THE FACTORS ASSOCIATED WITH PREVALENCE OF LIVER DISEASE AMONG SERO POSITIVE HBV AND AFB_1 INDIVIDUALS IN SELECTED HEALTH FACILITIES IN KITUI AND MAKUENI COUNTIES, KENYA

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The Factors Associated with Prevalence of Liver Disease among Sero Positive HBV and AFB_1 Individuals in Selected Health Facilities in Kitui and Makueni Counties, Kenya

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other university

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DEDICATION

This thesis is dedicated to the great matriach Angela Mutiku, my mother Felistus Mulau, my father Francis Kioko and to all the members of my family Veronica, Peter, John, Joshua, Laura and Ann Mutisya. To them I wish to say, “Determination is the price to pay for success”. I thank you for your torelance and As always, remember that, this far the Lord has brought us.

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ABBREVIATIONS AND ACRONYMS

ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
AFB_1	Aflatoxin B1
CI	Confidence interval
CTL	Cytotoxic T lymphocytes
CHIA	Chemiluminescence microparticle immuno assay
CV	Coefficient of variation
ELISA	Enzyme linked immunosorbent assay
EDTA	Ethylene diamine acetate
ERC	Ethical review committee
FAO	Food agricultural organization
FDA	Food drug administration
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HRP	Horseradish peroxidase
HB_eAg	Hepatitis B e Antigen
HB_sAg	Hepatitis B surface Antigen
HB_cAg	Hepatitis B core Antigen
LFT	Liver function test
N	Population size
n	Sample size
NPV	Negative predictive value

OR	Odds ratio
RR	Relative risk
Ẍ	Mean
χ^2	Chi square
Iu/ mL	international units per milliter
ppb	Parts per billion
SPSS	Statistical package for social sciences
SSC	Science steering committee
Sd	Standard deviation
ϭ̄	Standard deviation of a population data
TMB	Tetra methyl benzidine
t	T distribution
Z	Normal distribution
ALP	Alkaline phosphatase
LD	L- lactate dehydrogenase
PT	Prothrombin time
GGT	Gamma- glutamyltransferase
INR	International normalized ratios
NAFLD	Non alcholic fatty liver disease
AFBO	AFB_1 -exo-8, 9-epoxide
DNA	Deoxyribonucleic acid
CYP450	Cytochrome 450 enzyme system
OS	Oxidative stress

ROS	Reactive oxygen species
TLR	Toll like receptors
TEER	Trans-epithelial restistance

ABSTRACT

There are various causes of liver disease including viruses, trauma and chemical poisoning. The viruses causing liver disease includes hepatitis A, B, C and D while ingestion of aflatoxinB_1 contaminated food stuff may cause aflatoxicosis which manifests as hepatoxicity and in severe cases, sudden liver failure. An outbreak of aflatoxin poisoning and aflatoxicosis associated with aflatoxin B_1 contaminated maize grain and flour had been reported over the years in parts of Kitui, Makueni and Machakos counties of lower easten Kenya. This could have compounded a suspected existing problem of a viral disease burden including hepatitis B virus induced hepatitis among individuals within the population. A study was therefore conducted to determine the prevalence of liver disease due to sero prevalence of HBV and AFB_1 among the subjects, the relative risks of aquiring the disease in the study area and the association existing between liver disease and other suspected non causal contributory factors. The investigation was carried out in two steps, where the first was a case-control study where blood was analyzed for exposure and non exposure to AFB_1 and HBV while the second was an observational cross-sectional study where subject household grain and flour samples were collected and analyzed for AFB_1 in parts per billion (ppb). A non probability purposive sampling method was used to choose and divide the study area into strata with 19 health centers. The sample size (n) for the human case-control study was determined as 283 while Fisher *et al*, (1998) formula was used to calculate the minimum sample size for grain samples as 130, but was purposively adjusted to 283 to match the study participants. Individual serum samples were analysed for liver disease biomarkers, hepatitis B surface antigens, AFB_1 lysine albumen adducts and critical liver function enzyme indicators Aspertate amino transferase and Alanine amino transferase in the study. Study participant household grain and flour samples of 0.25kg each were collected and analyzed by Elisa method for AFB_1 in parts per billion (ppb). Structured questionnaire was used for data on suspected liver disease associated factors, including blood transfusion, unprotected sex, untreated water, unsterile body piercing instruments, and AFB_1 contaminated maize grain. A computer software SPSS® version 18.0 was used to statistically analyze the data. The case AST level range was 55.6 IU/mL to 344.50 IU/mL with a mean of 154.86 IU/mL (95%, CI; 147.52 to 162.20) p= 0.05, while the control AST level range of 9.85 to 332.50 IU/mL had a mean of 35.30 IU/mL (95%, CI; 27.86 to 42.76), p= 0.05. The case ALT level range was 58 to 444.51 IU/mL with a mean of 173.32 IU/mL (95%, CI; 159.13 to 187.5) p= 0.05, while the control ALT range was 8.20 to73.50 IU/mL with a mean of 28.41 IU/mL (95%, CI; 25.96 to 30.86) p=0.05). The case subjects in this study had a highly elevated AST levels with 100% (n=283), of samples having values greater than 40 IU/mL and 99.64% (n=282) of serum samples with ALT levels above 30 IU/ml. Case AFB_1 lysine albumin adducts level had a range of 15.5 to 135 pg/mg with a mean of 42.19 pg/mg (95%, CI; 38.45 to 45.93) p=0.05, while controls had a lower range of 3.5 to 60.5 pg/mg and a mean of 13.15 pg/mg (95%, CI; 12.3 to 15.0). In case cohort, 49.5% (n=140) of sample had HBsAg mean of 3481 IU/mL (95%, CI; 3037 to 3925) p= 0.05, while in controls 24% (n=68) of participants serum sample had a lower mean of 347.57 IU/mL (95%, CI; 278.5 to 416.80) p=0.05. The

prevalence of liver disease associated with HBV was 24.73%, due to dietary AFB_1 was 25.97%, while that due to combined AFB_1 and HBV infection was 1.24%. The relative risk (RR) due to HBV infection and dietary AFB_1 was 1.022 (95%, CI; 0.81 to 1.205) and 1.073 (95%, CI; 0.91 to 1.264), p=0.05 respectively. In conclusion, the etiologic agents HBV and dietary AFB_1 were both found endemic but the dietary AFB_1 induced hepatoxicity was more prevalent than HBV infections in the region. A vaccination campaign against HBV and training on better grain storage methods should be initiated to further lower AFB_1 toxicity incidence and hence the disease prevalence in lower eastern Kenya.

CHAPTER ONE

INTRODUCTION

1.1 Background information

Liver disease is known to have various caustive agents including viruses, toxins, autoimmune diseases and even physical injuries. Various studies have linked exposure to aflatoxins to chronic and acute hepato cellular injury leading to chronic liver diseases, including hepato cellular carcinoma. Aflatoxins are defined as toxic secondary metabolites produced by fungal strains of the genus *Aspergillus*, mostly *A. flatus, A. parasiticus, A. nomius* and *A.astellatus* (Lawley, 2013) that grow on a variety of substrates. 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 been shown to be a highly potent natural hepato-carcinogen and is usually the major aflatoxin produced by the aflatoxigenic fungal strains.

Aflatoxins have assumed significance due to their deleterious effects on human beings and animals including livestock and poultry. The aflatoxin problem was first recognized in 1960 when severe outbreak of a disease referred to as "Turkey 'X' disease" occurred in UK. In that epidemic, 100,000 turkey poults died. The cause of the disease was shown to be due to toxins in peanut meal infected with *Aspergillus flatus.* The toxins were named as aflatoxins (Arkell, 2015).

Naturally, aflatoxin producing fungi occur in certain food products in 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, chillies, 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 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 value of grain as animal feed and devalues it as a commodity for human consumption (Michell *et al.,* 2016). Because of the hepatoxicity of these aflatoxins, the duration of exposure is of particular public health concern. Both the Food and Agricultural Organization (FAO), and Agrofood 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 aflatoxin 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 in acute aflatoxicosis which manifests as hepatoxicity or in severe cases sudden liver failure (Golthardt *et al.,* 2009). To reduce the incidence of aflatoxin poisoning, codex alimentarius commission (CAC), has recommended that the levels of aflatoxins in food stuff for human consumption should be less or equal to 10 ppb (Codex Commission, 2008). Federal Drug Agency (FDA) has regulated that corn or corn products intended for animal feeds should have aflatoxin levels ≤ 20 ppb (FDA, 2009).

Aflatoxins have varying molecular weights with B_1 having the lowest followed by B_2, G_1 and G_2 hence they can be separated into individual components by thin layer chromatography. Aflatoxins also fluoresce strongly in the ultra violet light (ca 365 mm), with B_1 and B_2 producing blue fluorescence, whereas G_1 and G_2 produce green florescence (Vosough *et al.,* 2010). This has been used as a method of categorization of these toxic substances in human food.

As with aflatoxins, infection with hepatitis B virus leads to liver diseases including hepatocellular carcinoma, fulminant liver failure, liver cirrhosis (hardening of liver tissue), and membranous glomerular nephritis (MGN), with the attendant symptoms (Wang *et al.*, 2019; 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 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 new born develop full immunity to the disease (Bell & Nguyen, 2009). An estimated 30% of those infected do not show typical signs or symptoms (Bowyer *et al.,* 2011; Liaw *et al.,* 2010).

The enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are mainly found in the liver, but also found in red blood cells, heart cells, muscle tissue and other organs, such as the pancreas and kidneys. These enzymes were formerly called serum glutamic oxaloacetic transaminase (GOT) and serum glutamic pyruvic transaminase (GPT), respectively. These enzyme (AST/ ALT) levels are valuable aids primarily in the diagnosis of liver disease and can be used in combination with other enzymes to monitor the progress and even prognosis of various liver disorders. The normal concentrations in the blood are from 5 to 40 Iu/mL for AST and from 5 to 35 Iu/mL for ALT. However, when body tissue or an organ such as the liver or heart is diseased or damaged, additional AST and ALT are released into the bloodstream, causing levels of the enzyme to rise. Therefore, the amount of AST and ALT in the blood is directly linked in a dose-response relationship to the extent of the tissue damage. After severe damage, AST levels rise 10 to 20 times greater than normal, whereas ALT can reach higher levels (up to 50 times greater than normal). On the other hand, the ratio of AST to ALT (AST/ALT) sometimes can help determine whether the liver or another organ has been damaged (Bowyer *et al.,* 2011).

1.2 Statement of the problem

In Kenya, aflatoxin poisoning results in liver failure and deaths in up to 40% of the cases (Yard *et al.,* 2013). Aflatoxin poisoning is also referred to as aflatoxicosis. Usually this comes as a result of maize grain contamination with aflatoxigenic molds. According to FAO, maize is a staple foodstuff in Kenya and over 90% of the population relies on maize grain and maize products for food (Kang'ethe, 2011). In Kitui, Makueni, Machakos and other arid districts in the eastern part of Kenya, maize, the staple food crop grown is harvested and stored in traditional granaries.

There has been a reported outbreak of aflatoxin poisoning associated with consumption of contaminated white maize grain and maize meal in these districts for the last 30years (Wild and Gong, 2010). Aflatoxin producing fungi, including those of the genus *Aspergillus* grow on damp starchy foodstuffs such as groundnuts, pearl millets, maize grains and maize flour, oats and sorghum when temperature is favorable. Consumption of aflatoxin contaminated maize grain for years with or without co infection with HBV has been implicated` in liver disease with fatalities in these areas (Yard *et al.,* 2013).

In April 2004, an outbreak of acute hepatoxicity was identified among people living in Kenya's eastern and central provinces. An epidemiological investigation determined that the outbreak was as a result of aflatoxin poisoning from ingestion of contaminated white maize. As of 20th July, same year (2004), 317 aflatoxicosis cases and 125 deaths had occurred in the region making it one of the largest and most severe outbreaks of acute aflatoxicosis documented worldwide (Yard *et al.,* 2013). The outbreak covered more than seven districts encompassing an area approximately 40,149 km^2. Out of the 317 case-patients, 89% resided in four districts including Makueni, Kitui, Machakos and Thika. The latest estimated total population of these four districts is 4.45 million (KNBS, 2019).

An epidemiological study comparing the four districts including Makueni and Kitui showed that, Makueni and Kitui regions were heavily affected and represented 47% and 32% of Case-Patient respectively. This was followed by Machakos, 6% and Thika 4 % (CDC, 2004). A study of hospital data, (Ministry of health: Kitui district health report 1997- 2006) showed that between 1997 and 2006, the total admission in the then Kitui district hospital alone due to consumption of aflatoxin contaminated maize grain and maize meal was 670 individuals with a case fatality of 59.7% recorded by the time. Synergistic effects of AFB_1 and HBV leading to fatal liver disease including hepatocellular carcinoma (HCC), have been documented. While the occurrence of aflatoxins in the study area has extensively been researched and the prevalence of hepatitis B virus (HBV) in Kenya is known, the synergistic effect of the two factors in liver disease among residents needs elucidation, while the prevalence of liver disease in the study area is not known and needs to be

determined. The fact that the study area experiences frequent episodes of food shortage necessitated the study to be conducted as malnutrition compromises the immune response among individuals and favors rapid progression of disease.

1.3 Justification of the study

Kitui and Makueni are arid and semi arid in most parts, with a minimal 61mm of rainfall per annum (KNBS, 2009). Poor rainfall breeds poverty in most households with poverty index of 63.1% and 64.1% in Kitui and Makueni counties respectively (KAIS, 2007), which promotes poor sanitation methods, poor storage of maize grain, unprotected sex, non- treatment of water and hence constant poor health which may lead to blood transfusion. All these are factors associated with liver disease, since they may lead to serum presence of AFB_1 and HBV in subjects (Danuba *et al.,* 2013; CDC, 2011).

There have been episodes of AFB_1 poisoning in these areas (Yard *et al.,* 2013), but no study has focused on the prevalence of liver disease as a function of AFB_1 and HBV, and the synergistic effect, if any, of combined AFB_1 and HBV sero presence in the subjects. Furthermore, the risks of contracting the disease due to AFB_1 and HBV, and any possible link between non AFB_1 and HBV contributory or associated factors and liver disease has never been determined in those two counties. Such study will enable the county government and other stakeholders put mitigation measures in place to reduce cases of liver disease. Such mitigation measures could be a campaign for free introduction of anti- HBV vaccine which is already available.

1.4 Null hypotheses

Ho_1: There is no significant difference between prevalence of liver disease between those with HBV and those without.

Ho_2: There is no difference in prevalence of liver disease between those with combined serum presence of HBV, AFB_1 and those without.

Ho_3: There is no significant risk of liver disease among those exposed to HBV and AFB_1 in the study area

Ho_4: AFB_1 is not a major cause of liver disease in the area under investigation

1.5 Objectives

1.5.1 General objective

To determine the prevalence and distribution of liver disease due sero positivity of AFB_1 and HBV and the magnitude of association between non-HBV and AFB_1 contributory factors and liver disease in Kitui and Makueni counties.

1.5.2 Specific objectives

1. To determine AST, ALT, HBsAg, AFB_1 lysine albumin adduct level for participant blood samples in the study area.
2. To determine the serum prevalence of HBV among the participants in the study area.
3. To determine the serum prevalence of AFB_1 among participants in the study area.
4. To determine the prevalence of liver disease due to a combined serum presence of AFB_1 and HBV among the study participants in study area.
5. To dertemine the relative risk of liver disease among study participants due to HBV sero positivity, AFB_1 sero positivity and combined AFB_1 and HBV sero positivity in the study area.
6. To determine the magnitude of association between AFB_1 induced liver disease and maize grain and flour consumption among the participants in the study area.

1.6 Research questions

1. What is the level of AST, ALT, HBsAg, and AFB_1 lysine albumin adducts in study participants serum samples from the study area?
2. What is the prevalence of liver disease which is attributable to HBV sero positivity in area of study?

3. What is the prevalence of liver disease due to serum presence of AFB_1 among the participants in the study area?
4. What is the prevalence of liver disease due to combined effect of AFB_1 and HBV sero presence among study participants within the study area?
5. What are the relative risks of liver disease among study participants due to HBV, AFB_1 and combined HBV- AFB_1 sero positivity in the study area?
6. What is the magnitude of association between household maize grain and flour, and AFB_1 induced liver disease in the study area?

1.7 Variables of the study

The dependant variable in the study was the liver disease, defined as any condition affecting the proper function of the liver, while independent variables were aflatoxicosis due to dietary AFB_1 and infection due to HBV. The suspect contributory independent variables were identified as unprotected sex, blood transfusion, unsterile body peircing instruments, untreated drinking water, AFB_1-contaminated maize grain and maize flour used as food by the community, since they may have promoted infection with HBV and contributed towards aflatoxin B_1 toxicity respectively.

CHAPTER TWO

LITERATURE REVIEW

2.1 Anatomy and physiology of the liver

The human liver is the largest organ in the body weighing between 1.5 to 2 kg and it is divided into the right and the left lobes, with the right lobe being larger than the left. On anterior and the superior surfaces, the right and left lobe meet along the line of attachment of the falciform ligament. On the posterior and inferior surfaces, the lobar separation is more obvious and is represented by two fissures, the fissure for *ligamentum venosum* on the posterior surface and the fissure for the *ligamentum teres* on the inferior surface (Leiskau *et al.,* 2017). The quadrate lobe is on the inferior hepatic surface, while the coudate lobe is attached to the posterior surface. The human liver is protected by the ribs in the right upper quadrant and connected to both the systematic vascular system (hepatic artery and vein) and the portal vascular system (portal vein). The liver has unusual blood supply, with the hepatic artery carrying about 30% while the hepatic vein carries about 70% of the blood to the liver. This represents about 25% of human total cardiac blood output at rest. The liver filters and processes blood as it circulates through the body. It metabolises nutrients, detoxifies harmful substances, makes blood clotting proteins and performs many other vital functions. The cells in the liver contain proteins inform of enzymes that drive these bio chemical reactions (Leiskau *et al.,* 2017).

There are six major functions of the liver including metabolic, secretory, excretory, synthetic, storage, immunological and detoxification functions. The liver also plays an important role in the breakdown of unfunctional red blood cells and haemoglobin. Hemolysis takes place in multiple locations through out the body including the liver, spleen and bone marrow. Heme is broken down into biliverdin by the hepatocytes, which is then reduced to unconjugated bilirubin. Bilirubin is an important fluid as it helps excrete material not excreted by the kidneys and aids in the digestion of lipids through secreted bile salts and acids. The liver receives unconjugated bilirubin bound

to albumin from circulation. The unconjugated bilirubin then undergoes conjugation through the uridine diphosphate glucuromyl transferase (UGT) system to become hydrophobic. The newly conjugated bilirubin is then secreted through the bile canaliculi into the bile sac with some amount dissolving in the blood where it gets filtered for excretion by the kidneys. Most conjugated bilirubin fluid however enters the bile duct and gets excreted through the faecal matter. Some bilirubin is converted to unconjugated bilirubin by gut bacteria and reabsorbed to undergo the enterohepatic circulation again. The liver interacts with the endocrine and gastro intestinal systems by aiding in digestion and metabolism. It plays a role in hematology with clotting factors and protein synthesis as it stores fat soluble vitamins including vitamin A and K, while handling cholesterol homeostastis. The liver plays an important role in sex hormones metabolism as it produces carrier hormones which are important in reproduction and development. The immunological function of the liver is carried out by the specialised Kupffer and Pit cells which produce some antibodies against a multitude of infections caused by some viruses and bacteria (Kalra *et al.,* 2021).

