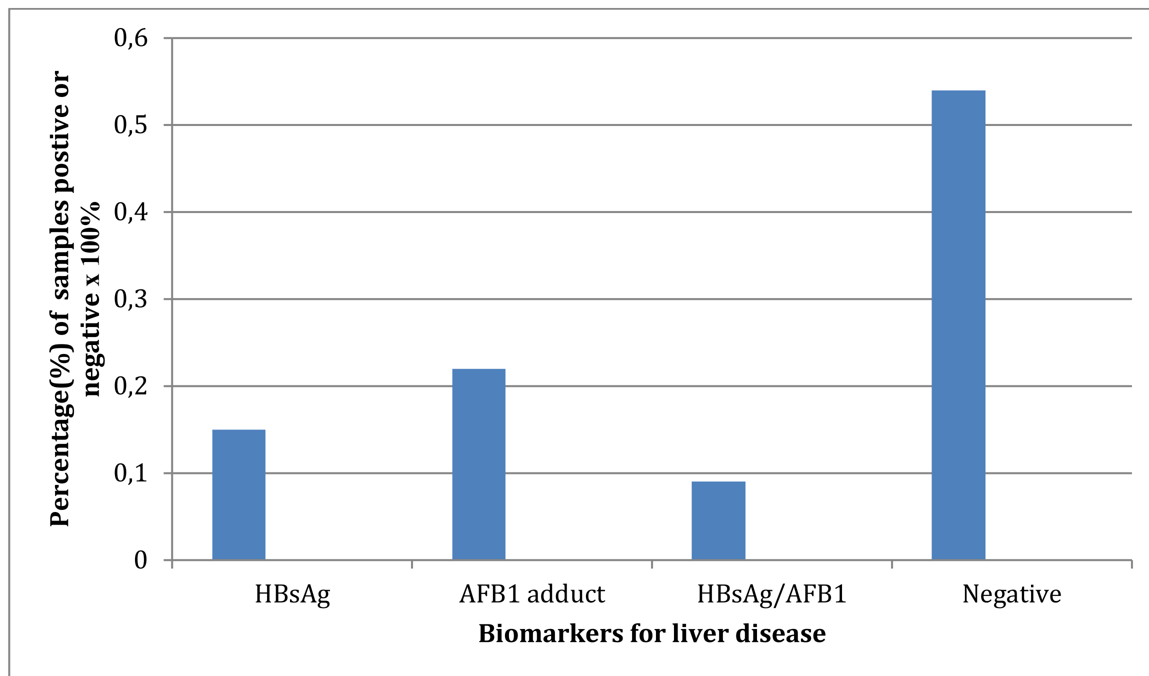

Figure 2: A control comparative bar chart for samples on disease biomarkers

Case cohort hepatitis B surface antigen (HBsAg) level in serum samples

Within the total case sample (N = 283), two categories of serum sample immerged on analysis; those positive for both HBsAg and AFB_1 lysine albumin adducts and those positive for HBsAg only. Among the subcohort identified with only HBsAg, 32.15% (n = 91), of the subject serum samples had positive evidence of hepatitis B surface antigens (HBsAg) at various levels, with a range of 0.50 x 10^3 Iu/mL to 9.80 x 10^3 Iu/mL and a mean of 4.289 x 10^3 Iu/mL, {95%; CI= (3.683 to 4.895) x 10^3}, $p \leq 0.05$. Within the same case-cohort, 20% (n = 57) of the sample was positive for both HBsAg and AFB_1 lysine albumin adducts. Among the case subject samples in this second subcohort, the HBsAg levels ranged from 0.7 x 10^3 Iu/mL to 8.5 x 10^3 Iu/mL, with a mean of 1.471x 10^3 Iu/mL {95%; CI= (1.206 to 1.736) x 10^3} $p\leq 0.05$. In this category of mixed infection, 20% (n=57) of sample had AFB_1 lysine albumen adducts range of 18.60 pg/mg to 102.80 pg/mg with a mean of 41.41 pg/mg {95%; CI= (36.20 to 46.63)} $p\leq 0.05$. Overall however, 52.29% (n = 148), of the case subject blood samples had evidence of active HBV infection as indicated by the quantified levels of hepatitis B surface antigens (HBsAg) with an HBsAg range of 0.50 x 10^3 Iu/mL to 9.80 x10^3 Iu/mL and a mean of 3.204 x 10^3 Iu/mL {95%; CI= (2.76 to 3.65) x 10^3} $p\leq 0.05$.

For the case subject HBsAg positive serum samples, the median mean was 2.20 x 10^3 Iu/mL. Any center HBsAg means above this median was considered high while any mean below was considered low for the purpose of comparisons.