When liver cells are damaged or destroyed, the enzymes in the cells leak out into the blood, where they can be measured by blood tests. Elevated liver function tests (LFTs) are found in approximately 8% of the general populations. These elevations may be transient in patients without symptoms, with the symptoms resolving after 3 weeks. However, diseases of the liver are diagnosed by an array of blood tests collectively termed as liver function tests (LFTs), which includes alanine transferase (ALT), aspertate transferase (AST), gammaglutamyl transferase (GGT), serum bilirubin, prothrombin time (PT), international normalized ratio (INR) and albumin (Lala *et al.*,2021). The tests are also used to assess the proper working of the liver in particular circumstances including:

1. Screening for liver infections such as hepatitis A, B, C and E
2. Monitoring the progression of a disease such as a viral or alcoholic hepatitis and determining the progress of certain type of medical treatment
3. Measuring the severity of certain diseases particularly scarring of the liver(cirrhosis)

4. Monitoring of possible side effects of certain medication

Alanine transferase (ALT), is an enzyme found in the liver that helps convert proteins into energy for the liver cells. When the liver is damaged, ALT is released into the blood stream thereby increasing the levels. Aspertate transferase (AST) is an enzyme that helps metabolize amino acids. It is usually found in low amounts in blood, thus an increase of AST beyond a certain limit may indicate liver damage, liver disease or muscle damage.

Alkaline phosphatase (ALP), is also an enzyme found in the liver and the bonemarrow which is important for protein metabolism by the liver. Higher than normal levels of ALP may indicate liver damage, a blocked bile duct or a certain bone disease. The test for elevated levels for this enzyme coupled with a similar test for gamma-glutamyltransferase (GGT) also found in the liver may indicate liver damage and blocked bile duct. Similarly, elevated levels of L-lactate dehydrogenase (LD) may indicate damage but may also indicate the use of blood thinning drugs including warfarin among others (Kalra *et al.,* 2021).

Albumin is one of several proteins made in the liver. These proteins are important for the immunological function of the liver. A test indicating less than normal set limit for albumin and total protein coupled with an increased prothrombin time (PT), defined as the time it takes for human blood to clot, may indicate liver damage or liver disease (Yin & Tong, 2009).

The two main transaminase enzyme measurements used frequently as liver function tests (LFTs) to check for liver disease are:

a. Aspartate aminotransferase (AST), which is an enzyme also found in muscles and many other tissues besides the liver.
b. Alanine aminotransferase (ALT) which is an enzyme almost exclusively found in the liver.

The test for elevated ALT and AST is reasonably sensitive and rapid, explaining its widespread use. When ALT and AST are found together in elevated levels in blood circulation, then liver damage is most likely the diagnosis (Lala *et al.,* 2021).

2.2 Pathogenicity of liver disease.

Liver disease is defined as any liver disorder affecting the proper function of the liver. It occurs when there is an impairment of liver functions (Yin & Tong, 2009). The eatiology of liver disease could be categorized into two parts: Infectious and non-infectious causes. The infectious liver diseases are those caused by a form of pathogen including a virus and a type of bacteria. These include hepatitis A, hepatitis B, hepatitis C, primary biliary cholangitis and primary sclerosing cholangitis. Non infectious liver diseases are not caused by any pathogen and hence not transmitable. These diseases include non alcoholic fatty liver (NAFLD), cirrhosis, hepatocellular carcinoma, alcoholic hepatitis, hemochromatosis, cell adenoma, Wilson disease and hyperoxaluria. The eatiologies for non infectious liver disease include;

a. Chronic alcohol abuse.
b. Poisoning by various substances, including mushrooms, phosphorous, aflatoxins, carbon tetrachloride and other organic solvents.
c. Paracetamol overdose which may occur at a lower level for chronic alcohol users
d. Drug toxicity associated medication including ciprofloxacin, doxycycline, erythromycin, isoniazid, and nitrofurantoin, among others.
e. Cocaine, ectasy and other illicit drugs.
f. Genetic factors, as in Wilson disease, hemochromatosis and hyperoxaluria

These factors differ from infectious liver diseases causative factors (eatiologies) which are particular pathogens including;

a. Hepatitis A virus
b. Hepatitis B virus
c. Hepatitis C virus
d. Adenovirus

e. Epstein barr virus
f. Cytomegalovirus
g. Hemorrhagic fever virus

2.3 Dietary aflatoxinB_1 toxicity

Aflatoxins elicit a variety of biological effects in humans and animals which include liver and kidney toxicity, genotoxicity, suppression of the immune system and aggravation of kwashiorkor in children (Zain, 2011). In humans, enlarged fatty livers are common at low doses of aflatoxin poisoning (Uslusoy *et al.,* 2011). Aflatoxin ingestion may result in acute hepatitis that presents either as jaundice or elevated liver enzymes, usually preceded with a prodromal feverlike illness. The clinical features give little indications as to the likely etiological agent. Photophobia, headache and cough may also be dominant in aflatoxin induced hepatitis. A serum sickness like illness occurs in about 60% of patients with acute aflatoxin induced hepatitis. This is also characterized by urticarial or maculo papular rash and arthralgia. This typically affects the wrist, knees, elbows and ankles. This is due to immune complex formations. Rheumatoid factors are frequently positive and are almost always self limiting and usually settle rapidly after the onset of jaundice. Aflatoxin induced hepatitis may produce other sub clinical problems including tinnitus and blurred vision (Samuel *et al.,* 2009).

Elevated liver enzymes have been found to be major indicators of liver fibroids in aflatoxin induced hepatitis. Aspartate amino transferase (AST) higher serum levels predict progression of liver disease more than alanine amino transferase (ALT) serum levels. Higher (AST) serum levels can therefore be used as biochemical indicators for advanced fibrosis or progression of liver diseases in aflatoxin poisoning which may also induce liver cirrhosis, steatosis and fibroids (Uslusoy *et al.,* 2011).

Aflatoxin poisoning can result to either acute toxicity or chronic toxicity. Various studies have estimated that the LD_{50} for AFB_1 for human cell lines is between 9.0mg/kg to 12mg/kg (Mckeen *et al.,* 2006). Further more acute AFB_1 toxicity is characterized by high fever, dark colored urine, vomiting and oedema of feet,

jaundice, rapidly developing ascitis, portal hypertension and a high mortality rate. The disease is common among the very poor, who are forced by economic circumstances to consume badly molded corn containing aflatoxins (Samuel *et al.*, 2009; Ellen *et al.*, 2013)

Chronic toxicity arises due to long term exposure to aflatoxins in the diet and the risk increases due to synergistic effect from increased alcohol consumption and a co infection with HBV. Aflatoxin B_1 has also been implicated as a cause of human hepatic cell carcinoma (HCC). However, subsequent studies in animals have demonstrated carcinogenicity to other organs other than the liver, including the kidneys, the pancreas, the bladder, bone viscera and the central nervous system, hence affecting the brain in some animal models (Fouad *et al.*, 2019). AflatoxinB1 chemically binds to DNA and causes structural DNA alterations with the result of a possible genomic mutation in mammalian cells (El-Amir *et al.*, 2012). Although these mutations are more pronounced in adults, ingestion of aflatoxins, viral diseases, and hereditary factors have been suggested as possible etiological agents of childhood cirrhosis. There is evidence to suggest that children exposed to aflatoxin contaminated breast milk and dietary items such as unrefined groundnut oil, may develop cirrhosis.

2.4 Mechanisms of aflatoxin B_1 toxicity

Consumption of aflatoxin B_1contaminated foodstuff may causes aflatoxicosis. The African and Asian continents are the leading regions affected by aflatoxicosis with recorded new cases of 7.7% (64,779) and 72% (609,596) per annum respectively. Together, this represents 80% of the world new cases per year (Benkerroum, 2020). Aflatoxin B_1 is also a major eatiology for hepatocellular carcinoma with estimated 25,200 to 155,000 new cases associated with it per year (Liu *et al.*, 2010). Among these new cases for hepatocellular carcinoma (HCC), 40% occurs in sub-Saharan Africa, where aflatoxin B_1 induced liver cancer alone accounts for one third of all new cancer cases (Gibb *et al.*, 2010). At country level, China with its large population has the highest incidence of hepatocellular carcinoma with majority of cases recorded at sourthen part of the country including Guangxi region where HBV

and aflatoxicosis, factors associated with HCC are endemic (McGlynn & London, 2005).

2.4.1 Mechanisms of AFB_1 genotoxicity

Chronic exposure to low doses of aflatoxins over a long time may cause chronic diseases, the most frequent and severe of which is cancer of various organs. Although dietary intake of aflatoxins has been classically associated with primary hepatocellullar carcinoma and bile duct hyperplasia, other organs, such as the kidney, the pancreas, the bladder, bone, and the viscera, have also been reported to develop cancers upon exposure to these mycotoxins (Fouad *et al.,* 2019). In addition, aflatoxins were reported to cause lung and skin occupational cancers via inhalation and direct contact, respectively (Marchese *et al.*, 2018). Infact, chronic exposure to aflatoxins causes a range of other severe diseases, including immunosuppression, teratogenicity, mutagenicity, cytotoxicity, and estrogenic effects in mammals (Klvana & Bren, 2019). Moreover, aflatoxins are believed to be involved in nutritional disorders, such as kwashiorkor and stunted growth, probably by interfering with the absorption of micronutrients including zinc, iron, vitamins and also protein synthesis together with metabolic enzyme activities (Turner, 2013). In domestic animals, feeds contaminated with sub-lethal doses of aflatoxins induce impaired productivity and reproduction, increased susceptibility to diseases, and reduced quality of the foods they produce (WHO, 2017). Despite the insidious character of chronic aflatoxin-induced diseases, their impact on public health globally is more severe and costlier than acute aflatoxicosis. Although aflatoxicosis outbreaks induce hundreds of deaths at once in an intermittent manner, they can be prevented or interrupted upon analysis of suspect foodstuffs even without evident mould growth, and their disposal effected if aflatoxin levels exceed the regulatory standard limits (Benkerroum, 2020).

The mutagenic effects of AFB_1 is mainly due to its intermediate metabolite AFB_1-exo-8,9 epoxide At the molecular level, aflatoxin B_1 has been demonstrated to alter the mechanical, chemical, and immune barriers that protect the intestinal mucosa against various external threats (Benkerroum, 2019). As a highly unstable molecule,

AFBO reacts with cellular macromolecules, including nucleic acid (DNA), proteins, and phospholipids, to induce various genetic, metabolic, signalling, and cell structure disruptions (Rushing & Selim, 2017). However, various studies have shown equally dramatic or higher effects of AFB_1 on cell function and integrity through the induction of oxidative stress (Ayala *et al.,* 2014). Once AFB_1 enters the human liver through the ingestion of contaminated foodstuffs, various isozymes of the cytochrome 450 enzyme systems react with it to form AFB_1-exo- 8,9-epoxide (AFBO) which reacts with cellular DNA to form unstable AFB_1-N^7-guanine adduct. The unstable adduct further reacts with isozymes in liver to form four (4) macromolecules (isomers) namely, apurinic DNA (damaged DNA), AFB_1-N^7-guanine, *trans*-AFB_1-FAPy-DNA adduct whose formula is *trans*-8,9-dihydro-8-(N5-formyl-2,5,6-triamino-4-oxo-N5-pyrimidyl)-9-hydroxy-AFB_1 and *cis*-AFB_1-FAPy-DNA adduct whose formula is *cis*- 8,9-dihydro-8-(N5-formyl-2,5,6-triamino-4-oxo-N5-pyrimidyl)-9-hydroxy-AFB_1. These AFBO generated adducts interferes with gene expression, DNA integrity, and genome stability thereby inhibiting DNA repair and hence genomic mutations expressed as cancers in various organs (Marin *et al.*, 2012, Benkerroum, 2019). Furthermore, AFB_1 is also acted upon by an isozyme of CYP 450 enzyme system, including CYP1A2 in liver through a different biochemical pathway, forming AFB_1-endo-8, 9-epoxide and reactive oxygen species both of which are stable macromolecules but which induce lesion on DNA macromolecule whose repair mechanism may be hindered by presence of HBV proteins for those co infected with HBV (Kew, 2003, Benkerroum, 2019).

2.4.2 Mechanisms of AFB_1 immunotoxicity

Oxidative stress and Reactive oxygen species both produced by reaction of AFB_1 and CYP 450 enzyme system isozymes in human liver, can do damage to cell wall lipid membranes to cause genotoxicity, immunotoxicity and acute intoxication by reacting with genomic DNA, other functional macromolecules and immunocompetent cells. Increased frequency and severity, and prolonged healing of infectious diseases, in addition to decreased vaccination efficacies provided evidence that aflatoxins disrupt both innate and acquired (adaptive) immunity (Coppock *et al.*, 2018). The general mechanisms of AFB_1 immunotoxicity is such that once AFBO is

formed by the action of various CYP450 isozymes, it interacts with immunocompetent cells through out the body affecting their proliferation and production of immune response mediators, thereby disrupting and dysregulating the innate and adaptive immunity where AFB_1 down regulates or up regulates the gene expressions of cytokines, chemokines and immunoglobulins (Igs). All this interferes with human body immune response (Benkerroum, 2019).

2.4.3 Mechanism of aflatoxin B_1 oxidative stress toxicity

Even though the mutagenic effect of aflatoxinB_1 has been primarily attributed to the formation of aflatoxin-N7-gua DNA adducts, recent studies have shown that it can also arise from the oxidative stress produced by AFB_1 metabolism. The oxidative stress radicals act on the DNA to induce the highly unstable oxidative DNA damage molecules or at times through the formation of by products from lipid peroxidation of membrane phospholipids (Ignatov *et al.,* 2017). Processing of AFB_1 in the liver by CYP450 enzymes induces oxidative stress, releasing excessive amounts of reactive oxygen species that can attack nitrogen bases and deoxyribose moieties of the DNA and generate more than hundred different DNA adducts (Evans *et al.,* 2004). The adduct 7,8-dihydro-8-oxo-20-deoxyguanosine (8-hydroxydeoxyguanosine, 8-oxo-dG, 8-OH-dG) derived from the oxidation of the DNA guanine residue by the hydroxyl radical generated by the oxidative stress radicals and which is also commonly used as a biomarker for oxidative DNA damage is also highly toxic and is associated with DNA mutagenesis in animals (Klaunig *et al.,* 2009, Benkerroum, 2019).

2.4.4 Mechanisms of AFB_1 innate immunity toxicity

When the physical barriers such as the skin and the intestinal epithelial cells are destroyed or damaged by AFB_1 toxicity, then the impairment of the barrier function against microbial and toxin invasions follows as a consequence and this has been demonstrated in vitro and in vivo studies. Indeed, the contact of AFB_1 with the skin of different animals has been shown to elicit various types of lesions spanning from the formation of intra-epidermal vesicles to squamous cell carcinoma (Rastogi *et al.,* 2006, Doi *et al.,* 2014). In a study where pigs were fed for four (4) weeks on feed

contaminated with mixtures of aflatoxins including AFG_2, AFG_1, AFB_1, and AFB_2, crusting and skin ulceration on the snout, lips, and buccal commissures was shown to be highly prevalent (Benkerroum, 2019). Aflatoxins, especially AFB_1 have also been shown to disrupt the integrity and function of the mechanical barrier of intestines by destroying the intestinal epithelial cells and the tight junctions (TJs) that cement them together or by interfering with the cell cycle progression. A study was contacted to determine how aflatoxin affected chicken cell cycle, where administration of 0.6 mg AFB_1 per kg to young chicken for three weeks stalled the cell cycle at the G2/M phase causing a reduction in the height of jejunum and in the villus height- crypt ratio, thereby impairing their function as a selective barrier (Yin *et al.,* 2016). These kinds of physiological changes dramatically alter the barrier function of the intestine to interfere not only with the innate immune response as a protective means against the invasion of pathogens or toxins, but also with nutrient absorption causing studed growth and disease. Studies have shown that aflatoxins are highly toxic to immune cells that play key roles in the innate immunity, such as monocytes, macrophages, dendritic cells, and natural killer cells. The toxins especially AFB_1 restrict their viability and function, as well as their genetic expression of cytokines and chemokines. Again, exposure of young chicken to AFB_1 was reported to repress the transcription of toll-like receptors (TLR) including TLR-2, TLR-4, and TLR-7, showing evidence of a suppressive effect on the innate immunity where these receptor proteins are involved in the recognition of external invaders by sentinel cells like the macrophages and dendritic cells, as a key step to trigger this type of immune response (Wang *et al.,* 2018). Aflatoxins AFB_1, AFB_2, and AFM_1 were reported to reduce viability and proliferation of many types of immune cells, which was an indication of a cytotoxic effect. It was shown that, a low dose of 10 ng/mL of AFB_1 reduced the antigen-presenting activity of porcine dendritic cells, although this reduction may not be associated with down regulation of the expression of TLRs or specific cytokines (Mehrzad *et al.,* 2014). In an invitro exposure study of human cell line (CacO-2), to 100 μM (micro moles) of AFB_1 for two (2) days, it was shown that the trans epithelial electrical resistance (TEER) decreased, with consequent increase in the paracellular permeability and decrease in cell viability, thus at the molecular level, aflatoxin B_1 has been demonstrated to alter

the mechanical, chemical, and immune barriers that protect the intestinal mucosa against various external threats (Romero *et al.,* 2016).

2.4.5 Mechanisms of AFB_1 neuro- degenerative toxicity

It is now known that in, addition to the classically known adverse health effects of aflatoxins, there is a body of evidence showing that chronic exposure to aflatoxins is also responsible for neuro degenerative disorders including dementia and alzheimers disease. Indeed, chronic infections with hepatitis B virus (HBV), where shown to potentiate AFB_1 general toxicity to more than sixty (60) times, the original measured toxicity value for aflatoxicosis (Henry *et al.,* 2002). Chronic HBV infection may induce the cytochrome p450 enyme system to metabolise AFB_1 to the mutagenic AFB1- 8,9-epoxide which inhibits hepatocyte regeneration and causes generation of oxygen and nitrogen reactive species.The AFBO and reactive oxygen species generated by CYP450 enzymes and aflatoxin-induced oxidative stress, respectively, react with functional macromolecules in neuronal brain cells where they inhibit lipid and protein synthesis to induce their degenerative toxicity (Wild *et al.,* 2015). Futhermore, aflatoxins were also reported to disrupt the structure and function of mitochondria of brain cells, which impedes oxidative phosphorylation and leads to their apoptosis (Verma, 2004). Furthur to the oxidative stress, aflatoxins induce neurodegenerative disorders by dysregulating the immune response of immunocompetent cells and creating pro inflammatory conditions in the central nervous system (Mehrzad *et al.,* 2017). When an HBV infection occurs together with AFB_1 toxicity the probability of induced p^{53} 249^{ser} and other mutations with the subsequent clonal expansion of cells containing the mutations increases. Nuclear excision repair which is normally responsible for removing the toxic AFB_1-DNA adduct is inhibited by the HBV protein favouring the persitance of the existing mutations. This contributes to uncontrolled cell cycling even within brain cells resulting in several disorders including brain tumors, dementia and alzheimers disease when P^{53} is non functional (Kew, 2003).

2.5 Hepatitis B viral infection and liver disease

Infection with Hepatitis B virus leads to hepatitis B disease which is grouped into two broad categories namely: Acute hepatitis B and Chronic hepatitis B diseases.

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 development of jaundice. It has been noted that itchy skin has been an indication as a possible symptom of all hepatitis virus types. The illness lasts for a few weeks and then gradually improves in most affected people. A few Cases may have more severe liver disease leading to sudden liver failure, and fatalities incase of treatment failure. The infection may be entirely asymptomatic and may go unrecognized (Liaw *et al.,* 2010).

Chronic hepatitis B either may 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).

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, known as hepatocytes. A functional receptor for the virus has been identified as a specific bile acid transporter called sodium taurocholate contransporting polypeptide (NTCP) (Yan *et al.,* 2012). There is however evidence that the receptor in hepatitis B virus is carboxypeptidase D (Glebe & 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 on 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).

Transmission of hepatitis B virus results from exposure to infectious blood or body fluids containing blood. Possible forms of transmission include sexual contact (Fairley and Read 2012), blood transfusions and transfusion with other human blood products (Buddeberg *et al.,* 2008). 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 among adults cannot be associated with any identifiable risk factor (Redd *et al.,* 2007).

The hepatitis B surface antigen (*HBsAg*) is most frequently used to screen for the presence of this 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. The 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 panels 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. 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 may be revealed by a biopsy (Lok & McMahon, 2007).

2.6 Global pattern of liver diseases due to HBV and HCV

Worldwide 350 million people are estimated to be infected chronically with HBV, while 170 million have hepatitis C virus (HCV), with 30% of the infections associated with liver cirrhosis and hepato cellular carcinoma in each category, however when other etiologies are factored in, the absolute estimated number of chronic liver disease (CLD) cases is 1.5 billion worlds wide (Cheemerla & Balakrishinan, 2021). The most prevalent type of CLD is NAFLD (59%), HBV (29%), HEV (9%) and ALD (2%). Other type of liver diseases including primary cholangitis, primary sclerosing cholangitis, alpha-1-antitrypsin deficiency, Wilson's disease and autoimmune hepatitis account for 1% of the cases (Cheemerla & Balakrishinan, 2021). Combined together however, the prevalence of liver disease due to HBV in Europe, North America and Australia is between 0.2% and 7% of the total population, while Eastern Europe, South America, Russia and the Mediterranean countries have a combined mean prevalence of between 3-11% (ECDC, 2010). In the US alone, the prevalence of liver disease due to chronic HBV and HCV is 0.5% and 1.8% respectively. This low prevalence has been attributed to National Vaccination Campaigns since 1991 in the US (Wesley *et al.*, 2010).

The highest disease burden is carried by tropical Africa, South East Asia and China with a combined disease prevalence of between 7-20%, while Africa alone accounts for an estimated prevalence of 5.3% (Andre, 2000). Studies in Kenya have shown an HB_SAg carrier rate of 10 to 15% (Muchiri *et al.*, 2012), with an estimated mean regional disease prevalence due to HBV, of 7% and a national prevalence of 5.1% (Mutuma *et al.*, 2011).

2.7 Conceptual frame work

A conceptual framework is an illustration of variables required in a study and how these variables relate to each other in visual or diagramatic form. In this study, the factors affecting the development of liver disease were classified into two categories namely the etiological factors which directly cause disease and affect the functions of the liver as an organ through a biochemical process and the disease associated factors which through a particular process, help transmit the pathological agents for liver disease. The factors associated with liver disease could also be described as disease environmental factors. Various studies have shown that some of the etiological factors for liver disease are: -

1. Hepatitis A, B, C, D and E which cause viral liver disease (hepatitis) in humans
2. Aflatoxins B_1, B_2, G_1, G_2 M_1 and M_2 of which aflatoxinB1 (AFB_1) is of particular interest since it is highly toxic and a natural carcinogen (Ramalho *et al.,* 2018).

Hepatitis B and C affect more people in the world today than hepatitis A, D and E. Furthur more, both hepatitis B and C chronic infection is the leading cause of hepatocellullar carcinoma (Martel *et al.,* 2015). Hepatitis A is more readily transmitted through the faeco- oral route in contaminated food and water than through sexual contact, while HBV is more efficiently transmitted sexually in both heterosexual and homosexuals (Brooks *et al.,* 2002). Hepatitis E just like hepatitis B and C is highly transmittable especially through a poorly screened blood, during emergency blood transfusion (Denner *et al.,* 2019). Some of the major environmental risk factors associated with liver diseases in various studies have been shown to be: -

1. Untreated water
2. AFB_1 contaminated cereals including maize grain and flour
3. Alcohol abuse
4. Poorly screened blood in emergercy blood transfusion
5. Unprotected sex with infected persons
6. Sharing of body pearcing instruments.

Acohol abuse has a persistence and harmful consequence to individuals including liver disease and alcohol dependence psychiatric disorders (Stickel *et al.,* 2017). While blood transfusion is a life saving procedure and while the procedure demands screening of blood, a nascent viral infection may not be detected by such procedure including Hepatitis B, C, and E.

The study through inclusion and exclusion criteria, sought to determine the prevalence of HBV and AFB_1 toxicity and also the extent to which the environmental variables contributed to the edemicity of liver disease among the residents of lower eastern Kenya. Figure 2.1 is a schematic diagram for a conceptual framework on particular independent variables for liver disease which was a dependent variable in the study.

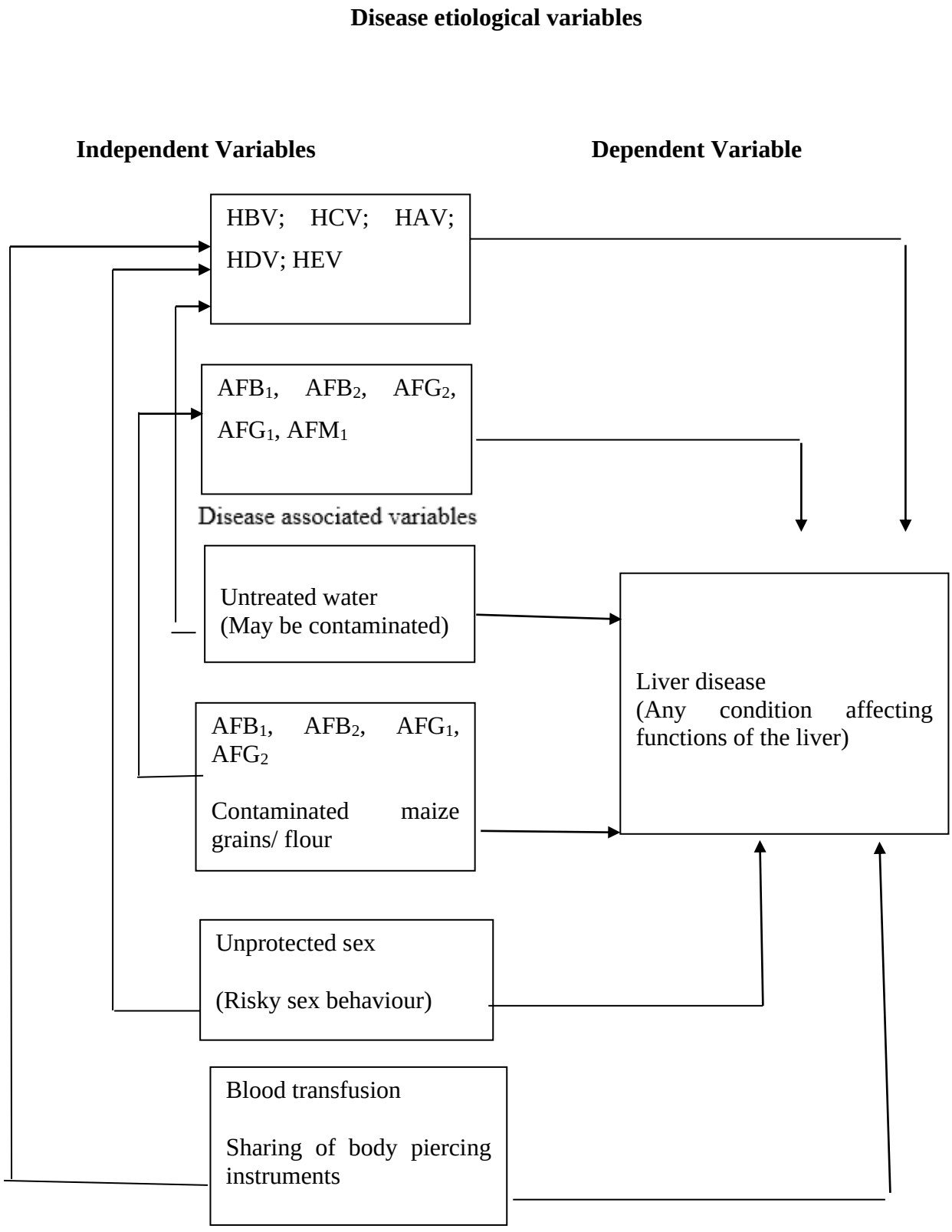

Figure 2.1: A Schematic Conceptual Framework

2.8 Assumption of the study

Part of this study will be cross sectional where maize grain and flour samples will be collected from the homes of both cases and controls households at a particular point in time. It will be assumed that the maize grain will have been grown, harvested and stored in the homes of both cases and controls and not bought in markets or bought from ouside the counties of study.

It is assumed that both cases and controls will have answered the selection criteria questionnaires truthfully so that those selected would not introduce confounding or other biases in the study.

2.9 Limitation of the study

Even though this study will particularly be screening for HBV and AFB_1 albumin adducts in serum samples of both cases and controls, there is a possibility that some of the cases had co-morbidities of liver disease including hepatitis A, C and E which would also raise AST- ALT ratio just like those with liver disease due to HBV and AFB_1 toxicity. Hence the AST- ALT ratio in this study may be used to diagnose liver disease but not a particular aetiology.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area

The study was conducted in lower eastern Kenya region in particular Kitui and Makueni counties including areas bordering Makueni and Machakos County since many people travel to Masaku referral hospital from Makueni County (Table 3.I). Non probability purposeful sampling method was used to choose these areas among the many Counties of lower eastern Kenya due to the region's frequent reports of aflatoxin B_1 induced aflatoxicosis (Mutegi *et al.*, 2018). The study area was stratified into two main strata even though a preliminary questionnaire-based survey showed that, a larger part of the population in upper parts of Makueni and Kitui Counties were being refered to a level five (5) health facility for treatment which Masaku referral health facility offered. Purposeful sampling technique was used to determine the nineteen (19) health facilities representing the health centers from where the subject serum samples were sourced. Serum samples were also sourced from Kitui and Makueni residents admitted at Masaku referral facility. A characteristic of the study area was that, it is arid and semi arid with seasonal rains and hence perennial food shortage stemming from low, unreliable and erratic rainfalls of about 71mm per annum (KNBS, 2019).

This lead to perennial crop failure and loss of livelihood, which predisposes the community to poverty, with poverty index for Kitui, Makueni and Machakos Counties being 63.1%, 64.1% and 60.8% respectively (KNBS, 2009; MFP, 2009). The poverty level was therefore a factor impacting negatively on health of the residents in these Counties leading to vices like alcohol abuse including the local brews (*Muratina*) and risky sexual behaviours (KAIS, 2007). Poverty leads to poor sanitation and lack of treated water for domestic use among the residents. According to studies by Ataei *et al*., (2019) and Anaedobe *et al.*, (2015) lower economic status, blood transfusion, sexual exposure and poor sanitation were among some of the sociodemographic variables which were contributory factors for HBV infection and

eventual progression to chronic liver disease for those unable to develop immunity to the disease.

Table 3.1: Distribution of the health facilities within the strata

Kitui	**Makueni**
Kavisuni	Kibwezi
Muthale	Emali
Mutomo	Sultan Hamud
Migwani	Mtito Adei
Kitui	Wote
Nuu	Masaku
Tei wa yesu	Kathozweni
Mathuki	Makindu
Mwingi	
Kyuso	
Mtito ndooa	

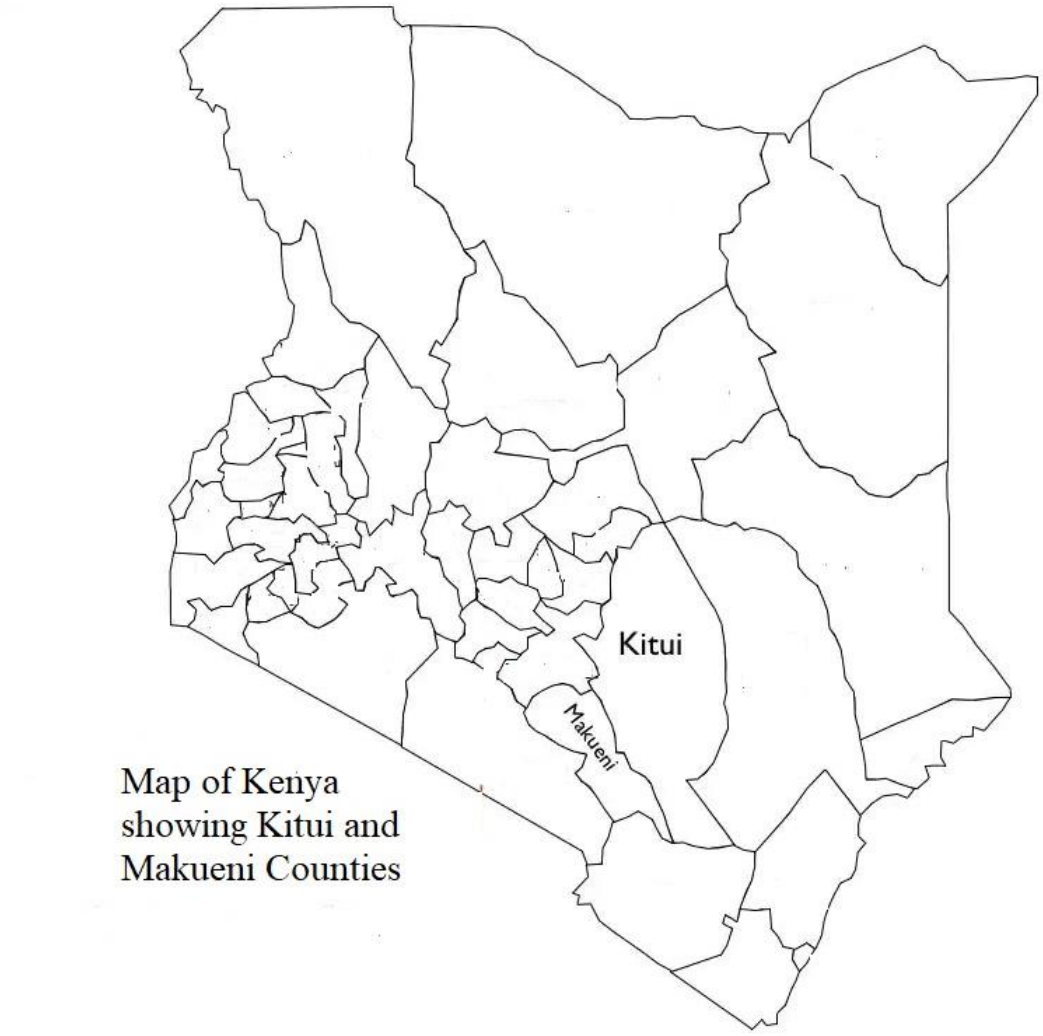

Figure 3.1: A map of Kenya showing Kitui and Makueni counties.

3.2 Study design

A study design is the formulation of a method and procedure to enable the collection and analysis of data hence this study was conducted in two stages. The first stage was a Case - Control study design for human subjects involving collection and laboratory analysis of blood samples for the levels of HB_SAg, ALT, AST and AFB_1-serum albumen adducts which determined the actual exposure and non exposure to HBV and AFB_1 among the subjects. For both Case and Controls, a structured questionnaire employing Likert five (5) point scale was administered to help determine by which percentage (%), in absolute terms the suspected contributory factors associated with liver disease including blood transfusion, unprotected sex,

untreated water, unsterile body piercing instruments and AFB_1- contaminated food stuff contributed to the actual disease among the Cases under the study. The second stage was an observational cross-sectional study involving the collection of case and control subject household maize grain and flour samples at a specific point in time and the eventual analysis of aflatoxin B_1 levels in parts per billion (ppb), from the same samples of the study area. This would link or delink case and control serum sample AFB_1 albumin adducts data to any consumption of AFB_1 contaminated maize grain and flour in area of study.

3.3 Study methodology

These are the specific procedures or techiniques used to collect, process, select and analyze data in the study. The methodology for the study was therefore quantitative since it involved formulation and testing of the hypothesis, collection, modeling and statistical analysis of an empirical data which was generated from the study. This included also the determination of the association between the independent variables and the dependent variable using appropriate tools.

3.4 Study populations

The study population comprised of case and control subjects purposefully sampled from the larger population of patients in Sub- County, County and Level 4 health facilities that had been diagnosed with liver disease for cases and without liver disease for controls as per the set criteria of between 12 yrs to 90 yrs of age. The age bracket would enable the investigator to capture reasonable representative case sample for both congenital and old age liver disease cases. Controls consisted of those patients admitted from non-liver ailments within the same categories of hospitals and health centers under investigation and who were matched in terms of sex, age and locality. Matching of cases and controls was necessary to reduce both selection and Barkison biases in the study.

3.5 Target population

The target human population for the Case-Control study comprised of the entire population of the two Counties under study, as obtained from the Kenya National Bureau of Statistics register for the most recent population censors (KNBS, 2019).

3.5.1 Defination of case and controls under study

Cases were defined as those who were clinically diagnosed with liver disease irrespective of the aetiologoical factor or causes and had been admitted to the health facility under the study, while the controls were those clinically diagnosed with non hepatic conditions, admitted in same facility, of same sex, geographical locality and of same approximated age as cases to reduce sample matching biases. For the controls, the investigator had also to rely on hospital diagnosis register to select the non hepatic controls patients.

3.6 Inclusion and exclusion criteria for human participants

3.6.1 Inclusion criteria

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

i. They were between ages of 12 to 90 years for all sexes. Such range limit was meant to capture liver disease cases including cogenital cases occurring early in life and those advanced in age
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 as per Appendix 4.
iv. They gave an assent to participate in the study in case of those who were below 18 years and an informed consent of the guardian or parent had been provided.
v. They had been clinically diagnosed to have liver disease irrespective of the etiological factor or agent and were inpatients in one of the selected health facilities.

vi. They did not belong to the vulnerable groups including prisoners, blind persons and pregnant women, mentally impaired or persons lacking consent capacity. This was to protect those with diminished outonomy for a free choice (Vanclay *et al.,* 2013).

3.6.2 Exclusion criteria

The human subject was excluded from this study if: -

i. They were between ages of 12 to 90 years for all sexes but in addition to liver disease had other severe illness which could not ethically allow them to participate in this study.
ii. They had been residents of the study area for at least one year, and they meet every other inclusion criterion but withdrew the consent before the serum samples were obtained.
iii. He or she had not given an informed consent to participate in the study in case of those above18 years.
iv. They gave an assent to participate in the study in case of those who were below 18 years and an informed consent of the guardian or parents had been provided but were discharged from the health facility before serum samples were drawn.
v. They had been clinically diagnosed to have liver disease but were outpatients in the selected health facility.
vi. 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. This was meant to ethically protect their outonomy to free will.

3.6.3 Inclusion criteria for control participants

i. They were between the ages of 12 to 90 years for all sexes to match the cases cohort.
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 18years.
iv. They gave an assent to participate in the study in case of those who were below 18 years in addition to informed consent of the guardian or parent.
v. They did not have clinical evidence of liver disease and were inpatient in one of the selected health facilities.
vi. They did not belong to the vulnerable groups including prisoners, blind persons, pregnant women, mentally impaired or persons lacking consent capacity.

3.6.4 Exclusion criteria for control participants

i. They had other severe illness which could not alow them to participate in study
ii. They withdraw consent before the commencement of the study
iii. They had not given an informed consent to participate in the study in case of those above 18 years.
iv. They had been cinically diagnosed to have liver disease irrespective of etiology
v. They belonged to the vulnerable groups including prisoners, blind persons, pregnant women, mentally impaired persons or persons lacking consent capacity.

3.6.5 Inclusion and exclusion criteria for household maize grain and flour stores

a) Inclusion criteria

For inclusion of a household grain store in this study, the following conditions were to be met: -

i. The households were also to be the homes of the study subjects and were to be situated within the study area.
ii. The households had to be stocked with either white maize or flour or both

iii. The home owner was to give an informed consent that the household store participates in the study by donating samples of grain or flour

b) Exclusion criteria

A home grain store was to be excluded from this study if: -

i. It was not situated within the study area
ii. It was stocked with other foodstuffs other than white maize or flour
iii. The home owner did not consent that his/her household store participates in the study

3.7 Case participants identification

Human subject cases were purposefully identified by use of non probability sampling method then selected from health facilities within the study area on condition that they had been clinically diagnosed with liver disease irrespective of the aetiological factor. Hospital admission registers at various County or Sub County health facilities in the Counties were also used to confirm admissions due to liver disease. For the Cases followed at Masaku level five (5) health facility, only those inpatients identified by an official register as residents of Kitui or Makueni were randomly selected after consenting to the study.

3.7.1 Control subjects' identification

Control subjects were selected by use of non probability purposeful sampling method from the sample of the population where the cases were drawn from. Appropriate numbers of Controls equal to the number of Cases were picked from the same health facility from 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.

Controls were matched to Cases as much as possible including using personal characteristics like age, sex and residency. Matching was however limited to three factors including age, sex and locality of the participants. It was also made to reduce

both selection and Berkison biases and also enable fair comparison of outcome data for both cohorts.