Among the centers with higher sample means for HBsAg levels, Mathuki health center with 0.70% (n=2) of positive subject serum sample had an HBsAg range of 1.8 x 10^3 to 9.8 x 10^3 Iu/mL and a mean of 5.80 x 10^3 Iu/mL, while Mwingi health center with also 0.7% (n=2) of the positive subject sample had an HBsAg range of 1.4 x10^3 to 7.50 x10^3 Iu/mL and a mean of 4.45 x 10^3 Iu/mL. E mali health center in same category but with 1.06% (n=3) of the subject positive sample had an HBsAg range of 0.70 x 10^3 to 8.50 x 10^3 Iu/mL and a sample mean of 4.13 x 10^3 Iu/mL. Table 5 shows those health centers which registered highest mean HBsAg levels irrespective of sample size (n) for serum samples. Makindu health center was among such category with 1.06% (n=3) of positive serum sample which had an HBsAg range of 1.30 x 10^3 to7.80 x 10^3 Iu/mL and a mean of 3.90 x 10^3 Iu/mL. Sultan health center had 2.12% (n=6) of serum samples positive for HBsAg and followed with a range of 0.95 x 10^3 to 7.80 x 10^3 Iu/mL and a mean of 3.86 x 10^3 Iu/mL, while Masaku health facility with 16.6% (n=47) of serum sample positive for HBsAg had a range of 0.50 x 10^3 to 9.80x 10^3 Iu/mL and a mean of 3.57 x 10^3 Iu/mL {95%; CI= (2.73 to 4.41)x10^3}, $p\leq 0.05$. Wote health center with 8.48% (n= 24) of serum sample positive for HBsAg had a range of 0.80 x 10^3 to 9.00 x 10^3 Iu/mL and was among centers which had

comparatively higher mean HBsAg values at 2.69 x 10^3 Iu/mL. Kathozweni health center with 3.18% (n=9) of positive serum sample had an HBsAg sample mean above the median mean at 2.44 x 10^3 Iu/mL and a level range of 0.85 x 10^3 Iu/mL to 6.50 x 10^3 Iu/mL.

Among the centers with HBsAg means below the median mean and therefore categorized as having lower mean HBsAg levels, Kibwezi and Kavisuni health centers had no case HBsAg positive serum samples, while Nuu health center with 0.7% (n=2) of positive subject sample had an HBsAg level range of 0.90 x 10^3 Iu/mL to 1.80 x 10^3 Iu/mL and a center lower mean of 1.35 x 10^3 Iu/mL. Mtito adei health center with only one positive HBsAg sample at 0.35% (n=1) had a mean of 1.60 x 10^3 Iu/mL while Kitui health center with 5.30% (n=15) of the HBsAg positive sample had a range of 0.60 Iu/mL to 9.20 Iu/mL and a mean of 1.70 x 10^3 Iu/mL. Tei wa Yesu health center with 1.41% (n=4) also had an HBsAg range of 0.70x 10^3 Iu/mL to 3.20 x 10^3 Iu/mL and a mean of 1.75 x 10^3 Iu/mL. Kyuso health center had a positive sample midline mean of 2.25 x 10^3 Iu/mL, and an HBsAg levels range of 1.30 x 10^3 Iu/mL to 3.20 x 10^3 Iu/mL. All the other health centers had serum sample HBsAg means above this midline. Table 5 is a summary of the case serum samples size (n), range, median, and mean HBsAg levels per health centre. It also shows the HBsAg positive samples (n) out of the original sample for each center in the study.

Table 5: Case subject sample mean HBsAg levels per health center

Heath center	sample n	range Iu/mL (10^3)	mean Iu/mL (10^3)	median Iu/mL(10^3)	sd
Mutomo	12	0.50—9.80	2.85	1.40	2.994
Tei wa yesu	4	0.70—3.20	1.75	1.55	0.906
Kitui	15	0.60—9.20	1.70	3.10	2.880
Mtito ndooa	4	1.50—8.50	3.26	1.53	3.023
Mwingi	2	1.40—7.50	4.45	4.45	3.050
Kyuso	2	1.30—3.20	2.25	2.25	0.950
Migwani	5	1.10—6.80	3.44	2.30	2.283
Kavisuni	0	0.00	0.00	0.00	0.000
Nuu	2	0.90—1.80	1.35	1.35	0.450
Muthale	7	0.15—9.50	3.14	2.20	2.720
Mathuki	2	1.80—9.80	5.80	5.80	4.00
Kibwezi	0	0.00	0.00	0.00	0.000
Wote	24	0.80—9.00	2.69	1.55	2.392
Sultan H.	6	0.95—7.80	3.86	3.15	2.560
Masaku	47	0.50—9.80	3.57	1.95	2.928
Kathozweni	9	0.85—6.50	2.44	1.80	1.641
Makindu	3	1.30—7.80	3.90	2.60	2.808
Emali	3	0.70—8.50	4.133	3.20	3.252
Mtito adei	1	0	1.60	1.60	0.000

Comparatively though, Nuu health center with 0.7% (n=2) of positive serum sample had the lowest mean HBsAg value at 1.35 x 10^3 Iu/mL followed by Mtito adei health center which was in category of those centers with lower sample HBsAg means, but with only 0.35% (n=1) of positive serum sample, at 1.60 x10^3 Iu/mL lower than that of Kitui health center which had 5.3% (n=15) of positive serum samples having an HBsAg range of 0.60 x 10^3 Iu/mL to 9.20 x 10^3 Iu/mL and a mean of 1.70 x 10^3 Iu/mL. Figure 3 is a bar chart comparison of the case subjects HBsAg mean levels per center with Mathuki, Mwingi and E mali health centers having higher HBsAg center means, while Nuu, Mtito adei , Kitui and Tei wa Yesu centers had lower HBsAg means in serum samples analysed.

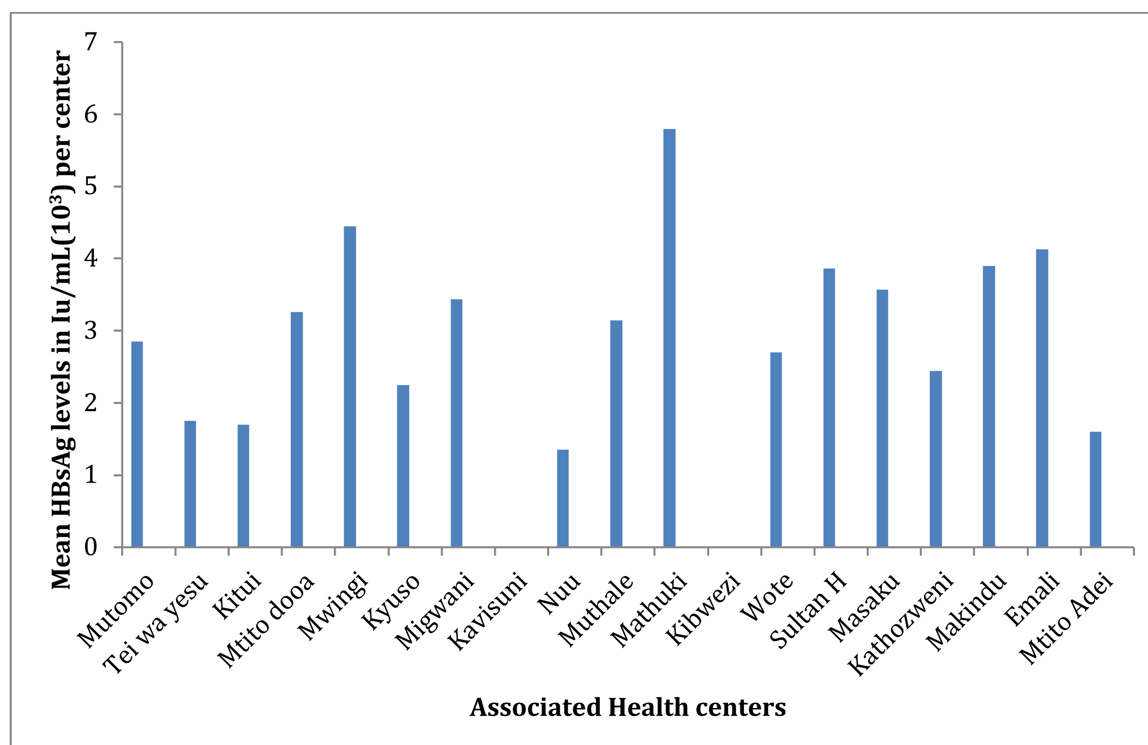

Figure 3: Case subjects serum mean HBsAg levels per health center

Control cohort hepatitis B surface antigen (HBsAg) levels in blood sample

The control cohort had serum samples positive for both HBsAg and AFB_1 lysine albumin adducts but some serum samples were positive for HBsAg only. Within the sample, (N = 283), 15% (n = 42), of serum samples had positive evidence of HBsAg only, with levels ranging from 150 Iu/mL to 990 Iu/mL, and with a mean of 506.3 Iu/mL, (CI = 427.8 to 584.8), at 95% confidence level ($p \leq 0.05$). The control cohort also had 9% (n = 26), of the serum sample positive for both hepatitis B surface antigens and AFB_1 lysine albumin adducts at various levels(Table 6). Among this sub group, 9% (n=26), of sample was positive for AFB_1 lysine albumin adducts with a range of 3.5 pg/mg to 22.4 pg/mg and a mean of 10.35 pg/mg (CI= 8.56 to 12.15) at 95% confidence level ($p\leq 0.05$), while hepatitis B surface antigens (HBsAg) had a range of 50 to 150 Iu/mL, with a mean of 86.35 Iu/mL (CI = 76.99 to 95.70), at 95% confidence level ($p\leq 0.05$). Overall however, 24% (n = 68), of the control subject serum sample was positive for low levels of HBsAg in serum samples with a level range of 50 Iu/mL to 990 Iu/mL and a mean of 347.57 Iu/mL (CI= 278.35 to 416.80) at 95% confidence level, $p\leq 0.05$. Table 6 shows the HBsAg range and the medians per health center. In controls the median mean for HBsAg was 0.15 Iu/mL and therefore ten (10) health centers had mean HBsAg greater than the median while eight (8) centers had means below the median and hence considered to have low mean levels for HBsAg. However, among the control health centers with the low serum sample mean HBsAg in this cohort, Kavisuni and Kyuso centers each with 0.35% (n=1) of the positive subject sample had a mean of 0.70 $x10^3$ and 0.90 x 10^3 Iu/mL respectively, considered low even without a clinical disease among the subjects from whom the serum sample was drawn.