3.8 Sampling procedure

3.8.1 Human subjects sampling procedure

Non-probability purposive sampling method was used to pick human subjects on basis of clinical diagnosis of liver disease for Cases and non liver disease for Controls as per section 3.8.2 below. 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 samples in laboratories by use of code numbers.

3.8.2 Sample size for the case -control design

The sample size (n) for the cases- control design was determined by use of the modified Schelsselmans (1982) formular for matched case- control studies by Charan & Biswas, (2013). Thus: -

$$n = \frac{(r + 1).\acute{P}\,(1 - \acute{P})\,(Z_{\beta} + Z\alpha/2)^2}{1\,(P1 - P2)^2}$$

Where,

n = sample size in the Case group

r = ratio of controls to cases, usually equal to one (1) for equal number of Cases and Controls.

$\acute{P} = P_1 + P_2 / 2$ = mean proportion exposed equal to "proportion of exposed cases plus proportion of controls divided by by two.

Z_{β} = standard normal variate for 80% power of study is 0.84 while for 90% power of study is usually given as 1.28

$Z\alpha/_2$ = standard normal variate for level of significance at 95% confidence level usually is 1.96.

$P_1 - P_2$ = difference in proportions expected based on previous studies in the area.

Hence fixing the power of the study at 80% and determining the expected proportions in the case and control groups from study populations to be 0.3274 and 0.2226 respectively, then: -

r = 1, $P_1 = 0.2226$, $P_2 = 0.3274$, P = 0.275, $Z_\beta = 0.84$, while $Z\alpha/_2 = 1.96$.

Thus substituting,

$$n = \frac{2.}{1} \quad \frac{0.275\,(1 - 0.275)\,(0.84 + 1.96)^2}{(0.3275 - 0.2226)^2}$$

n= 281.63

This was the minimum sample for the cases group. Thus, the study adopted a maximum of n = 283 for cases and n = 283 for controls respectively for a more representative sample.

Based on the three categories of health facilities, mainly Sub- County, County and refferals (level 4 and 5), 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-counties, counties and level 4 & 5 health facilities respectively (Table 3.4). 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 county, county and level 4 and 5 hospitals (Table 3.2, 3.3, 3.4 and3.5). 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.

This implied that the minimum total number of subjects, both Cases and Controls in the study was to be 283×2 = 566. Since this sample was representative, the study enrolled those admitted purposefully since following outpatients was not practical in this case (Jaaskelainen *et al.,* 2018)

Table 3.2: Case subject sample (n) per Sub County health facility

Category I				
Sub county	**Health centre**	**Mean admission /month /centre**	**Admissions for 6 months (t)**	**Case sample(n)**
(N=47)	Kavisuni	8	48	3
	Migwani	24	144	10
	Nuu	15	90	6
	Tei wa Yesu	10	60	4
	Mtito Ndooa	18	108	7
	Mathuki	12	72	5
	Sultan Hamud	30	180	12
	Total (T)	**117**	**702**	**47**

Table 3.3: Case subject sample (n) per County level health facility

Category II				
County level	**County level facility**	**Mean admission /month /centre**	**Admissions for 6 months (t)**	**Case sample (n)**
(N=94)	Kitui	90	540	21
	Muthale	60	360	15
	Mutomo	70	420	17
	Mwingi	40	240	10
	Kyuso	10	60	2
	Kibwezi	12	72	3
	Makindu	30	180	7
	Emali	15	90	4
	Mtito Adei	13	78	3
	Kathozweni	50	300	12
	Total (T)	**387**	**2322**	**94**

Table 3.4: Case subject sample (n) per level 4 or 5 health facility

Category III				
Level 4 or 5 hospitals	**Health facility**	**Mean admission per month per centre**	**Admissions for 6 months (t)**	**Case sample (n)**
N=142	Wote	2100	12600	45
	Masaku	4500	27000	97
	Total (T)	**6600**	**39600**	**142**

Table 3.5: Hospital category and minimum case and control subject's summary

No	Category of health facility	Number of health centers	Number of subjects(n)	Minimum number of: - cases	controls
1	Sub –county	7	94	47	47
2	County	10	189	94	94
3	Referal hospitals (Level 4 and5)	2	283	142	142
	Totals (T)	**19**	**566**	**283**	**283**

Table 3.6: Distribution of subjects enrolled by hospital's name in the two Counties

No.	Kitui county Name of centre	Minimum no. of (n) cases	(n) controls	Makueni county Name of centre	Minimum no. of (n) cases	(n) controls
1	Kavisuni	3	3	Kibwezi	3	3
2	Muthale	15	15	Emali	4	4
3	Mutomo	17	17	Sultan Hamud	12	12
4	Migwani	10	10	Makindu	7	7
5	Nuu	6	6	Wote	45	45
6	Mwingi	10	10	Mtito Adei	3	3
7	Kitui	21	21	Kathozweni	12	12
8	Kyuso	2	2	Masaku	97	97
9	Tei wa Yesu	4	4		283	283
10	Mtito ndooa	7	7			
11	Mathuki	5	5			

3.8.3 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 best practice. The phlebotomist used sterile hand gloves, and then assembled equipment including a tourniquet, methanol and 70% alcohol swabs cotton wool for skin disinfection, to be applied over punctured site and red rubber topped 10 ml vacutainer tubes to where 4ml of blood each was collected. Methanol was used as a local anaesthesia in this procedure. Laboratory specimen labels and forms for recording the case and control sample codes were also used in this procedure.

The subjects were prepared by also obtaining a verbal consent even if informed consent had been obtained before as per the ethical guidelines issued for study clearance. The case and control study participants were matched for age and locality (residency), while blood was collected within shortest time span to avoid temporal variations in serum parameters of interest.

The phlebotomist prepared by performing a hand hygiene procedure and putting on gloves. The median cubital vein within the cubital area of the forearm was selected while the puncture site was disinfected using 70% alcohol as a tourniquet was applied above selected puncture site to enable the procedure as follows: - The phlebotomist anchored the vein by holding the patients arm and placing a thumb below the venepuncture site. Once the veins were more visible the puncture needle attached to a syringe of 4ml was introduced to the vein swiftly at an angle of 30^0 or less. Once sufficient blood was drawn, the tourniquet was released before withdrawing the needle. The needle was withdrawn and sterile cotton wool placed on the affected site.

Four (4ml) of blood was transferred to a red rubber topped vacutainer tube in a rack and placed in a blood transporting box immediately. In the above procedure, direct use of vacutainer tubes with standardized suction to draw blood were avoided due to risk of vein collapsing, especially in the elderly. The used needles and syringes were discarded to a puncture – resistant sharps container for proper disposal.

The labels were to have the subject number, date of birth, date and time of blood collection but not the name of the person for ethical and confidentiality reasons (WHO, 2010).

3.8.4 Storage and transport of blood samples

Four(4ml) of blood from each case and control subject was drawn into a10ml sterile red topped vacutainer tube which was coated with an additive clot activator (thrombin), and a gel for easy serum separation during centrifuge and placed in blood transport boxes**,** which contained ice cubes**.** The blood boxes maintained the blood samples at a temperature of between 1^0 to $6\ ^0$ C. The samples were then transported

after each collection session to the laboratory for centrifugation to obtain serum from clots. The serum was stored in a deep blood freezer and frozen as "Fresh Frozen serum" (FFS) at Kabete pathology laboratory (UON) at between -25^{0}C to -40^{0}C, such blood component was to have a shelf life of one (1) year (Robeck, 2011).

3.8.5 Serum from frozen blood component

The serum used was whole blood minus white blood cells (WBC), red blood cells (RBC) and the fibrinogen clotting factors (Wang *et al.*, 2002; Elizabeth, 2007). The 4ml fresh serum was thawed after fleezing using a thawing bath at 30^{0}C to 37^{0}C for between 30 to 45 minutes, then centrifuged again to get at least 3ml of serum each for laboratory diagnostic analysis and determination of HB_S Ag, ALT, AST and AFB_1 - albumin adduct levels to enable data comparison on cases and those contols exposed but had no liver disease.

3.8.6 Determination of aflatoxin B1, AFB1 serum albumin adducts, AST, ALT and HBsAg in blood samples

i. Preparation of samples and AFB_1-albumin standards

The direct competitive ELISA Kit manufactured by Glory® Science of 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 had set 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 concentration of 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 (Mutungi *et al.*,2008).

ii. Preparation of AFB1- albumin adduct-enzyme conjugates.

The ELISA kit had an already prepared AFB_1-albumin adduct-enzyme conjugate which was used for tests, in both micro-titre and standard wells.

iii. 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.

iv. Analysis procedure

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-titre 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 manufactured by Glory® Science of USA was added to all the wells of both the standards and the sample, covered with an aluminium 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 1000ul distilled water, plus 0.25ml of poly oxy ethelene sorbitan monohydrate), and dried by tapping with a blotting paper (Nardini *et al.*, 2017).

An enzyme substrate manufactured by Glory® Science of 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 (Farzan *et al.,* 2007).

The intensity of colour in all wells was determined by measuring absorbance at 450nm, using an ELISA reader from Uniskan II® Lab systems of Finland.

The absorbance value data for standards and serum samples was entered into computer software, "R-ridasoftwin® version 1.60 of R-bio pharm of 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 into parts per billion (Mutungi *et al.,* 2008).

3.8.7 Determination of HBsAg levels in serum samples

The quantification of HBsAg was done by automated analyzers available commercially namely Architect QT® manufactured by Abbot laboratories of United Kingdom. This investigation used Architect QT® 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 HB_SAg tests per hour.

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

The subsequent chemiluminescent reaction was measured in relative light units (RLU), which are converted to HB_SAg units, using a previously graduated Architect HB_SAg 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 HB_SAg 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.

A sample of blood serum 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. The laboratory procedure for determination of AST, ALT and AFB_1 for subject's household maize grain and flour are attached (Appendix 6 and 8).

3.8.8 Disposal of used samples

Both the aflatoxin contaminated maize grain, flour and infected blood samples were biohazard substances. The samples were to be stored for one (1) year after lab analysis and closure of the study for any re analysis should a need arise. These were thereafter incinerated as was, and still is the practice with this class of biohazard substances (Udofia *et al.,* 2017).

3.9 Sample size for subject household maize and flour samples

The study population of household grain stores comprised of all home grain storage facilities in Kitui and Makueni Counties. The population frame was informed by the total Cases and Controls from the sampled health facilities in Kitui and Makueni Counties. To determine if a link existed for case and control serum AFB_1 albumin lysine adducts to diet, sampling of case and control household maize grain and flour was undertaken to enable the analysis of the data.

The minimum sample size (n) was then determined by using Fisher *et al.,* (1998), formulae as detailed here: thus, $$n \geq \frac{z^2 \text{x p x q}}{d^2}$$

Where n was to be the minimum sample size required, q = (1 - p), z = 1.96, the normal deviation at 95% confidence level, p = prevalence of condition under study, which was aflatoxin contamination of maize grain in the study area, and d = 0.05, the absolute precession required for the study at 95% confidence level and 5% significance level.

The mean prevalence level of aflatoxin contamination at study area was 9.3%, (Lauren *et al.,* 2004; CDC, 2004), and was used to determine the sample size. Factoring in the value of q = (1 – p), as 0.907, and p = 0.093, then $n \geq (1.96)^2 (0.093) (0.907) / (0.05)^2 = 129.61$. The minimum sample size was to be 130. For this sample to be representative, the number was purposefully increased to n= 283**,** to match the total households of the Cases (n_1) and also same number for the Controls (n_2). Table 3.7 shows the distribution of both the case and the control maize grain and flour sample.

Table 3.7: Distribution of study participants' household maize grain and flour samples

strata	**case (n_1)**	**control (n_2)**	**($n_1 + n_2$)**
Kavisuni	03	03	06
Muthale	15	15	30
Mutomo	17	17	34
Migwani	10	10	20
Kitui	21	21	42
Nuu	06	06	12
Tei wa Yesu	04	04	08
Mathuki	05	05	10
Mwingi	10	10	20
Sultan Hamud	12	12	24
Kyuso	02	02	04
Mtito Ndooa	07	07	14
Wote	45	45	90
Kathozweni	12	12	24
Masaku	97	97	194
Makindu	07	07	14
Emali	04	04	08
Mtito Andei	03	03	06
Kibwezi	03	03	06
	283	**283**	**566**

3.9.1 Sampling procedures for maize and flour samples

Purposive sampling technique was used in this procedure. The number of Case and Control households in the study was used to determine the number of grain and flour samples to be used and this was reproduced in the sample selected. From each of the sampled 283 household stores, 0.25kg each of maize grain and maize flour

respectively was collected. These samples were given free from household owners but some were bought at a market fee of Sh50.00 per sample. An automatic spear type sampler sourced from Pneumac®Agricultural services of UK primed to scoop 0.25kg was used. To avoid contamination, samples were separately sealed in khaki paper bags sourced from Mafuko® industries of Kenya and transported to the laboratory at Bora Biotech in Kenya for quantitative analysis of the levels of AFB_1 in both maize grain and flour respectively. All the samples were stored in dry conditions and at a temperature range of 15ºC to 20ºC before analysis.

3.9.2 Determination of aflatoxin B_1 level in subject maize grain and flour samples

The direct competitive ELISA kit used in the process was for total determination of aflatoxin B_1 whose manufacturer, Bora test® biotech of Kenya had approximated that it could offer aflatoxin B_1 detection rate of between 88% - 100% for maize products (Gathumbi *et al.*, 2001).

i. Preparation of samples and aflatoxinB_1 standards

Maize grain samples were fine milled and aflatoxin B_1 extraction done using the AOAC official method 990.32, with modifications (AOAC, 1995).

Extracts were diluted in methanol PBS (10:90), solution before use. A calibrated aflatoxin B_1 standard manufactured by Bora test® of Bora Biotech Kenya, whose concentration was 10 ug/ml, was used to prepare diluted aflatoxin standards of 0 ppt, 37 ppt, 111ppt, 333 ppt and 1000 ppt, for ELISA analysis, by diluting the calibrated standard with methanol: PBS (10:90) solution as follows; - six test tubes were be arranged in a test tube rack and marked neat (N) S_1, S_2, S_3, S_4 and S_5 with sticker labels. Ten (10ul) of calibrated aflatoxin standard, whose composition was aflatoxin B_1 in methanol was pipetted into the neat test tube and mixed with 1000 ul of (10:90) , methanol in phosphate buffered saline (PBS) solution. In S_1 2000 ul of 10% methanol in PBS, was pipetted, and 20 ul of aflatoxin standard solution in neat (N) added. In S_2 1000 ul of 10% methanol in PBS was pipetted and 500 ul of S_1 was

added. In S_3, 1000 ul of 10% methanol PBS was pipetted and 500 ul of S_2 added. Similarly, in S_4, 1000 ul of 10% methanol in PBS was pipetted and 500 ul of S_3 added. In S_5 only 1000ul of 10% methanol in PBS was pipetted, with no addition of aflatoxin B_1 standard solution. An already prepared "Bora test® solution" was used for this purpose (Mutungi *et al.,* 2017).

ii. Preparation of aflatoxinB_1 enzyme conjugate

A working dilution of aflatoxin B_1 enzyme conjugate was prepared by diluting a neat aflatoxin B_1 horse radish peroxide sourced from Bora test® Bora Biotech of Kenya with methanol in Phosphate buffered saline (PBS) at a ratio of 1:10,000 (Mutungi *et al.,* 2017).

iii. Preparation of an enzyme substrate

A working dilution of an enzyme substrate solution was prepared by mixing (1:1), portion of citric acid buffered solution (pH 3.8) containing 325ul of 30% hydrogen peroxide per litre of solution and one portion of a solution of 50.4mg tetra methyl benzidine (TMB) in an acetone- methanol (1:9) solution.

iv. Analysis procedure

Fifty (50) ul, of diluted aflatoxin standards was pipetted to the antibody coated micro-titre wells of the assay in the order S_5; S_4; S_3; S_2 and, S_1, representing standard dilutions of 0 ppt; 37ppt; 111ppt; 333 ppt; and 1000 ppt respectively. Similarly, 50ul of sample was pipetted into adjacent wells of coated micro titre wells. Aliquots (50ul) of diluted aflatoxin B_1 horseradish peroxide conjugate (Enzyme conjugate) was added to all wells of both the aflatoxin B1 standards and the sample extracts, covered with aluminium foil and incubated at room temperature (28ºC), for two hours. The plates were then emptied and washed with saline tween solution (8.55gm sodium chloride, dissolved in 1000ul distilled water, plus 0.25 ml of poly oxythelene sorbitan monohydrate), and dried by tapping with a blotting paper.

An enzyme substrate solution (HRP and TMB), was added and the plates incubated in the dark for 10 minutes. The enzyme reaction was stopped by adding a 100 ul of 1m sulphuric acid simultaneously into all micro-titre wells.

The intensity of colour in both standards and sample extract wells was determined by measuring the absorbance at 450nm, using ELISA reader sourced from Uniskan II®, lab systems of Finland. The absorbance value data for standards and sample extracts was entered into computer software, "R-ridasoftwin® version 1.60 of R-bio pharm Germany," which used percentage absorbance values against known standard aflatoxin B_1 concentration to draw standard curve. The software automatically generated the aflatoxin B_1 levels of the sample in parts per billion (ppb) using the curve. These were recorded in the data collection tools shown as Appendix10 (Mutungi *et al.,* 2017).

3.9.3 Laboratory quality control

For comparison purposes, part of 10% of the sample of maize grain and floor were given to a certified and accredited laboratory (Kabete veterinary pathology labs) for analysis of aflatoxin B_1 levels. These results were compared statistically with those obtained from the analysis of the rest of samples from Bora® biotech, Kenya.

3.10 Data collection tools

Data was collected by use of two different methods including, the use of a structured questionnaire and by the direct laboratory analysis of samples collected from the field. The principal investigator used a questionnaire to collect biodata of the participants, as well as data on possible dietary exposure to aflatoxin B_1 and other suspected contributory factors to hepatitis B viral infection and eventual liver disease. Three (3) types of data capture forms were used to avoid possible loss or mix up of data. One was used to record aflatoxin B_1 levels in white maize grain and flour, collected from subject household stores within the study area (appendix 9) and the other to record data on serum parameters in the study including levels of AST, ALT, AFB_1 lysine albumin adducts and HBsAg on test results (appendix 10). The

third form captured data from the questionnaire on suspect associated factors to liver disease (appendix 7).

3.11 Reliability and validity of the data collection tool

The accurancy and the consistency of the questionare used were critical for validity and reliability of the tool and the opinions and attributes it was made to measure. The questionnaire employed the Likert five (5) point scales. To test the validity and reliability of the instrument, the coefficient of reliability also called Cronbach alpha value (*a*) was determined where,

$$\alpha = \frac{N\,\hat{C}}{V + (N-1)\,\hat{C}}$$

Where N= Number of items,

$\hat{C}$ = Average inters item covariance among items

V= Average variance

By use of SPSS and by adjusting the items within the questionnaire, the Cronbach alpha (*a*) for the questionnaire was determined as, α= 0.789. This value compared favourably to accepted Cronbach alpha range of between 0.7 and 0.95 (Tavakol *et al.,* 2011).

3.11.1 Data management

After raw data was recorded into a determined format, it was then cleaned and stored in soft copy by way of a removable data discs. Data cleaning was done by querying, collating, coding, flagging out inconsistencies and omitting of incompatible responses in case of questionnaires. In addition, an item that was not responded to was considered as missing to avoid confounding biasis. Coding was done in such

way that if a Case was coded as two (2), then the blood, maize grain and flour samples were coded as 2a, 2b, and 2c respectively. This trend was followed for both Cases and Controls and for all the samples. The data was then keyed into a computer programme using particular software (SPSS, version 18.0, lead technology 2007, USA) and saved in a data disc for storage. For purposes of data protection, a computer pass word known only to the principal investigator (PI) was used and still is being used. In addition, the data capture forms and questionnaires were kept safely in a cabinet safe until the completion of the study.

3.11.2 Data analysis

Data was extracted from the questionnaires filled and administered separately for the already identified Cases subjects as per the clinical records in health facilities and analyzed for the total number subjects who answered "yes" or "no" to having being consumers of home-grown maize grain and flour, had blood transfusion, unprotected sex, used body piercing instruments (needles and syringes) and used untreated water at the county under investigation. A computer software SPSS version 18.0 was used to determine correlation coefficient (r) between the dependent and independent variables at 95% confidence level. A coefficient of determination (r^2) between the risk factors (dependent variables) and liver disease was also determined using same software method.