Table 6: Control subjects mean HBsAg levels in serum samples per health center

Health center	sample n	range Iu/mL(10^3)	mean Iu/mL(10^3)	median Iu/mL(10^3)	sd
Mutomo	10	0.085—0.990	0.411	0.40	0.310
Tei wa yesu	1	0.00	0.060	0.06	0.00
Kitui	3	0.095—0.150	0.132	0.15	0.026
Mtito ndooa	0	0.00	0.00	0.00	0.00
Mwingi	4	0.07—0.80	0.500	0.57	0.297
Kyuso	1	0.00	0.900	0.90	0.00
Migwani	4	0.55—0.85	0.683	0.67	0.108

Kavisuni	1	0.00	0.780	0.78	0.00
Nuu	3	0.20—0.55	0.333	0.25	0.155
Muthale	3	0.15—0.95	0.483	0.35	0.339
Mathuki	1	0.00	0.150	0.15	0.00
Kibwezi	0	0.00	0.00	0.00	0.00
Wote	13	0.05—0.50	0.228	0.25	0.144
Sultan H	5	0.20—0.49	0.348	0.35	0.097
Masaku	17	0.06—0.98	0.304	0.10	0.333
Kathozweni	3	0.085—0.150	0.108	0.09	0.030
Makindu	0	0.00	0.00	0.00	0.00
Emali	0	0.00	0.00	0.00	0.00
Mtito Adei	0	0.00	0.00	0.00	0.00

Migwani health center with 1.41% (n=4) of positive serum sample and HBsAg level range of 0.55 x 10^3 to 0.85 x 10^3 Iu/mL had a mean of 0.683 x 10^3 Iu/mL{95%; CI= (0.577 to 0.788) x 10^3} $p \leq 0.05$, while Mwingi health center in same category, had 1.41% (n=4) of positive subject samples with HBsAg range of 0.07 x 10^3 Iu/mL to 0.80 x 10^3 Iu/mL but a lower center mean of 0.50 x 10^3 Iu/mL{CI= (0.208 to 0.791)x 10^3} at 95% confidence level ($p \leq 0.05$). Muthale health center with 1.06% (n=3) of positive serum samples had an HBsAg level range of 0.15 x 10^3 to 0.95 x 10^3 Iu/mL and a mean of 0.483 x 10^3 Iu/mL{95%; CI= (0.099 to 0.866) x 10^3} $p \leq 0.05$. This was followed by Sultan H center with 1.77% (n= 5) of positive serum sample and an HBsAg level range of 0.20 x 10^3 to 0.49 x 10^3 Iu/mL but a mean of 0.348 x 10^3 Iu/mL {95%; CI=(0.263 to 0.433) x10^3} $p \leq 0.05$. This was considered high for a clinically HBV disease free group.

Table 6 shows the control subject mean HBsAg levels in blood samples per health center. It was noted that the health centers with highest numbers (n) of positive samples in control cohort had much lower mean HBsAg values. This included Masaku health center with 6.0% (n=17) of positive serum samples which had an HBsAg level range of 0.06 x 10^3 to 0.98 x 10^3 Iu/mL and a mean of 0.304 x 10^3 Iu/mL{95%; CI= (0.146 to 4.623) x10^3} $p \leq 0.05$, while Wote center with 4.59% (n=13) of positive serum sample had an HBsAg level range of 0.05 x 10^3 to 0.50 x 10^3 Iu/mL and a mean of 0.228 x 10^3 Iu/mL{95%; CI= (0.150 to 0.306) x 10^3} $p \leq 0.05$, among the others.

Among the centers with lowest mean HBsAg levels, Makindu, Emali, Mtito Adei, Kibwezi and Mtito ndooa, each had 0%(n=0) of positive serum sample while Kathozweni with 1.06% (n=3) of positive sample had an HBsAg levels range of 0.085 x 10^3 Iu/mL to 0.150 x 10^3 Iu/mL and a mean of 0.0108 x 10^3 Iu/mL.

Figure 4 bar chart compares the control subjects' sample HBsAg means per center with Kyuso and Kavisuni health centers having the highest control HBsAg means, while Tei wa yesu and Kathozweni had the lowest HBsAg means among the health centers with subjects positive serum samples.

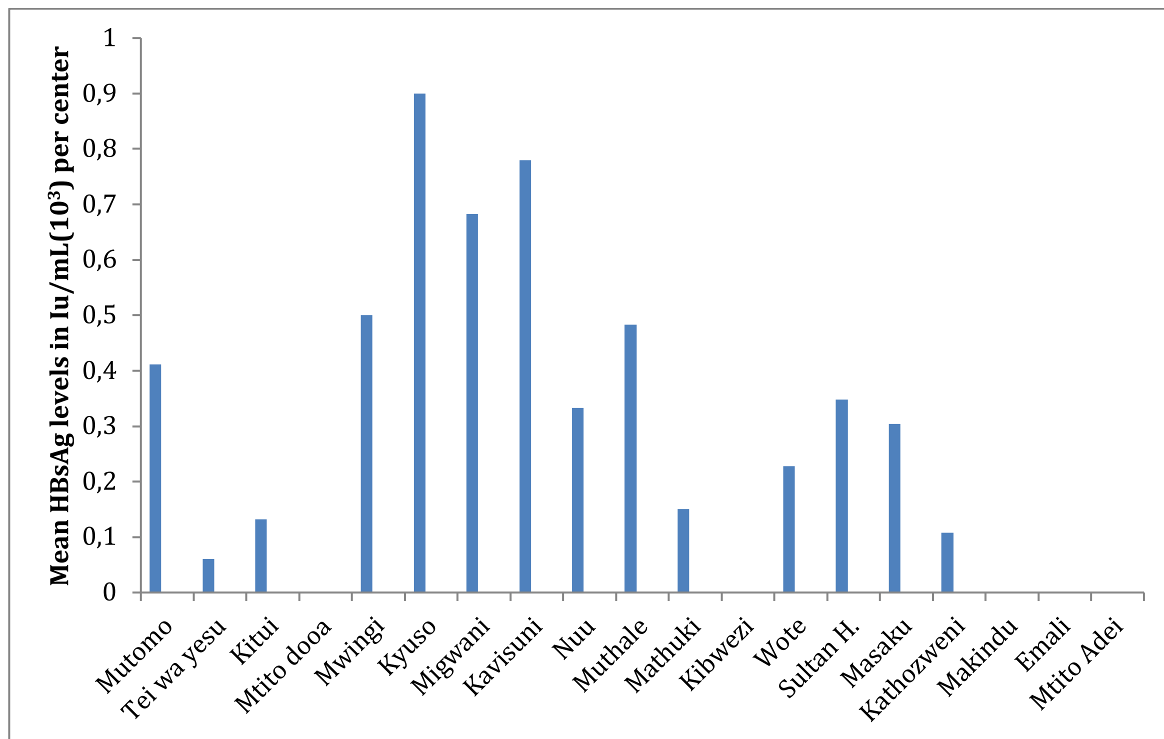

Figure 4: Control subjects mean HBsAg levels in blood samples per health center

Case cohort AFB_1 lysine albumin adducts level in blood samples.

Among the total number of case samples (N = 283), 36% (n = 101), positive subject samples had an AFB_1 lysine albumen adducts level with a range of 15.5 pg/mg to 135 pg/mg, and a mean of 43.64pg/mg (CI= 38.91 to 48.37), at 95% confidence level ($p \leq 0.05$). Additionally, within the same case cohort, 20% (n = 57), of serum samples analyzed tested positive for both hepatitis B surface antigens (HBsAg) and AFB_1 lysine albumin adducts. For this sub cohort, 20% (n=57), of the subject serum sample had AFB_1 lysine albumin adducts with a range of 18.60 pg/mg to 102.80 pg/mg, and a mean of 41.41 pg/mg (95%; CI= 36.195 to 46.624) $p \leq 0.05$, while HBsAg levels ranged from 700 Iu/mL to 8500 Iu/mL, with a mean of 1471 Iu/mL (CI=1205.7 to 1736.3) at 95% confidence level ($p \leq 0.05$) . Overall however, 55.83% (n=158) of the case subject sample had AFB_1 lysine albumin adducts range of 15.5 pg/mg to 135.0 pg/mg with a mean of 42.93 pg/mg (CI=39.36 to 46.51) at 95% confidence level ($p \leq 0.05$).

Among the centers with higher mean for AFB_1 lysine albumin adducts, Mathuki health center with 0.706% (n=2) of the serum samples, had a subject AFB_1 lysine albumin adducts level range of 68.0 pg/mg to 82.50 pg/mg with a mean of 75.75 pg/mg (95%; CI= 65.70 to 85.80) $p \leq 0.05$, while Kathozweni health center with 3.15% (n=9) of subject serum samples had a range of 23.8 pg/mg to 102.80 pg/mg with a mean of 63.80 pg/mg(95%; CI= 46.20 to 81.43), $p \leq 0.05$. E mali health center with 1.06% (n=3) of the serum sample was in same category, with a subject serum AFB_1 lysine albumin adducts range of 18.50 pg/mg to 64.80 pg/mg, but with a center adducts mean of 42.80 pg/mg (CI= 21.40 to 64.20) at 95% confidence level($p \leq 0.05$). Table 6 shows the case subject AFB_1 lysine albumin adducts level per center.

For health centers with lower mean subject AFB_1 lysine albumin adducts in this cohort, Mtito ndooa with 1.06% (n=1) of the sample had a range of 18.80 pg/mg to 25.00 pg/mg and an AFB_1 lysine albumin adducts mean of 21.53 pg/mg (95%; CI= 18.61 to 24.45) $p \leq 0.05$, while Kyuso health center with 0.35% (n=1) of the sample had a mean of 23.80 pg/mg. Tei wa Yesu in this category with 0.71%(n=2) of the sample had an AFB_1 adducts range of 19.80 pg/mg to 31.50 pg/mg and a mean of 25.65 pg/mg(CI= 17.56 to 33.74) at 95% confidence level, $p \leq 0.05$. Table 7 shows the case subjects AFB_1 lysine albumin adducts levels, range, means and median per health center

Table 7: Case subjects AFB_1 lysine albumin adduct level in blood samples per health center

Health center	sample (n)	range pg/mg	mean pg/mg	median pg/mg	sd
Mutomo	11	15.80—93.80	34.06	25.80	24.84
Tei wa yesu	2	19.80—31.50	25.65	25.65	5.840
Kitui	9	16.80—47.80	33.28	33.80	9.344
Mtito ndooa	3	18.80—25.00	21.53	20.80	2.583
Mwingi	8	18.50—96.50	44.83	28.65	30.23
Kyuso	1	0.000	23.80	23.80	0.000
Migwani	7	19.80—63.00	38.08	32.00	17.090
Kavisuni	2	19.80—56.50	38.15	38.15	18.350
Nuu	5	15.80—71.80	38.84	32.00	21.030
Muthale	10	19.50—66.50	34.24	27.90	15.930
Mathuki	2	68.00—82.50	75.25	75.75	7.250
Kibwezi	1	0.000	33.80	33.80	0.000
Wote	24	17.50—64.50	34.15	31.25	11.797
Sultan H	7	19.80—74.50	43.66	43.00	21.040
Masaku	52	15.50—97.80	47.38	43.00	22.500
Kathozweni	9	23.80—102.8	62.13	63.80	26.980
Makindu	5	24.00—55.00	40.62	38.80	11.410
Emali	3	18.50—64.80	42.03	42.80	18.910
Mtito Adei	3	53.30—135.0	68.50	68.50	35.417

Figure 5 shows a comparison on case subject mean AFB_1 lysine albumin adducts between health centers with Mathuki health center having higher subject mean of 75.75 pg/mg, while Mtito ndooa health center had the lowest AFB_1 lysine albumin mean at 20.80 pg/mg of albumin.