Laboratory data on AFB_{1}- albumen serum adducts and HB_S Ag levels, ALT and AST levels, was analyzed for means ($\bar{X}$), ranges, standard deviation (Sd), difference between means and confidence intervals of the means (CI) at 95% confidence level or 5% significance level.

A multiplicative and additive regression analysis was done to determine any synergistic effect (γ) on induction of liver disease due to a combined sero presence of HBV/AFB_1 in participants under investigation. A computer software SPSS version 18.0 was used for this procedure.

Prevalence of liver disease due to sero presence of HBV, AFB_1 and the combined sero presence of HBV- AFB_1 in study participants from the two Counties was also

determined. The odds ratio (OR) representing odds of contracting liver disease in these Counties was determined by use of a contingency table. The odds ratio was a measure of the relative risk (assuming the rare disease scenario), in this study (Robert *et al.*, 2012).

Aflatoxin B_1 (AFB_1), levels of household grain and flour samples in parts per billion (ppb), as determined by a computer software (R-ridasoftwin® version 1.60, R-biopharm, Germany), was analyzed for means($\bar{X}$), ranges, standard deviations (Sd) and confidence intervals (CI) of the means at 5% significance level by use of a computer SPSS version 18.0 software (Peersman, 2014).

3.12 Computations of odds ratio, relative risk and prevalence

The computations of odds ratio, relative risk, and prevalence involved construction of a contingency table each for the exposure and non exposure of case and control subjects to the major disease factors including HBV, AFB_1 or a combination of both HBV and AFB_1, then substituting the values after which the computations were done (Rothman, 2012).

Table 3.8: Contigency table on Exposure and Non-exposure to HBV

Status	Liver disease	Non-Liver disease	Total
Exposed to HBV	a	b	a + b
Non-exposed to HBV	c	d	c +d
	a +c	**b +d**	**a +c +b +d**

Table 3.9: Contigency table on Exposure and Non- exposure to AFB_1

Status	**Liver disease**	**Non-Liver disease**	**Total**
Exposed to AFB_1	a	b	a + b
Non-exposed to AFB_1	c	d	c +d
	a +c	**b +d**	**a +c +b +d**

Table 3.10: Contingency table on Exposure and non-exposure to a combination ofAFB_1 and HBV factors

Status	Liver disease	Non-Liver disease	Total
Exposure to combined HBV +AFB_1	a	b	a + b
Non-exposure to combined HBV+AFB_1	c	d	c +d
	a +c	**b +d**	**a +c +b +d**

The prevalence of a disease in the study refered to the ratio of the number of cases of a disease in a population at a specified time to the number of persons in the population at that specified time. This could be written as, "Prevalence = Number of positive samples / Total number of samples tested" (Rothman, 2012).

The odds ratio in this study was also a measure of relative risk (assuming the rare disease scenario), of liver disease associated with exposure to HBV due to contaminated water, blood transfusion, unprotected sex or consumption of AFB_1 contaminated grain or the combined effect of HBV and AFB_1(Robert *et al.*, 2012).

A questionnaire was used to determine the most probable factor associated with the infection with HBV among notable variables including blood transfusion, injecting of drugs, unprotected sex and contaminated water. Sero prevalence of liver disease due to HBV would then be determined. Similarly, sero prevalence of AFB_1 in the human subject was determined and recorded.

From the contingency table above, the ratio $\frac{a}{c}$ = odds of exposure when one has the disease, while $\frac{b}{d}$ = odds of exposure when one has no disease (Andrade, 2015).

Hence the odds ratio (OR) was given by

$$\frac{a}{c} \div \frac{b}{d} = \frac{ad}{bc}$$

The interpretation of the odds ratio in the study was done as per Zsumilas, (2010), criteria, thus

i. If $OR = \frac{ad}{bc} > 1$, then the odds of exposure was positively associated with the adverse outcome (disease) compared to the odds of not being exposed.

ii. If $OR = \frac{ad}{bc} < 1$, then the odds of exposure was to be negatively associated with the adverse outcomes (disease) compared to the odds of not being exposed. This may have meant a protective effect.

iii. If $OR = \frac{ad}{bc} = 1$, then this meant that there was no association between the suggested exposure and outcome (disease). It could mean that the number of Cases in exposure category was equal to the number of Cases in the non-exposure group (Ranganathan *et al.*, 2015).

3.13 Ethical considerations and clearance

This was sought and granted by the Scientific Steering Committee (SSC), and the Ethical Review Committee (ERC) based at Kenya Medical Research Institute through a protocol No. 2988 (Appendix 1 and 2). Authorization to conduct research was sought from National Commission for Science, Technology and innovation (NACOSTI). Permission was additionally sought from the medical officers in charge of each health facility where a blood sample from human subjects was collected. The subject's informed consent was sought and recorded (Appendix 4). They were informed about the voluntariness of participation and the study procedures, including risks of participation and discomforts. The benefits of participation to the individual

and the community was also discussed and explained to the participant (Vanclay *et al.,* 2013)

Informed consent was sought from subjects before they were enrolled in the study. The principal investigator ensured confidentiality of all the data collected and also ensured that the data was used only for the intended purposes and not otherwise (Coughlin, 2006)

3.14 Consent explanation

The subjects were informed of the purpose of the study as an investigation to find out the prevalence of liver diseases in Kitui and Makueni Counties as a result of hepatitis B infection and aflatoxin poisoning in the population. An explation for the study was that, liver diseases arose due to malfunction of the liver. This occurred due to several reasons including but not limited to hepatitis B virus infection or aflatoxin B_1 poisons which could arise from consumption of mouldy grains and chronic alcoholism. Infection with hepatitis B virus and concurrent consumption of mouldy grains may have lead to rapid development of liver disease. The study was therefore meant to provide information that was to assist in prevention of liver diseases in the future (Vanclay *et al.,* 2013).

3.15 Conditions for withdrawal

The subject participation in this study was voluntary and they were free to reject a request to participate without giving reasons. Subjects were encouraged to read, understand and to ask questions. In case a recruited subject could not read or write, the document was read for them to enable them understand what was contained in the document before agreeing to participate. Parents were to give consent that their children participate in the study before they were included. A child who did not ascent to participate in the study was not included even if the parent had consented. The subjects were informed that they could withdraw from the study at anytime without giving any reason.

3.16 Informed consent

Having read, understood and accepted to participate in the study, then the following was requested from the subjects: 4 millilitres of blood drawn from each of them and 0.25 kg each of maize grain and flour from their homes. They were asked questions concerning their age and gender, where they obtained white maize grain and flour from and other questions as per the questionnaire (Appendix7). They were free to answer any or all of the questions and were not required to give reasons as to why they would decline to answer. This exercise was conducted once with no follow up activity (Coughlin, 2006).

3.17 Risks and discomforts

Subjects were informed that, during and immediately after drawing of the blood, they would experience some little pain at the puncture point. The puncture area could swell and take several minutes before bleeding stops. Subjects could faint and at times, though rarely may get infection through the site of puncture. In addition subjects could be inconvenienced by the exercise. To ensure safety, blood was drawn by a trained technician and a phlebotomist who had licence to practice. In case local phlebitis and pain occurred, the doctor in charge of the centre was always requested to manage it. All equipment used was properly sterilized. Needles and syringes once used were not to be re-used. In addition, the exercise was conducted in a hospital laboratory and the highest care was taken which ensured subjects safety (Vanclay *et al.,* 2013).

3.18 Benefits to individual and community

There were no material or financial benefits for participating in this study. This information was passed to the participants. However, the findings of the study were to benefit the entire community as it would help improve general public health and prevent occurrence of hepatitis B virus infection and aflatoxicosis which were often suspected to be the major causes of liver disease in the area of study.

3.19 Cost on individuals

The Subjects did not spend any money while participating in this study. The maize grain and flour samples requested from the homes of the subjects as part of this investigation was compensated for at market rates at Sh50.00 per sample of 0.25kg translating to Sh100.00 for the two 0.25kg samples. Some participants willingly donated free samples for the investigation.

3.20 Study individual's well fare

Since the participants were to spent time answering to the questioneire, some light refreshments including a packet of 250ml of milk were provided. In addition, any participant in the control group who posted abnormal results was referred to the clinician in charge of the health facility for treatment and appropriate clinical management.

3.21 Confidentiality

For purposes of confidentiality, subject names were not recorded on samples even though the names were known (Vanclay *et al.*, 2013). The blood, maize grain and flour samples collected did not have marks or labels (identifiers) that could enable anyone trace from whom it was collected since codes were used to guarantee anonymity. Again, data generated was coded and hence was not traceable to subjects. Data generated from analysis of samples was stored in electronic files with appropriate password for security, while used questionnaires were stored in metal cabinet safes for further reference. Blood, maize grain and flour samples were only used for this study and no other person or authority was allowed to handle or use them.

3.22 Expected application of results

(i) Specific individual benefits

The study was to confer indirect benefit to the patients in that, establishment of the actual prevalence of HBV infection was to justify a vaccination campaign which

could protect those relatives of patients and non patients not infected, including spouses and children.

(ii) Public benefits

The public benefit in the study was to be realized through public health intervention strategies by the County or the National government by advising on proper storage and drying of maize grain to avoid aflatoxin contamination and hence aflatoxicosis. An HBV prevalence of concern could have triggered a serious vaccination campaign against HBV in Kitui and Makueni to reduce the prevalence since immunization had been shown to be the most effective means of prevention (Ventola, 2016).

CHAPTER FOUR

RESULTS

4.1 Quantitative laboratory sample results

4.2 Case and control aspertate amino transferase (AST) levels in serum samples

The aspertate amino transference (AST), an enzyme found in blood, body tissues, and the liver, and whose level is indicative of liver damage was analyzed from Case subject samples (N=283). The AST values individual range was 55.6 Iu/mL to 344.5 Iu/mL, with an overall mean of 154.86 Iu/mL, (CI; 147.52 to162.20 Iu/mL), at 95% confidence level ($p \leq 0.05$). The mean AST levels per health centre for the case subject's cohort ranged from 107.63 Iu/mL in Kavisuni health centre to 212.33 Iu/mL in Kathozweni health center in Makueni County. A non parametric Chi square test results indicated a significant association between the health centres where samples were drawn and AST levels in blood samples from the case subjects in all the health centers in Makueni and Kitui counties. The observed Chi square test value was, $\chi^2_{(0.05,\ 18df)} = 28.678$), while the study calculated test value was $\chi^2_{(0.05,18df)} = 70.536$). The mean standard deviation, a measure of dispertion for the data mean in the case AST levels was 55.66 Iu/mL with a range of 18.45 to 91.29 Iu/mL and a median of 54.89 Iu/mL (Table 4.1). The control group overall mean AST level per health center was 35.31 Iu/mL (CI; 27.86 to 42.76 Iu/mL) at 95% confidence level ($p \leq 0.05$). The control mean AST level range was 19.10 to 101.16 Iu/mL while the overall mean standard deviation for AST means level in control subject sample was 20 Iu/mL. The comprehensive individual health facility data results are tabulated in table 4.2. The comparative case-control median AST levels are tabulated in figure 4.1.

Table 4.1: Distribution of case cohort serum sample and mean AST level per health facility

health facility	AST range Iu/mL	sample (n= 283)	mean Iu/mL	sd Iu/mL
Mutomo	55.60-310.00	17	150.85	86.98
Kavisuni	85.60-142.70	03	107.63	30.70
Migwani	79.80-205.9	10	142.99	46.63
Tei wa Yesu	85.80-190.50	04	144.42	45.00
Kitui	75.80-285.00	21	147.65	65.58
Mtito Ndooa	82.50-284.60	07	138.55	69.66
Mwingi	65.00-242.50	10	133.15	54.89
Kyuso	23.50-149.60	02	136.55	18.45
Nuu	90.00-208.50	06	129.58	43.01
Muthale	72.40-215.80	15	129.41	45.12
Mathuki	126.50-205.60	05	157.74	32.17
Kibwezi	98.90 -149.60	03	126.40	25.62
Wote	73.60 - 220.70	45	132.84	36.53
Sultan Hamud	75.00 - 310.00	12	168.84	77.70
Masaku	73.50 - 340.00	97	170.35	65.04
Kathozweni	112.00-344.50	12	212.32	84.32
Makindu	95.50-265.00	07	149.55	61.56
Emali	118.50-318.00	04	183.82	91.29
Mtito Adei	85.00-230.50	03	172.76	77.26

Note: sd= standard deviation

Table 4.2: Distribution of control serum samples and mean AST level per health facility

health facilty	AST range Iu/mL	samples (n=283)	mean Iu/mL	sd (σ)
Mutomo	15.55-70.00	17	36.83	15.89
Kavisuni	24.90-54.30	03	36.90	15.43
Migwani	13.40-72.40	10	36.44	18.99
Tei wa Yesu	16.40-332.50	04	101.16	154.32
Kitui	13.60-42.50	21	29.14	8.80
Mtito Ndooa	13.20-36.30	07	23.51	9.50
Mwingi	9.35-70.20	10	32.32	19.54
Kyuso	32.10-65.10	02	48.60	23.33
Nuu	15.30-45.50	06	31.97	13.70
Muthale	19.40-75.20	15	34.06	14.40
Mathuki	19.80-48.70	05	32.54	10.66
Kibwezi	13.70-23.90	03	19.10	5.14
Wote	12.80-52.30	45	27.46	10.60
Sultan Hamud	12.80-55.40	12	29.04	13.11
Masaku	13.30-61.31	97	29.24	10.16
Kathonzweni	15.20-42.90	12	31.56	8.57
Makindu	1 7.30-54.60	07	32.32	12.76
Emali	17.30-39.70	04	28.58	9.16
Mtito Andei	19.40-41.30	03	30.13	10.96

Note: Sd = standard deviation

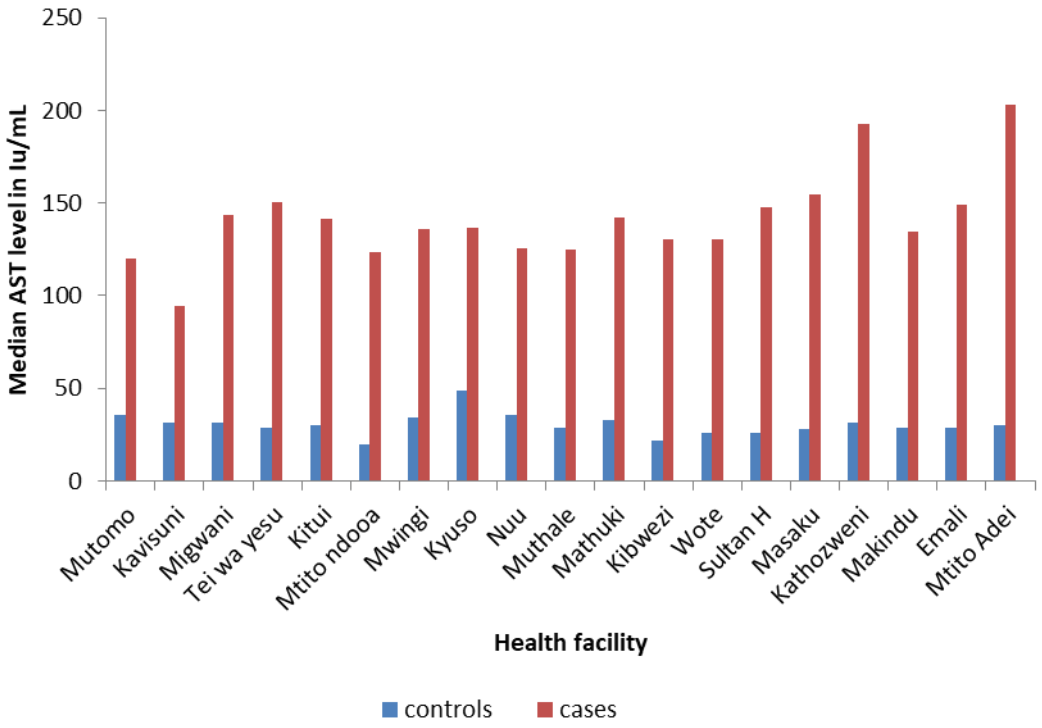

Figure 4.1: Case-control comparative serum AST median levels per health facility

4.3 Case and control cohort alanine amino transferase (ALT) levels in blood samples

The analysis of case cohort sample, (N = 283), yielded an overall mean level of 173.32 Iu/mL (CI; 159.132 to 187.508 Iu/mL), at 95% confidence level (p=0.05). The median mean was 172.02 Iu/mL(Table 4.3). The non parametric Chi square test value for association between the mean case ALT values and the health centers indicated calculated value of χ^2 = 109.124, (p = 0.05). The observed test Chi square value was given as $\chi^2_{(0.05,\ 18df)}$ = 28.678, (p=0.05). The control subject ALT level overall mean was 28.41 Iu/mL (CI; 25.958 to 30.862 Iu/mL) at 95 % confidence level (p=0.05) with a median mean of 27.77 Iu/mL (Table 4.4). Figure 4.2 shows the comparative medians for the cohorts.

Table 4.3: Distribution of Case cohort serum samples and ALT range per health facility

health centre	ALT range Iu/mL	sample (n=283)	mean Iu/mL	sd (ϭ)
Mutomo	75.70-420.00	17	176.09	106.43
Kavisuni	80.90-195.60	03	134.10	57.79
Migwani	68.50-385.00	10	170.92	104.23
Tei wa Yesu	68.50-245.60	04	153.60	80.96
Kitui	55.40-412.50	21	179.97	107.72
Mtito Ndooa	65.00-390.00	07	162.99	106.12
Mwingi	64.50-389.00	10	172.02	92.35
Kyuso	92.80-215.00	02	153.90	86.40
Nuu	74.50-189.80	06	130.10	43.07
Muthale	72.60-402.60	15	167.79	87.93
Mathuki	86.50-415.80	05	219.66	120.37
Kibwezi	72.60-152.90	03	102.67	43.78
Wote	12.4-401.80	45	161.84	83.99
Sultan Hamud	90.50-395.00	12	189.92	91.45
Masaku	58.00-405.00	97	195.13	90.30
Kathonzweni	101.50-363.50	12	188.74	73.26
Makindu	98.00-388.50	07	177.59	103.39
Emali	90.80-444.50	04	239.95	159.38
Mtito Andei	112.8-380.50	03	216.10	143.92

Note: Sd= standard deviation

Table 4.4: Distribution of control cohort serum sample and ALT range per health facility

health facilty	range Iu/mL	sample (n=283)	mean Iu/mL	sd (ϭ)
Mutomo	13.40-64.50	17	33.87	13.79
Kavisuni	20.90-51.80	03	34.17	15.91
Migwani	11.10-64.70	10	32.16	18.31
Tei wa Yesu	14.30-32.30	04	26.00	8.45
Kitui	13.20-38.45	21	25.47	7.81
Mtito Ndooa	10.50-32.50	07	20.50	8.25
Mwingi	8.20-67.80	10	29.94	20.03
Kyuso	24.50-61.30	02	42.90	26.02
Nuu	13.90-43.20	06	30.92	13.34
Muthale	14.50-73.50	15	31.79	15.29
Mathuki	11.90-39.40	05	25.62	11.97
Kibwezi	11.50-21.90	03	16.83	5.07
Wote	9.60-49.60	45	24.41	10.51
Sultan Hamud	9.60-49.70	12	26.52	13.40
Masaku	9.60-52.70	97	25.70	10.55
Kathonzweni	11.80-40.30	12	29.78	8.83
Makindu	16.40-45.70	07	29.97	10.69
Emali	15.80-33.40	04	25.65	7.29
Mtito Andei	17.20-38.90	03	27.77	10.86

Note: sd = standard deviation

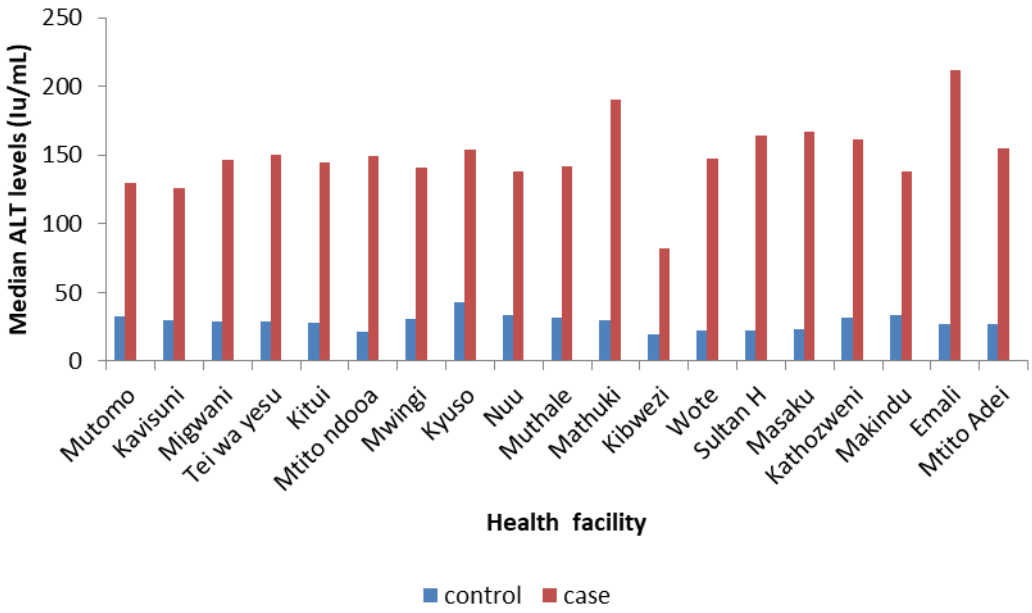

Figure 4.2: Case and control comparative median ALT serum levels per health facility

4.4 Samples positive for HBsAg and AFB_1 serum albumin adducts, the biomarkers of liver disease

In case cohort sample 47% of subject serum samples were positive for HBsAg, while 49.5% was positive for aflatoxin B_1 lysine albumin adducts (AFB_1 lysine albumin adducts). In the same cohort, 7% 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 1.06% of the serum samples testing negative for both HBsAg and AFB_1 lysine albumin adducts (Table 4.5).