Within the same case cohort, a total of 55.83% (n = 158), of case subject serum samples had tested positive for AFB_1 lysine albumin adducts, suggesting a mixed etiology for liver disease.

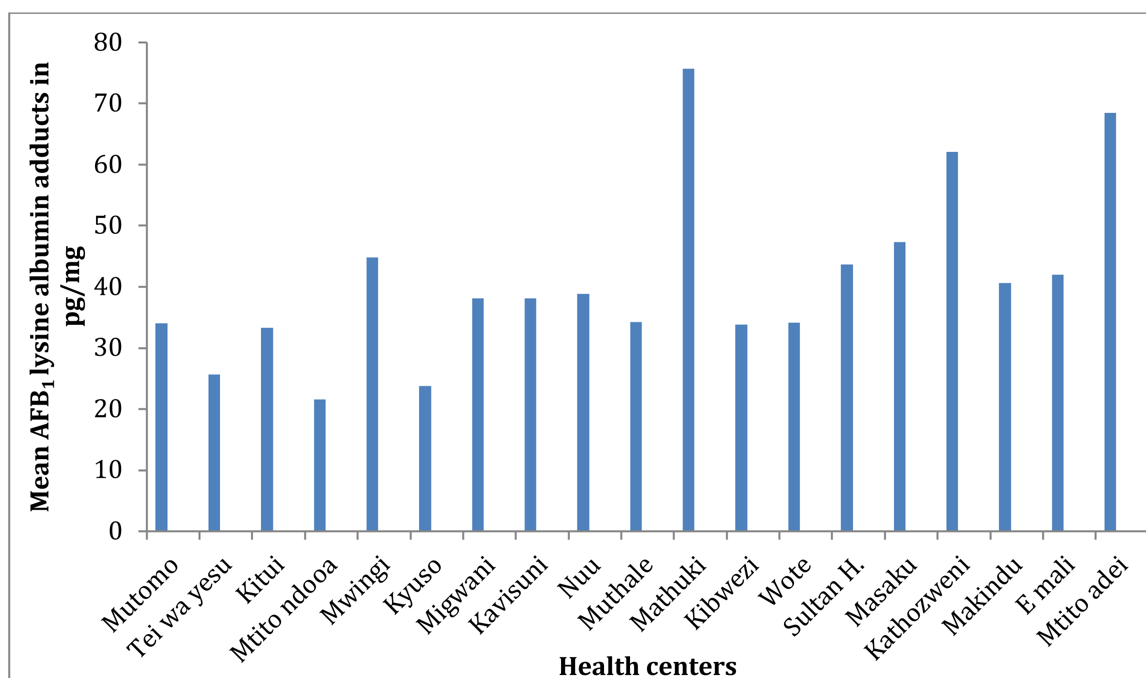

Figure 5: Case mean AFB_1 lysine albumin adducts level in blood samples per center

Control cohort AFB_1 lysine albumin adducts level in blood samples

Among the control cohort sample (N=283), 22% (n = 62), of the sample had tested positive for AFB_1 lysine albumin adducts with a range of 4.30 pg/mg to 60.50 pg/mg and a mean of 15.95 pg/mg (CI = 13.22 to 18.67) at 95% confidence levels ($p \leq 0.05$). Within the same cohort, 9% (n = 26), of the control serum samples had evidence of both HBsAg and AFB_1 lysine albumin adducts at various levels (Table 8).

Table 8: Control subjects mean AFB_1 lysine albumin adduct level in serum samples per center

Health center	positive sample (n)	range pg/mg	mean pg/mg	median pg/mg	sd
Mutomo	8	6.50—12.00	9.03	7.90	2.130
Tei wa yesu	2	5.50—16.50	11	11	5.500
Kitui	7	9.50—29.50	19.73	19.50	6.387
Mtito ndooa	1	0.00	4.50	4.50	0.000
Mwingi	3	5.80—15.60	12.30	15.50	4.596
Kyuso	0	0.00	0.00	0.00	0.000
Migwani	1	0.00	8.40	8.40	0.000
Kavisuni	0	0.00	0.00	0.00	0.000
Nuu	1	0.00	8.00	8.00	0.000
Muthale	5	5.50—32.50	18.12	18.80	10.452
Mathuki	2	17.00—28.50	22.75	22.75	5.750
Kibwezi	0	0.00	0.00	0.00	0.000
Wote	11	4.50—23.40	12.25	13.00	5.269
Sultan H.	1	0.00	9.50	9.50	0.000
Masaku	33	3.50—40.50	13.90	10.80	9.262
Kathozweni	8	6.40—60.50	23.125	15.70	18.281
Makindu	3	5.80—10.40	8.33	8.80	1.906
E mali	1	0.00	7.50	7.50	0.00
Mtito adei	1	0.00	11.40	11.40	0.00