Table 4.5: Case subject samples positive and negative for biomarkers of liver disease

Biomarkers	Sample (n)	Ratio	%
HBsAg	133 positives	0.470	47
AFB_1 lysine albumin adducts	140 positives	0.495	49.5
AFB_1 lysine adducts + HBsAg	7 positives	0.0247	2.47
All above	3 negatives	0.0106	1.06
Totals	**283**	**1.000**	

For control subjects 15% of serum samples were positive for HBsAg, while 22% of the sample was positive for AFB_1 lysine albumin adducts. In the same cohort, 9% of the serum sample was positive for both HBsAg and AFB_1 lysine albumin adducts. Control cohort had 54% of the subject serum sample testing negative (Table 4.6)

Table 4.6: Control subjects' samples positive and negative for biomarkers of liver disease

Biomarker	Sample (n)	Ratio
HBsAg	42 positives	0.1484
AFB_1 lysine albumin adducts	62 positives	0.22
AFB_1 lysine albumin adducts + HBsAg	26 positives	0.09
All above	153 negatives	0.54
Total	**283**	**1.00**

4.5 Case- control cohort hepatitis B surface antigen (HBsAg) levels in serum samples

The analysis results for the total case serum sample (N = 283), was placed in two categories-

The sample positive for both HBsAg and AFB_1 lysine albumin adducts and that positive for HBsAg only. The sample positive for HBsAg was 49.46% (n = 140), of the total subject serum samples and 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 3.481 x 10^3 Iu/mL, {95%, CI; (3.037 to 3.925) x 10^3}, $p \leq 0.05$. The median level was 2.20 x 10^3 Iu/mL for the same cohort (Table 4.7). Within the same case cohort, 2.5% (n = 7) of the sample was positive for both HBsAg and AFB_1 lysine albumin adducts. Among the case subject sample in this second sub cohort, the HBsAg levels ranged from 1.2 x 10^3 Iu/mL to 1.8 x 10^3 Iu/mL, with a mean of 1.474 x 10^3 Iu/mL {95%, CI; (1.312 x 10^3 to 1.636 x 10^3) }, $p \leq 0.05$. In this category, 2.5% (n=7) of sample had AFB_1 lysine albumen adducts range of 18.80 pg/mg to 72.50 pg/mg with a mean of 43.80 pg/mg (95%, CI; 27.37 to 60.23), $p \leq 0.05$.

The control cohort had serum samples positive for both HBsAg and AFB_1 lysine albumin adducts with 15% (n = 42), of serum samples positive for HBsAg 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 4.8). The analysis results for the case and control median levels for hepatitis B surface antigens (HBsAg) are compared in figure 4.3

Table 4.7: Case mean HBsAg levels for positive serum sample per health facility

heath center	positive (n= 140)	range Iu/mL (10^3)	mean Iu/mL (10^3)	sd
Mutomo	11	0.50—9.80	3.37	3.02
Tei wa yesu	04	1.5—5.50	2.95	1.61
Kitui	13	0.60—9.20	3.6	2.90
Mtito ndooa	05	1.20—8.50	2.85	2.83
Mwingi	02	4.50—7.50	6.00	1.50
Kyuso	02	1.80—3.20	2.5	0.70
Migwani	03	1.50—6.80	4.60	2.26
Kavisuni	0	0.00	0.00	0.00
Nuu	02	1.80—2.20	2.0	0.20
Muthale	06	1.50—9.50	3.50	2.77
Mathuki	03	1.50—9.80	4.37	3.84
Kibwezi	01	0.00	2.20	0.00
Wote	24	0.90—9.00	3.01	2.27
Sultan Hamud	05	1.6—7.80	4.44	2.43
Masaku	49	0.50—9.80	3.64	2.86
Kathozweni	07	1.7—6.50	2.88	1.61
Makindu	02	2.60—7.80	5.20	2.60
Emali	01	0.00	3.20	0.00
Mtito adei	0	0.00	0.00	0.00

Table 4.8: Controls mean HBsAg levels for positive serum samples per health facility

Health center	positive (n=68)	Range Iu/mL (10^3)	Mean Iu/mL (10^3)	Sd
Mutomo	10	0.085—0.990	0.411	0.310
Tei wa yesu	01	0.00	0.060	0.00
Kitui	03	0.095—0.150	0.132	0.026
Mtito ndooa	00	0.00	0.00	0.00
Mwingi	04	0.07—0.80	0.500	0.297
Kyuso	01	0.00	0.900	0.00
Migwani	04	0.55—0.85	0.683	0.108
Kavisuni	01	0.00	0.780	0.00
Nuu	03	0.20—0.55	0.333	0.155
Muthale	03	0.15—0.95	0.483	0.339
Mathuki	01	0.00	0.150	0.00
Kibwezi	00	0.00	0.00	0.00
Wote	13	0.05—0.50	0.228	0.144
Sultan Hamud	05	0.20—0.49	0.348	0.097
Masaku	16	0.06—0.98	0.304	0.333
Kathozweni	03	0.085—0.150	0.108	0.030
Makindu	00	0.00	0.00	0.00
Emali	00	0.00	0.00	0.00
Mtito Adei	0	0.00	0.00	0.00

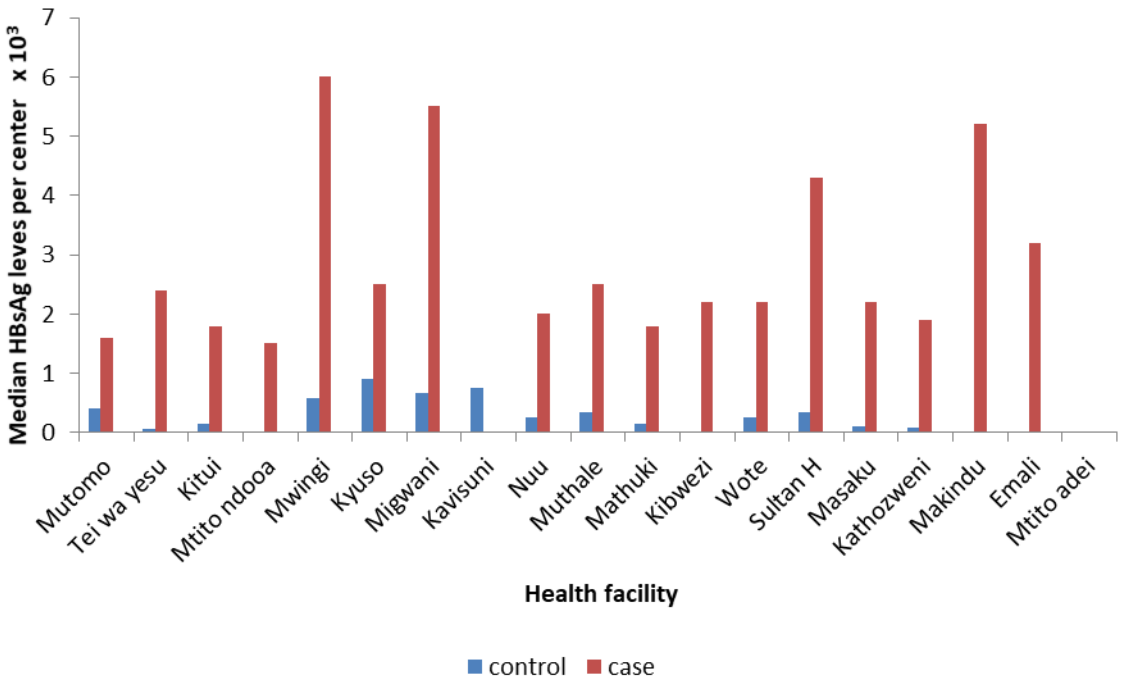

Figure 4.3: A comparative case-control median HBsAg levels in serum samples per health facility

4.6 Case and control AFB_1 lysine albumin adducts level in serum samples

The analysis results for AFB_1 lysine albumin adducts level for case samples was 51.94% (n = 147) positive, with a range of 15.5 pg/mg to 135 pg/mg, and a mean of 42.19 pg/mg (95%, CI; 38.45 to 45.93), $p \leq 0.05$. For this sub cohort, 2.5% (n=7), of the subject serum sample had AFB_1 lysine albumin adducts with a range of 18.80 pg/mg to 72.50 pg/mg, and a mean of 43.80 pg/mg (95%; CI; 27.20 to 60.23), $p \leq 0.05$ (Table 4.9). Among controls, 9% (n = 26) of serum samples had evidence of both HBsAg and AFB_1 lysine albumin adducts at various levels while 31% (n=88) of serum samples were positive for AFB_1 lysine albumin adducts with a range of 3.5 pg/mg to 60.50 pg/mg and a mean of 14.30 pg/mg (95%, CI; 12.23 to 16.36), $p \leq 0.05$ (Table 4.10). Figure 4.4 is a comparative median level for case and control samples.

Table 4.9: Case mean AFB1 lysine albumin adducts levels for serum samples per health facility

Health Center	positive (n= 147)	range (pg/mg)	Mean(x=39.81) (pg/mg)	sd (pg/mg)
Mutomo	06	15.80—93.8	34.48	26.81
Tei wa yesu	01	0.00	31.50	0.00
Kitui	07	16.80—47.8	29.84	11.26
Mtito ndooa	03	18.80—25.0	21.53	2.583
Mwingi	08	21.80—96.5	45.26	29.87
Kyuso	00	0.000	0.00	0.000
Migwani	07	22.5—61.8	34.47	13.14
Kavisuni	02	19.80—56.5	38.15	18.35
Nuu	04	15.80—71.8	36.15	21.30
Muthale	10	19.50—68.5	36.11	15.69
Mathuki	02	68.00—82.5	75.25	7.250
Kibwezi	02	21.0—33.80	27.40	6.40
Wote	20	17.50—64.5	31.84	11.59
SultanHamud	08	19.80—74.5	43.57	19.68
Masaku	50	15.5—102.8	47.91	23.74
Kathozweni	06	23.8—85.50	54.65	22.81
Makindu	05	24.0—55.0	40.62	11.41
Emali	03	18.50—64.8	42.03	18.91
Mtito Adei	03	53.3—135.0	85.67	35.417

Note: Sd= standard deviation

Table 4.10: Control mean AFB_1 lysine albumin adducts levels for serum samples per health facility

Health center	Positive (n=88)	Range pg/mg	Mean($\bar{X}$=11.1) pg/mg	Sd pg/mg
Mutomo	08	6.50—12.00	9.03	2.130
Tei wa yesu	02	5.50—16.50	11.00	5.500
Kitui	07	9.50—29.50	19.73	6.387
Mtito ndooa	01	0.00	4.50	0.000
Mwingi	03	5.80—15.60	12.30	4.596
Kyuso	00	0.00	0.00	0.000
Migwani	01	0.00	8.40	0.000
Kavisuni	00	0.00	0.00	0.000
Nuu	01	0.00	8.00	0.000
Muthale	05	5.50—32.50	18.12	10.452
Mathuki	02	17.0—28.50	22.75	5.750
Kibwezi	00	0.00	0.00	0.000
Wote	11	4.50—23.40	12.25	5.269
SultanHamud	01	0.00	9.50	0.000
Masaku	33	3.50—40.50	13.90	9.262
Kathozweni	08	6.40—60.50	23.12	18.281
Makindu	03	5.80—10.40	8.33	1.906
E mali	01	0.00	7.50	0.000
Mtito adei	01	0.00	11.40	0.000

Note: Sd= standard deviation

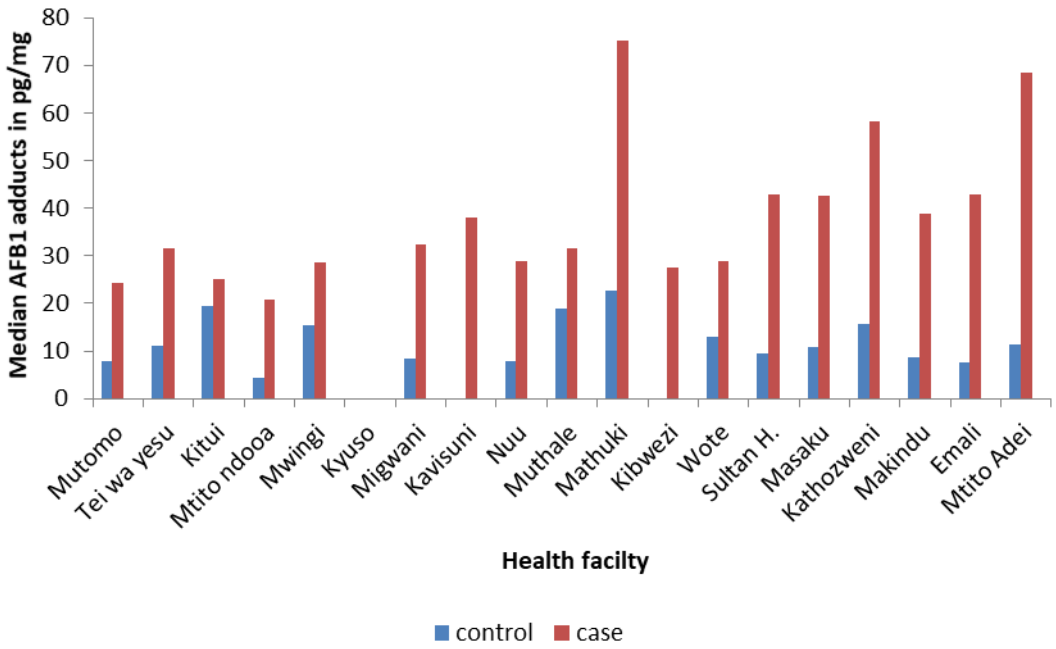

Figure 4.4: Comparative case - control median AFB_1 lysine albumin adducts level in serum samples per health facility

4.7 De ritis ratio values for both case and control serum samples

The overall mean De ritis ratio range for the case cohort sample (N = 283), was between 0.7606 and 1.4647, with a mean value of 0.9866, (CI; 0.9157 to 1.0579), at 95% confidence level ($p \leq 0.05$). The median ratio was 0.9681 for the case cohort. This is tabulated below as mean De ritis ratio values from various health centers for both case and control serum samples (Table 4.11). For the Control cohort, the De ritis ratio range was 1.0308 to 1.2132 with a mean De ritis ratio value of 1.120 (CI; 1.097 to 1.143), at 95% confidence level ($p \leq 0.05$). The median De ritis ratio for the control cohort was 1.1120 (Table 4.11).

Table 4.11: Mean De ritis ratio for case and control serum sample per health facility

health facility	case ratio	control ratio
Mutomo	0.8950	0.8940
Tei wa Yesu	1.0727	1.1119
Kitui	0.9583	1.1461
Mtito Ndooa	0.9543	1.1589
Mwingi	0.8406	1.1460
Kyuso	1.0133	1.1861
Migwani	0.9990	1.2132
Nuu	0.8825	1.0859
Muthale	0.8701	1.1112
Kibwezi	1.4647	1.0524
Wote	0.9741	1.1819
Sultan Hamud	1.0615	1.1239
Masaku	0.9685	1.1950
Kathozweni	0.9681	1.0867
Makindu	1.2474	1.0554
E-mali	0.8319	1.1660
Mtito Adei	1.1263	1.0308
Mathuki	0.8577	1.1045
Kavisuni	0.7606	1.0874

Note: ratio = AST/ALT

A total of 91.8% (n = 260), out of the serum sample (N= 283) had the De ritis ratio greater than one (ratio >1), while 8.13% (n = 23) of the same sample had registered a De ritis ratio less than one (ratio < 1). For the case subject cohort, 69.61% (n = 197), of the sample had a De ritis ratio less than one (ratio < 1), while 30.4% (n = 86) of the sample had De ritis ratio greater than one (ratio>1) in this particular study (Table 4.12). None had a ratio value equal to one (ratio =1).

Table 4.12: Cohorts with De ritis ratio less, equal to and greater than one (1)

Cohort	Ratio< 1	Ratio= 1	Ratio> 1	Total
Cases	197	0	86	283
Controls	23	0	260	283
Totals	**220**	**0**	**346**	**566**

4.8 Case hospital admissions due to HBV and AFB_1 induced liver conditions

The causal factors for hospital admission for this cohort are tabulated in table 4.13 below.

Table 4.13: Case subject admissions due to HBV and aflatoxinB_1 (AFB_1)

Disease factor	Rate %	Subjects (n)	Status
HBV	47.00	133	Exposed to HBV
AFB_1	49.47	140	Exposed to AFB_1
HBV + AFB_1	2.47	7	Exposed to both factors
Unknown	1.06	3	Evidence of disease but causes unknown
Totals	**100**	**283**	

4.9 Control hospital admissions due to non-HBV and AFB_1 toxicity conditions

The non liver disease non exposed control subjects where 54 % (n=153) while those with mixed aetiology were 9 % (26). Other causal factors for hospital admissions are tabulated below (Table 4.14).

Table 4.14: Controls subjects exposed to both HBV and AFB_1 but admitted due to other

Disease Factor	Rate %	Subjects (n)	Status
HBV	15	42	Non disease/exposed
AFB_1	22	62	Non disease / exposed
HBV + AFB_1	9	26	Non disease / exposed
Non – disease	54	153	Non disease /Non Exposed
Total	**100**	**283**	

4.9.1 Odds ratio and relative risks of HBV infection in the two counties

On examination of samples for hepatitis B virus antigen (HBsAg), the total sample for both case and controls (N = 566), exposed and testing positive was 23.49% (n = 133), while 22.9% (n = 130) had evidence of exposure but did not have the disease.

However, the total exposed to HBV was 46.46% (n = 263), while the total unexposed was 53.53% (n= 303). On disease category, 26.50% (n = 150), had liver disease but had not been exposed to hepatitis B virus (HBV). Therefore, 27.03 % (n= 153), of the study subjects had no evidence of disease and had not been exposed to HBV. The tabulation of data values was done in a contingency table 4.15

Table 4.15: Contingency table for HBV associated liver disease

Status	Disease	Non-disease	Total
Exposed	133 (a)	130 (b)	263
Non exposed	150 (c)	153 (d)	303
Total	**283**	**283**	**566**

The risk ratio (RR) from the contingency table above therefore worked out as value ratios as follows: RR $= \frac{a}{a+b} / \frac{c}{c+d} = \frac{133 \ x \ 303}{263 \ x \ 150} = 1.022$

While the relative risk (RR) was 1.022, (95%, CI; 0.8663 to 1.2052), p= 0.05, the odds ratio

(OD) was determined as: - $OD = \frac{ad}{bc} = \frac{133 \times 153}{150 \times 130} = 1.043$

Therefore, OD was 1.043, (95%, CI; 0.750 to 1.450), p= 0.05

4.9.2 Prevalence of liver disease due to sero presence of HBV in the two counties

The prevalence of liver disease due to sero presence of HBV in this study was determined as a ratio of those patients who had the physical disease and were exposed to HBV (n = 140), to the total population randomly picked for the study (N=566).

Thus, this was $\frac{140}{566}$ = 0.2473. This was a sero prevalence of (24.73%) in the two Counties.

4.9.3 Odds ratio and relative risk due to AFB_1 toxicity in the two counties

Analysis of the total case and controls sample (N = 566), for AFB_1 lysine albumin adducts gave 24.73% (n = 140) as exposed, while 22.96% (n = 130), were exposed but had no physical evidence of aflatoxicosis. The total exposed for AFB_1 lysine albumin adducts for the two groups was 40.8% (n = 270). Those non- exposed to AFB_1 but had liver disease in was 25.26% (n = 143), while 27% (n = 153) showed no evidence of exposure and had no liver disease (Table 4.16).

Table 4.16: Contingency table for AFB_1 induced aflatoxicosis

status	disease	non-disease	total
Exposed	140 (a)	130 (b)	270
Non exposed	143 (c)	153 (d)	296
Total	**283**	**283**	**566**

The relative risk (RR) from the contingency table above was determined as

$$RR = \frac{\frac{a}{a+b}}{\frac{c}{c+d}} = \frac{140 \text{ x } 296}{270 \text{ x } 143} = 1.073$$

Thus the RR ratio was 1.073, (95%; CI; 0.9101 to 1.2649), p= 0.05, while the odds ratio from the above contingency table was determined as:-

$$OD = \frac{ad}{bc} = \frac{140 \ x \ 153}{143 \ x \ 130} = 1.152,$$

Therefore, OD was 1.152, (95%, CI: 0.91 to 1.265), p= 0.05

4.9.4 Prevalence of liver disease due to sero presence of AFB_1 among the study participants

The prevalence of liver disease due to subject's sero presence of AFB_1 lysine albumin adducts in serum was determined as a ratio of the patients with physical

disease due to AFB_1 toxicity who were exposed (n=147) to the total selected population sample (N=566).

This was therefore $\frac{147}{566}$ = 0.2597 and was equivalent to a disease prevalence of 25.97

% due to dietary AFB_1 exposure among the population under study.

4.9.5 Prevalence of liver disease due to AFB_1 toxicity and HBV co infection

Out of the total sample of case and controls (N = 566), 1.24% (n = 7), had evidence of exposure to both AFB_1 as shown by presence of AFB_1 lysine albumin adducts and HBV since samples were co-infected with hepatitis B virus (HBV) as shown by presence of HBsAg. Out of the total sample exposed to HBV and AFB_1, 24.20% (n= 137), those exposed to same disease factors but had no evidence of disease was 22.9% (n = 130). A total of 75.79% (n= 429) were not exposed to both HBV and AFB_1 factors. Those not exposed to compined disease factors but had evidence of liver disease were 48.76% (n = 276). Only 27.03% (n = 153), of this cohort was non-exposed and had no evidence of liver disease. Table 4.17 is data contingency table for combined disease factors

Table 4.17: Contingency table for combined HBV and AFB_1 disease factors

status	disease	non-disease	total
Exposed	7 (a)	130 (b)	137
Non exposed	276 (c)	153 (d)	429
Total	**283**	**283**	**566**

The risk ratio (RR) in this case was determined as a mean of the known risk ratios of the individual disease factors in this study since it involved two independent risk factors to liver disease, including HBV and AFB_1 risk ratios, thus: -

Relative risk (RR) = 1.0475, (95%, CI; 0.5073 to 2.163), $p \leq 0.05$.

The odds ratio (OD) for the combined odds that a subject would be infected with HBV and have AFB_1 toxicity was estimated also as a mean of individual Odds for the disease factors, thus;

The Odds ratio (OD) was $\frac{2.195}{2.} = 1.097$

4.9.6 Prevalence of liver disease due to HBV and AFB_1 co infection

The prevalence of liver disease due to sero presence of HBV and AFB_1 was determined as a ratio of those cases with physical disease and with evidence of combined exposure to HBV and AFB_1 (n=7) to the total population in the randomly picked total sample (N=566). Thus: -

This was $\frac{7}{566} = 0.0124,$

This was 0.0124 x 100% = 1.24%

4.10 Comparative mean case and control household maize grain and flour AFB_1 levels

The Case household maize grain sample had lowest aflatoxinB_1 level of 0.00 ppb. The highest level was 33.00 ppb with a mean of 12.25 ppb (95%, CI; 10.55 to 13.96 ppb), $p \leq 0.05$. The Case household maize flour sample with the lowest AFB_1 levels had 0.00 ppb, while highest level was 48.30 ppb. This range had a mean of 16.06 ppb (95%, CI; 14.23 to 17.89 ppb), $p \leq 0.05$. The control subject household maize grain sample, had lowest AFB_1 level at 0.00 ppb, while the highest level was 13.50 ppb, with a mean of 5.03 ppb (95%, CI; 4.65 to 5.41 ppb), $p \leq 0.05$. Control maize flour AFB_1 level range was from 0.00pb to 15.60ppb with a mean of 7.60 ppb, (95%, CI; 7.188 to 8.011 ppb), $p \leq 0.05$ (Table 4.18)

Table 4.18: Comparative mean case and control maize grain and flour AFB_1 levels

Cohort	N	Minimum (ppb)	Maximum (ppb)	Mean (ppb)	Sd (σ)
Case household grain	283	0.00	33.00	12.25	8.08
Case household flour	283	0.00	48.30	16.06	1.83
Control household grain	283	0.00	13.50	5.03	3.23
Control household flour	283	0.00	15.80	7.60	3.53

4.10.1 Case- control maize grain AFB_1 level in samples associated with the health facilities

Cases sample had 87.28% (n = 247) contaminated with AFB_1. The mean AFB_1 level in case grain samples was 12.25 ppb (95%, CI; 10.55 ppb to 13.96 ppb), $p \leq 0.05$ (Table 4.19).

Controls had a mean of 5.029 (95%, CI; 4.65 to 5.41), $p \leq 0.05$ (Table 4.20).

Table 4.19: Case household maize grain sample mean AFB_1 levels per health facility

Health sample facility (n=283)		Mean ($\bar{X}$=12.25ppb)	Range (ppb)	Sd (ppb)
Mutomo	17	9.01	0.00-23.50	7.71
Tei wa Yesu	04	12.50	3.00-21.50	9.12
Kitui	21	9.59	0.00-22.50	7.63
Mtito Ndooa	07	9.19	0.00-19.00	6.87
Mwingi	10	18.36	4.00-33.00	9.18
Kyuso	02	7.40	3.50-11.30	5.52
Migwani	10	13.29	3.50-21.30	6.34
Kavisuni	03	8.70	0.00-14.80	7.73
Nuu	06	15.27	0.00-26.8	8.99
Muthale	15	10.91	0.00-28.50	8.61
Mathuki	05	13.16	5.30-21.00	6.24
Kibwezi	03	10.37	5.50-18.30	6.92
Wote	45	8.54	0.00-31.30	7.74
Sultan Hamud	12	9.86	0.00-21.00	7.54
Masaku	97	11.29	0.00-31.00	7.83
Kathozweni	12	10.39	0.00-31.00	9.39
Makindu	07	17.84	2.50-29.00	8.27
E-mali	04	15.32	4.00-23.30	8.20
Mtito Adei	03	21.83	17.50-30.00	7.08

Table 4.20: Control household maize grain sample mean AFB_1 levels per health facility

Health facility sample (n=283)		**Mean($\bar{x}$=4.93)** (ppb)	**Range** (ppb)	**Sd** (ppb)
Mutomo	17	7.15	0.00-13.00	4.27
Tei wa Yesu	04	6.02	0.00-11.80	5.28
Kitui	21	5.25	0.00-11.00	3.16
Mtito Ndoa	07	7.63	3.80-11.00	2.83
Mwingi	10	6.32	2.50-13.50	3.21
Kyuso	02	7.65	7.00--8.30	0.92
Migwani	10	4.79	0.00--9.00	3.20
Kavisuni	03	2.43	0.00--5.30	2.68
Nuu	06	5.02	0.00-11.00	4.37
Muthale	15	4.72	0.00-11.00	3.09
Mathuki	05	6.00	0.00-- 8.50	2.23
Kibwezi	03	2.83	0.00-- 5.50	2.75
Wote	45	4.54	0.00-10.30	2.71
Sultan Hamud	12	4.56	0.00-- 9.00	2.47
Masaku	97	5.07	0.00-13.00	3.21
Kathozweni	12	3.20	0.00--7.50	2.69
Makindu	07	4.16	0.00--7.80	2.84
E-mali	04	4.08	0.00-10.00	4.25
Mtito Andei	03	2.17	0.00--4.50	2.25

The comparative median values for case and controls sample aflatoxin B_1 are shown in figure 4.5

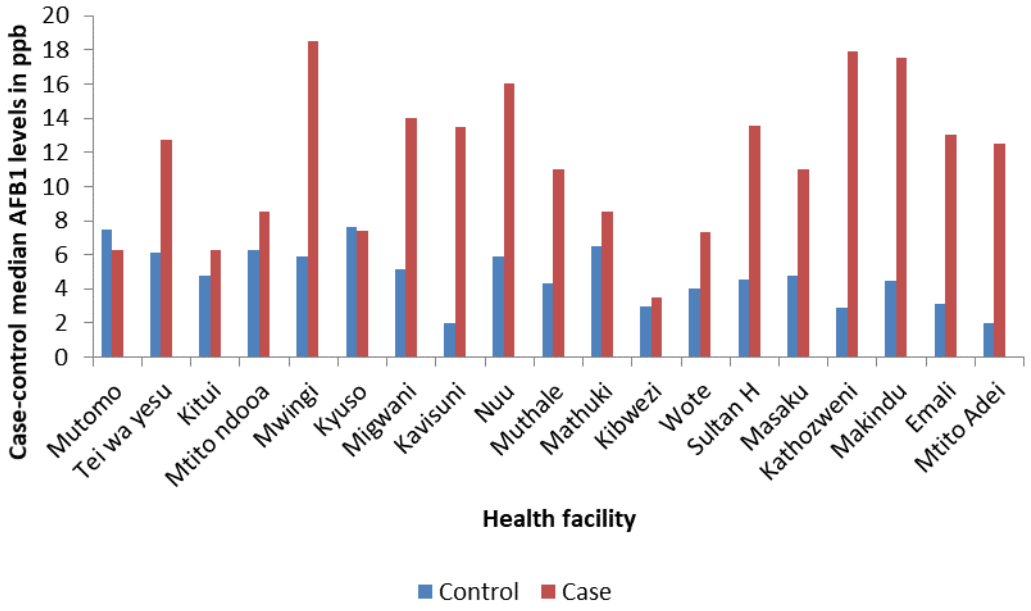

Figure 4.5: Case and control comparative median household maize grain aflatoxin B_1 levels per health facility

4.10.2 Case-control household maize flour aflatoxin B1 levels associated with the health facilities

The total maize flour sample (N= 283) had 95.76% (n = 271) contaminated with aflatoxin B_1, while 4.24% (n = 12) had no evidence of aflatoxin B_1 content (Table 4.21). The overall mean aflatoxin B_1 levels in case flour samples were 16.06 ppb (CI; 15.24 to 18.90 ppb) at 95% confidence level ($p \leq 0.05$). The control cohort household maize flour sample (N = 283), had 96.46% (n = 273), of the sample contaminated with aflatoxin B_1. The total sample had 3.54% (n = 10) with zero levels of AFB_1. The mean ($\bar{X}$), aflatoxin B_1 levels for control cohort household flour samples was 7.60 ppb (CI; 7.19 ppb to 8.02 ppb), at 95% confidence level ($p \leq 0.05$), with a median mean of 7.53 ppb (Table 4.22).

Table 4.21: Case subjects mean household maize flour sample AFB_1 levels per health facility

health facility	sample (n=283)	mean $\bar{X}$=16.96(ppb)	range (ppb)	sd (ppb)
Mutomo	17	13.95	3.00-41.00	10.65
Tei Wa Yesu	04	16.00	3.00-21.00	7..76
Kitui	21	13.79	2.00-33.50	10.69
Mtito Ndooa	07	15.93	2.50-41.00	12.91
Mwingi	10	21.40	6.50-43.50	10.67
Kyuso	02	15.90	7.30-24.50	8.60
Migwani	10	19.34	7.00-43.50	11.09
Kavisuni	03	19.00	9.50-27.50	7.38
Nuu	06	23.02	9.50-40.00	10.80
Muthale	15	15.25	0.00-33.58	10.05
Mathuki	05	13.96	3.00-29.50	9.79
Kibwezi	03	6.93	0.00-15.00	6.18
Wote	45	14.43	0.00-43.80	12.04
SultanHamud	12	16.12	4.00-31.50	8.99
Masaku	97	15.55	0.00-45.50	11.18
Kathozweni	12	21.12	0.00-41.50	9.50
Makindu	07	17.73	0.00-33.00	10.45
Emali	04	26.07	2.50-48.30	20.07
Mtito Andei	03	16.87	16.80-22.8	2.78

Table 4.22: Control household maize flour mean aflatoxinB_1 levels per health facility

health sample center (n=283)		Mean $\bar{X}$= 7.50(ppb)	Range (ppb)	Sd (ppb)
Mutomo	17	7.81	0.00-13.80	3.54
Tei wa Yesu	04	6.60	0.00-13.80	5.65
Kitui	21	8.84	0.00-15.50	3.74
Mtito ndooa	07	6.57	0.00-3.80	1.88
Mwingi	10	7.53	3.50-11.80	2.99
Kyuso	02	7.65	4.80-10.50	4.03
Migwani	10	7.34	0.00-13.50	3.99
Kavisuni	03	5.03	3.80-6.80	1.56
Nuu	06	6.07	3.50-9.00	1.83
Muthale	15	7.46	3.50-12.80	2.87
Mathuki	05	8.56	4.00-11.50	2.91
Kibwezi	03	8.20	4.30-13.00	4.42
Wote	45	7.66	2.50-14.30	3.26
Sultan Hamud	12	7.26	3.30-11.50	2.58
Masaku	97	7.44	0.00-14.80	3.54
Kathozweni	12	8.21	0.00-15.80	5.85
Makindu	07	7.99	2.30-13.80	4.78
E-mali	04	9.20	3.50-12.00	3.91
Mtito Andei	03	7.20	3.80-12.80	4.89

Note: sd = standard deviation

Figure 4.6 compares the case and control median aflatoxinB_1 levels for both case and controls household maize flour sample in ppb.

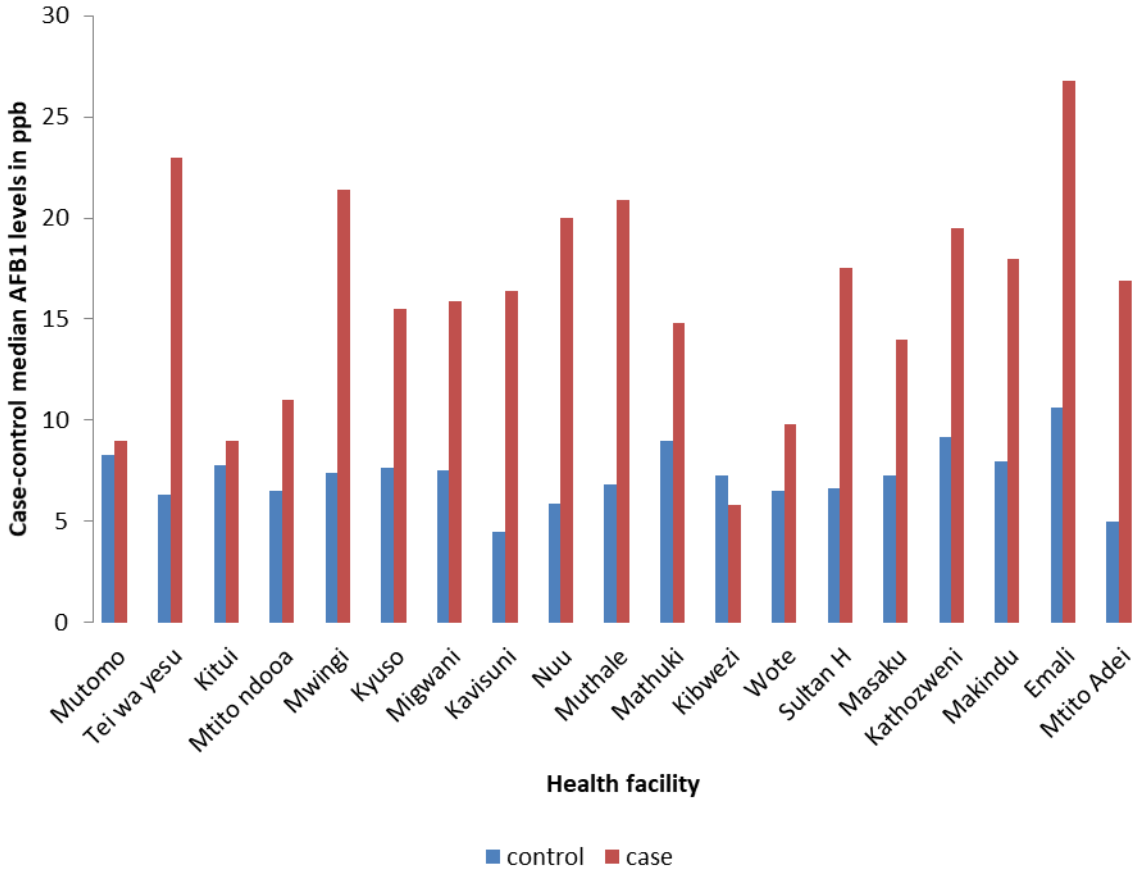

Figure 4.6: Median case and control household maize flour aflatoxin B_1 levels per health facility

4.10.3 Case and control household maize grain samples with AFB_1 levels exceeding 10ppb

The total case and control household maize grain sample (N = 566) had 29% (n=168) with aflatoxin B_1 levels exceeding 10 ppb, the codex allimentarius commission (1995a) allowable limit for aflatoxin B_1 levels in maize grain and flour for human consumption. For the total case cohort (N = 283), 53.0% (n = 150) of the subject house hold samples had aflatoxin B_1 levels exceeding 10 ppb while for control household maize grain sample associated with non-liver disease subjects, 3.4% (n = 18) had aflatoxin B_1 levels exceeding 10 ppb (Table 4.23).

Table 4.23: Case and control household maize grain samples with AFB1 levels exceeding 10ppb

Maize grain	N	exceeding 10ppb	%
		(n)	%
Case	283	150	53%
Controls	283	18	6.4%
Total	**566**	**168**	

For the case cohort, the maize grain sample associated with the subject households whose owners had liver conditions was higher at 53.0% (n = 150). The control household sample had 3.4% (n = 18) out of the total sample.

4.10.4 Case and control household maize flour samples with AFB_1 level exceeding 10 ppb

The total case and control sample (N = 566), for subject household maize flour which exceeded 10 ppb, the Codex commission (1995a), allowable food aflatoxin B_1 limit was 41.2% (n = 233). The case cohort sample drawn from households associated with persons with liver disease had 57.24% (n = 162) of sample exceeding 10ppb aflatoxin B_1 levels out of the total sample. The control maize flour sample associated with those households whose subjects was without liver disease had 25.1% (n = 71) of samples with aflatoxin B_1 levels exceeding 10ppb (Table 4.24).

Table 4.24: Case and control household maize flour samples with aflatoxin B_1 level above 10ppb.