Within this sub cohort, sample serum hepatitis B surface antigens (HBsAg) levels had a range of 50 Iu/mL to 150 Iu/mL with a mean of 86.35 Iu/mL (95%; CI= 76.99 to 95.69) $p \leq 0.05$, while AFB_1 lysine albumin adducts had a level range of 3.5 pg/mg to 22.4 pg/mg with a mean of 10.35 pg/mg (CI= 8.56 to 12.15) at 95% confidence level ($p \leq 0.05$). Overall however, 31% (n=88) of the control subject serum samples were positive for AFB_1 lysine albumin adducts with a level range of 3.5 pg/mg to 60.50 pg/mg and a mean of 14.30 pg/mg (CI= 12.23 to 16.36) at 95% confidence level ($p \leq 0.05$). Table 8 shows the control subjects mean AFB_1 lysine albumin adduct levels per health center.

Among the control centers with higher mean AFB_1 lysine albumin adducts level, Kathonzweni health center with 2.83%(n=8) of positive serum samples had an AFB_1 albumin adducts range of 6.4 pg/mg to 60.5 pg/mg and a mean of 23.125 pg/mg (95%; CI=10.46 to 35.80) $p \leq 0.05$, while Mathuki center with 0.71% (n=2) of positive serum sample had an AFB_1 adducts range of 17.00 pg/mg to 28.50 pg/mg with a mean of 22.75pg/mg (95%; CI=14.78 to 30.72) $p \leq 0.05$. In this category, Kitui health center with 2.47% (n=7) of positive serum samples had a range of 9.50 pg/mg to 29.50 pg/mg with an AFB_1 lysine albumin adducts mean of 19.73 pg/mg (CI=14.99 to 24.46) at 95% confidence level ($p \leq 0.05$). Among centers with lower mean AFB_1 lysine albumin adducts, Mtito ndooa and E mali health centers with 0.35% (n=1) each, of positive serum sample, had an AFB_1 lysine albumin adduct mean of 4.50 pg/mg and 7.50 pg/mg respectively. Makindu center in this lower mean category, had a sample range of 5.8 pg/mg to 10.4 pg/mg with a mean of 8.33 pg/mg (CI= 6.17 to 10.49) at 95% confidence level ($p \leq 0.05$). Figure 6 shows a bar chart comparison of AFB_1 lysine albumin adduct means per center, with Kathozweni and Mathuki health centers having highest mean AFB_1 albumin adducts at 22.75 pg/mg and 23.13 pg/mg respectively, while Mtito ndooa had the lowest mean AFB_1 serum adducts at 4.5 pg/mg.

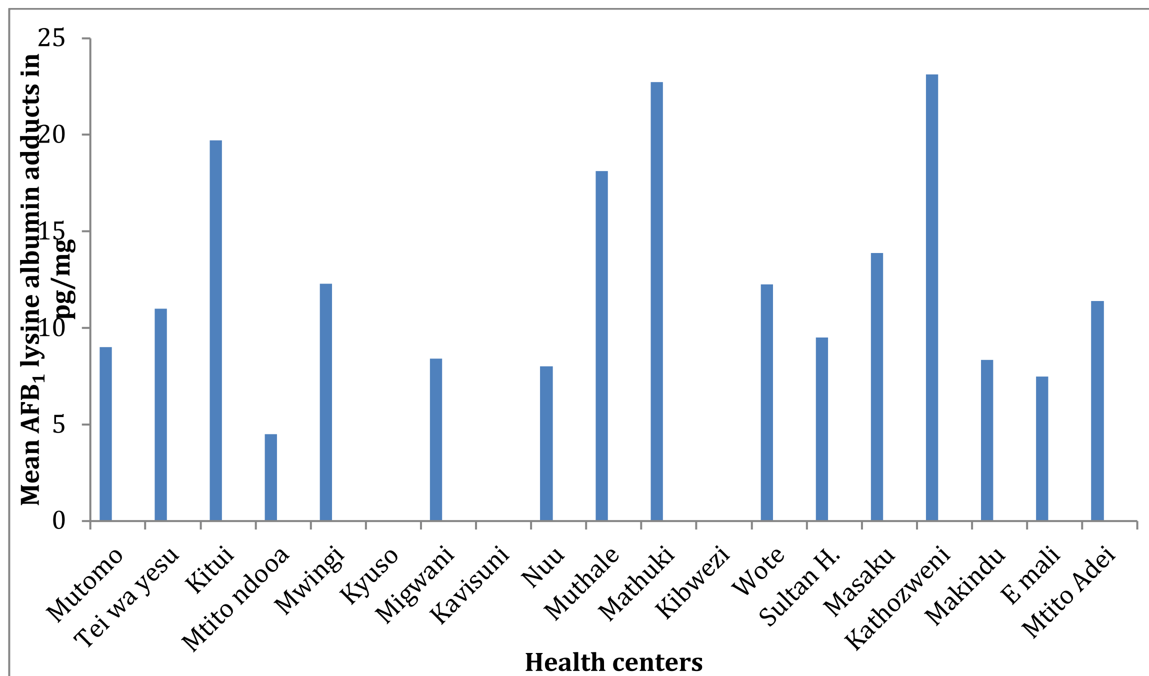

Figure 6: Control subjects mean AFB_1 lysine albumin levels per health center

DISCUSSION

The current work is the first study to evaluate the link between the elevated levels of serum HBsAg and AFB_1 lysine albumin adducts as biomarkers to liver disease in lower eastern Kenya.

Lower eastern Kenya has had an aflatoxicosis outbreak severally in the near past, with the outbreak of April 2004 generating 317 cases and causing 125 deaths (Lewis *et al.,* 2005). A cross sectional study by the team revealed a high level of AFB1 contamination of maize grain which was and still his the stable food in this region (Lewis *et al*., 2005). Aflatoxin B1 is also metabolized primarily in the liver by cytochrome p-450 system, forming the highly reactive AFB_1-8, 9-epoxide which binds to hepatic cell's DNA. This bio chemical reaction forms AFB_1-formalmidopyrimidine DNA adducts which alters normal cell function, making the macro molecule highly carcinogenic (Turner *et al.,* 1998). Studies have shown that, the half life (1/2) of serum albumin adducts stored under normal temperature is 20 days, hence any chronic exposure to dietary AFB_1 leads to high circulatory concentration of AFB_1 lysine albumin adducts leading to chronic liver damage, liver disease or sudden aflatoxicosis, depending on a patient's immune system in dealing with the toxin (Scholl & Groopman, 2008). In this study the case subjects had more positive serum samples 55.83% (n=158) testing for AFB_1 lysine albumin adducts than control subjects at 31.0% (n=88), who had no liver disease. Similarly, the case subject mean AFB_1 lysine albumin adducts was higher at 42.93 pg/mg (95%; CI=39.36 to 46.51) $p \leq 0.05$, than the controls at 14.30 pg/mg (95%; CI=12.23 to 16.36) $p \leq 0.05$. According to Turner, *et al.,*(2013), formation of AFB_1 serum albumin adducts is dose dependent, with accumulation of the lysine albumin adducts correlating well with exposure to dietary AFB_1 in many cases. This observation agrees with this study, which is suggestive of high exposure to dietary AFB_1 among the case subjects in the region. The lower mean level for AFB_1 lysine albumin adducts in controls who had no evidence of liver disease suggested a certain cumulative threshold for ingested AFB1 as one causal agent for the liver disease had not been met. This suggested that AFB1 induced hepatoxicity in case subjects in this study were dose and level dependent. This agrees with studies by Turner *et al. (*2013).

This observation again agrees with other studies by Muthomi *et al.,* (2009) and Muhia *et al.,* (2008) which found that subjects in this region were exposed to higher levels of aflatoxin B1 from dietary maize grain. Other studies have shown that products of exposure to dietary AFB_1 and other mycotoxins, end up in the liver and the circulatory system, including AFB_1 lysine albumin adducts and are highly toxic to the human body, at times

causing acute jaundice and liver failure (WHO, 2018; Redd *et al.,*2009). This agrees with the current study, which found varying levels of AFB_1 lysine albumin adducts in both case and control serum samples.

Exposure to HBV may lead to acute or chronic hepatitis B infection depending on age and the immune status of an individual whose diagnosis relies heavily on the presence of HBsAg as a biomarker of liver disease in subject serum samples (Terrault *et al.,* 2016). The lower mean of HBsAg in control subject samples, which were disease-free of 3.476×10^2 Iu/mL suggested that subjects were under incubation for HBV or had recovered after an exposure to an HBV infection. This was supported by studies by Dufour (2006), who observed that HBsAg was detectable in chronic, passive, or acute hepatitis B infections, even soon after recovery. In the same study, 20% (n=57) of case subjects serum samples were positive for both HBsAg and AFB_1 lysine albumin adducts, while the control subjects had 9.0% (n=26) of the sample positive for the combined AFB_1 serum adducts and HBV infection. In this study, the case subjects had a higher number of samples with mixed infection than the controls. This suggested that a combined infection of HBsAg and AFB_1 toxicity had a synergistic or an additive effect on liver damage and hence, liver disease among the subjects. This view is supported by studies, including that which exposed the synergistic effect of dietary AFB_1 and HBV in transgenic mice on hepatocellular carcinoma (Kew, 2003). Further, since the control subjects were not among those admitted to health centers for liver disease and did not have physical symptoms of the disease, this was suggestive of a recent exposure or recovery from an HBV infection.

CONCLUSION

This is the first case-control study in lower eastern Kenya to link the serum levels of HBsAg and AFB_1 lysine albumin adducts to liver disease among the subjects. This evaluation study exposed dietary AFB_1 and HBV as endemic multifactor etiology for liver disease in lower eastern Kenya. The residents of the study area were found to be highly exposed to dietary AFB_1 and hepatitis B virus as determined by the quantified AFB_1 lysine albumin adducts and HBsAg values in serum samples presented in the study. While the control subjects were disease-free, some had evidence of a recent exposure to the two liver disease factors with HBsAg means of 347.57 Iu/mL (95%; CI= 278 to 416.80) $p \leq 0.05$ and AFB_1 lysine albumin adducts mean of 14.30 pg/mg (95%; CI=12.23 to 16.36) $p \leq 0.05$. Within the case and control cohorts, the percentage (%) of subject serum samples testing positive for AFB_1 serum albumin adducts were more (36.13%), than those testing positive for HBsAg (30.83%). It is therefore concluded that, even though both dietary AFB_1 toxicity and HBV infection were endemic in the area, comparatively dietary AFB_1 toxicity was much more prevalent in the region than hepatitis B infections and that the two factors had an additive effect to liver disease

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