Maize flour	N	exceeding 10ppb	%
Case	283	162	57%
Controls	283	71	25%
Total	**566**	**233**	

4.10.5 Differences between case participants mean and median level household AFB_1 for both maize grain and flour sample per health facility

In all centers associated with case participant's household maize flour samples, the **median** aflatoxinB_1 levels were higher than the median case participant's household maize grain levels (Fig.4.7). Further, all the case household maize flour samples (n = 283) had **mean** aflatoxin B_1 level values higher than that of case household maize grain mean aflatoxin B_1 levels (Table 4.25).

Table 4.25: Case sample comparative mean household maize grain and flour aflatoxin B_1 levels per health facility

Health centre	sample (n= 283)	case AFB_1 (ppb) maize grain($\bar{X}$=11.65)	case AFB_1(ppb) maize flour($\bar{X}$=18.15)
Mutomo	17	9.01	12.26
Tei wa Yesu	04	12.50	16.00
Kitui	21	9.59	13.79
Mtito Ndooa	07	9.19	15.93
Mwingi	10	18.36	21.40
Kyuso	02	7.40	15.90
Migwani	10	13.29	19.34
Kavisuni	03	10.93	19.00
Nuu	06	15.52	23.02
Muthale	15	10.70	15.27
Mathuki	05	11.02	13.96
Kibwezi	03	5.77	6.93
Wote	45	8.68	14.43
SultaHamud	12	10.90	16.12
Masaku	97	11.24	15.56
Kathozweni	12	15.21	21.00
Makindu	07	15.47	17.73
E-mali	04	12.23	26.08
Mtito andei	03	14.33	18.87

The aflatoxin B_1 median levels for both maize grain and flour for the case cohort is shown figure 4.7 below.

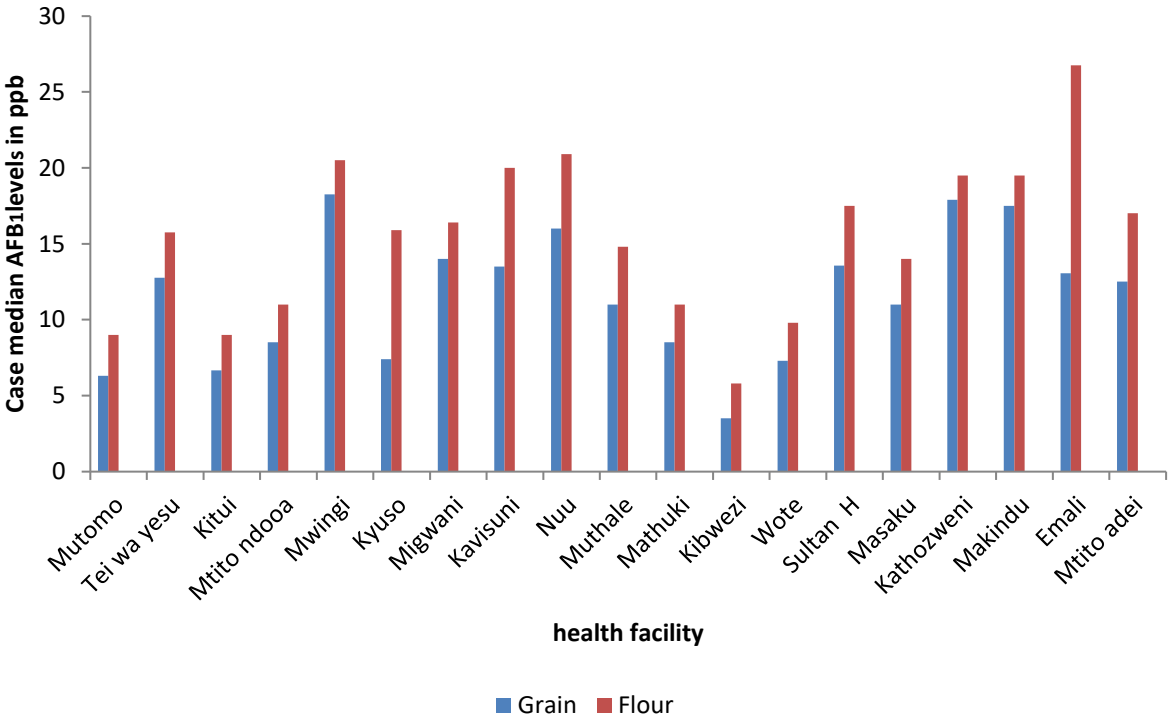

Figure 4.7: A comparative case median household aflatoxin B_1 levels between maize grain and flour samples per health facility

4.10.6 Differences between control samples mean household AFB_1 for both maize grain and flour sample per health facility

The mean for control household maize flour samples was 7.51 ppb (95%, CI; 7.08 to 7.93), $p \leq 0.05$. That of maize grain was 4.93 ppb (95%, CI; 4.22 to 5.63 ppb), $p \leq 0.05$, with a range of 2.17 ppb to 7.65 ppb (Table 4.26).

Table 4.26: Control comparative mean AFB_1 levels for household grain and flour samples per health facility

Health centre	sample (n=283)	Mean AFB_1 maize grain ($\bar{X}$= 4.93)	Mean AFB_1 maize flour ($\bar{X}$=7.51)
Mutomo	17	7.15	7.81
Tei wa Yesu	04	6.03	6.60
Kitui	21	5.25	8.84
Mtito ndooa	07	7.63	6.57
Mwingi	10	6.32	7.53
Kyuso	02	7.65	7.65
Migwani	10	4.79	7.34
Kavisuni	03	2.43	5.03
Nuu	06	5.02	6.07
Muthale	15	4.72	7.46
Mathuki	05	6.00	8.56
Kibwezi	03	2.83	8 .20
Wote	45	4.54	7.66
Sultan Hamud	12	4.56	7.26
Masaku	97	5.07	7.44
Kathozweni	12	3.20	8.21
Makindu	07	4.16	7.99
E-mali	04	4.08	9.20
Mtito Andei	03	2.17	7.20

The median for maize flour sample AFB_1 level range was 7.30 ppb with a range of 5.03ppb to 9.20ppb. That of maize grain sample was 4.79 ppb with a range of 2.17 to 7.65 ppb (Fig.4.8).

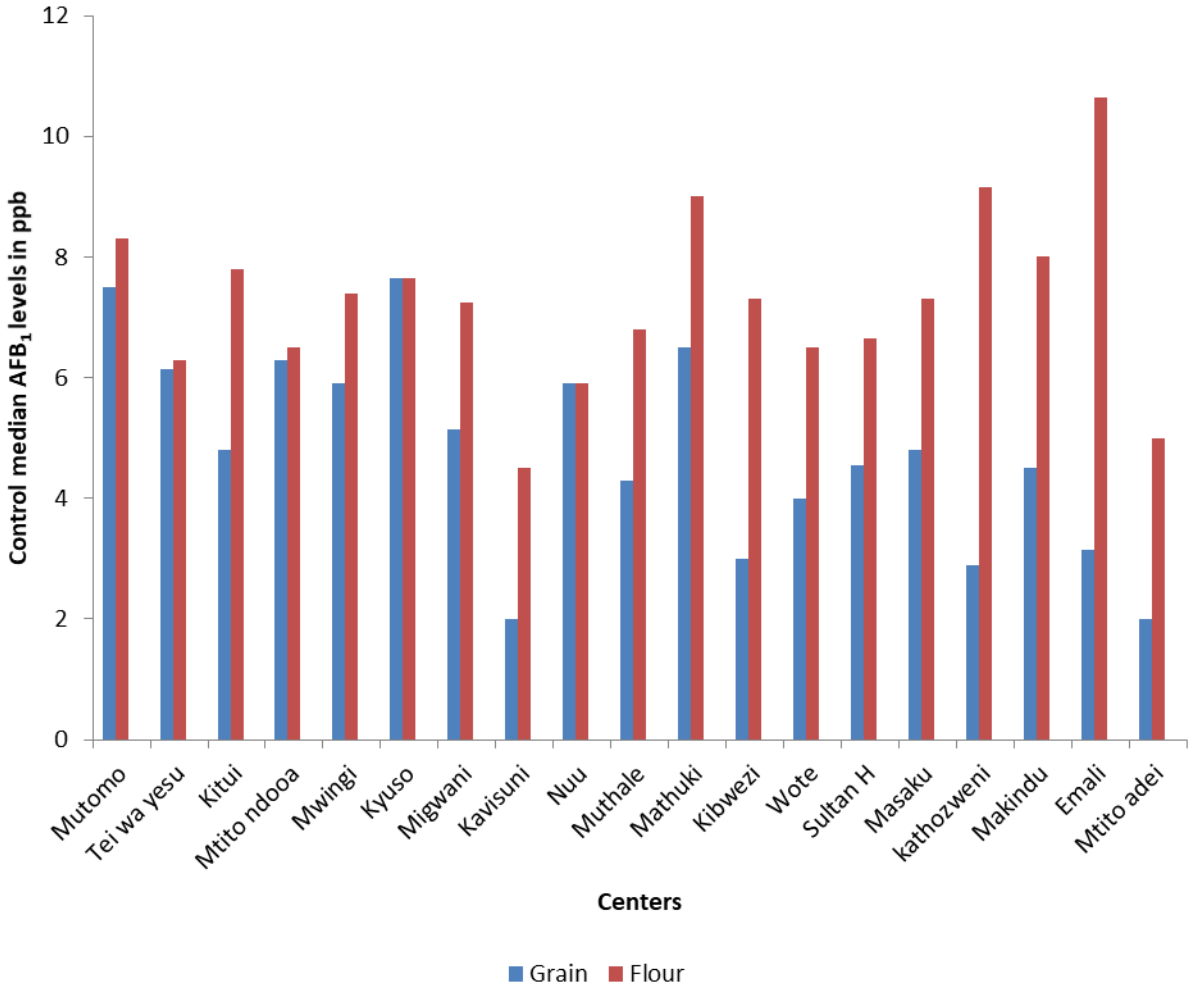

Figure 4.8: A control comparative median AFB_1 level between household maize grain and flour samples per health facility

4.11 Qualitative results of the questionnaire on independent variables and liver diseases

A total of 283 questionnaires were analyzed following various parameters including age brackets, gender, marital status and frequency of hospital visits due to liver conditions and related complaints by patients.

Other questions and the frequency of responses were analyzed and results tabulated.

4.11.1 Age bracket of respondents

The analysis of the sample population (N=283), gave an age range of 12 yrs to 90yrs with a mean age of 60.2 yrs (CI= 58.80 to 61.60) at 95% confidence level ($p \leq 0.05$), for the case cohort (Table 4.27).

Table 4.27: Respondents age groups among the case study participants

age bracket (yrs)	subjects (n)
Between 12. --17.5yrs	17
Between 17.5--35.5yrs	32
Between 35.5--58.5yrs	139
Between 58.5--71.5yrs	87
Between 71.5--90.5yrs	8
Total	**283**

The frequency distribution bar chart gave a normal distribution of patient age group among those who participated in the questionnaires survey (Fig.4.9).

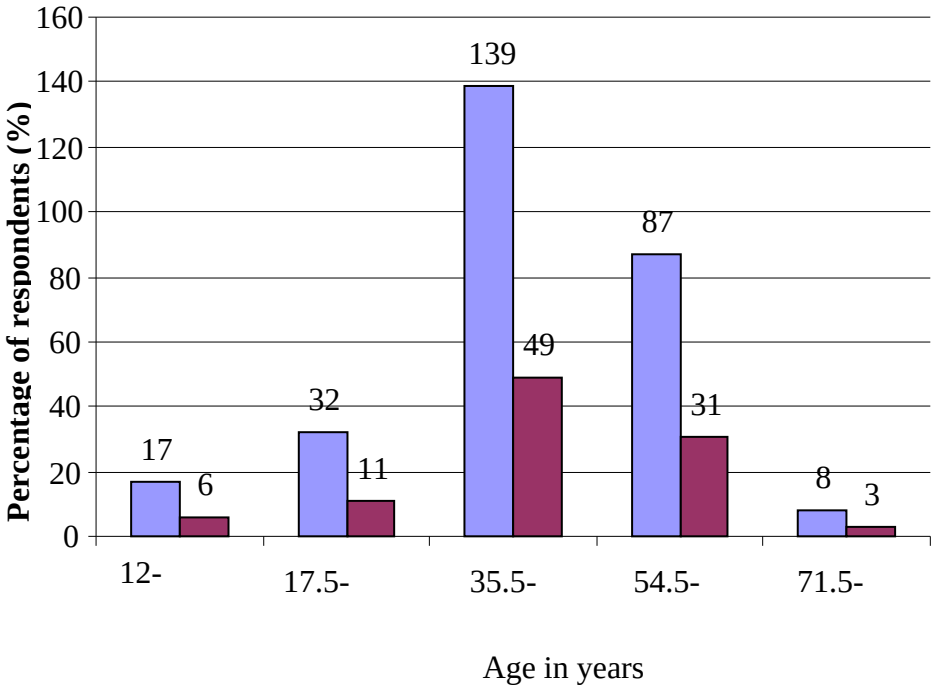

Figure 4.9: Distribution of the respondent's age groups

4.11.2 Marital status of the respondents

The analysis of the participants responses had 71% (n=201), being the respondents who had been admitted to health facilities and where married. Figure 4.10 below shows the frequency of respondents of various categories of respondents.

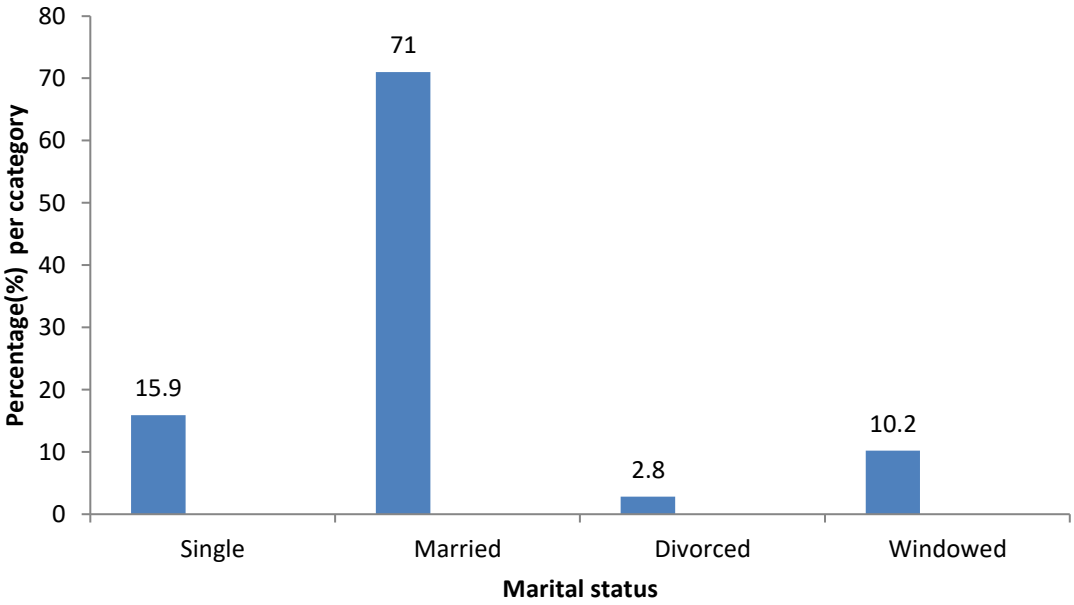

Figure 4.10: Distribution frequency of marital status per category

4.11.3 Frequency of hospital visits due to liver disease

The analysis of the questionnaires had 55.8% (n=158) visiting the health centers only once on liver condition complaint. Table 4.28, below shows the numbers and the percentage (%) for each category of the health facility visits.

Table 4.28: Study participant's hospital visits per category

Category preference	**subjects sample (n)**	**%**
Once only	158	55.8
Twice only	84	29.7
More than 3 times	8	2.8
	2.8	
Unable to remember	25	8.8
	8.8	
None at all	8	2.8
	2.8	
Total	**283**	**100%**

4.12 Questionnaire results on suspect disease variables

4.12.1 Blood transfusion among respondents

The analysis results showed that 63.6% (n=180) of subjects out of the total sample (N=283) had respondend to the questionnaire on blood transfusions. Table 4.29 shows the frequency distribution of the procedure among the sampled patients

Table 4.29: Distribution frequency for blood transfusion among respondents

Category	**Frequency**	
Preference	**Numbers (n)**	**Percentage (%)**
Once	74	26.14
Twice	59	20.84
Four times	39	13.80
Don't know	8	2.80
Did not answer	103	36.40
Total	**283**	**100**

4.12.2 Unprotected sex and non use of condoms

The analysis of responses gave 30% (n=85) of the questionnaire respondents out of total sample (N= 283) who were willing to answer particular questions on sex behaviors and use of condoms. Out of the total sample, 70% (n=198) of patients declined the questionnaire section of the respondents asking about whether they used condoms or not. A total of 5.7% (n=16), agreed to have used them always during sex, while 9.2% (n=26) admitted to have used them rarely. Those who admitted to have used them most of the time were 15.2% (n= 43).

4.12.3 Unsterile body piercing instrument use among respondents

The study participants response analysis had 44.52% (n=126) responding positively to the questionnaire. Table 4.30 is the summary on use of unsterilized body piercing instruments

Table 4.30: Response summary on use of unsterilized body piercing instruments

Respondents	Frequency	
Category	**Numbers (n)**	**Percentage (%)**
Always	30	10.6
Most of the time	54	19.1
Rarely	42	14.8
Declined	157	55.6
Total	**283**	**100**

4.12.4 Respondents use of maize grain in the study area.

The question on use of maize grain as food had 100% (n=283) of respondents answering to the questionnaires. The highest number of respondents 51.2% (n=145), indicated use of maize grain as stable food four (4) times per week. Table 4.31 tabulates the preference of maize grain use by the respondents.

Table 4.31: Preference table for maize grain as food by the respondents

Category	Subjects (n)	Percentage (%)
Twice	8	2.80
Twice	28	9.90
Four times	145	51.2
Five times	44	15.50
Six times	58	20.50
Total	**283**	**100**

4.12.5 Respondents preferred storage methods for maize flour in study area

The question on consumption and storage of maize flour as food in the study area attracted two hundred and three (N=283) respondents. One hundred and eighty seven (n=187) preferred storing maize flour in paper bags. Forty seven (n=47) stored maize flour in plastic containers and forty nine (n=49) preferred storring maize flour in plastic guuny bags. Table 4.32 shows the percentages (%) storage preference frequency for the respondents.

Table 4.32: Storage preference for maize flour by the respondents

Respondents	**Frequency**
Storage preference	**Percentage %**
plastic gunny bags	17.3
plastic container	16.6
paper bags	66.1
Total	**100**

4.12.6 Respondents use of untreated river water

The qustionnnaire response rate on use of untreated water was 100% (n=283), and the tabulated results are shown on table 4.33

Table 4.33: Water source preference for respondents

Respondents preference	**Frequency**	
water source	**Numbers (n)**	**Percentage (%)**
open dams	153	54.1
streams	88	31.1
boreholes	24	8.5
tank water	18	6.4
Total	**283**	**100**

4.12.7 Questionnaire score results between independent and dependant variables (liver disease)

The qualitative analysis of the questionnaires on various liver disease dependant variables including blood transfusion, unprotected sex, untreated water, unsterile body piercing instruments, aflatoxin B_1 contaminated maize grain and aflatoxin B_1 contaminated maize flour gave the results as tabulated in table 4.34 below.

Table 4.34: Independent variable mean scores and standards deviations

Independent variable	N	Mean score	Sd
Blood transfusion	283	2.1	1.0
Unprotected sex	283	2.6	1.3
Untreated water	283	2.6	0.9
Unsterile instruments	283	3.1	0.9
AFB_1 maize	283	3.3	0.8
AFB_1 flour	283	3.3	0.7

4.12.8 Correlation coefficient between independent and dependant variables

The results of determination of correlation coefficient (r) between dependant variable (liver disease) and independent variables including blood transfusion, unprotected sex, unsterile body piercing instruments, untreated water, aflatoxin B_1 contaminated maize grain and AFB_1 contaminated are tabulated in table 4.35.

Table 4.35: Pearson correlation coefficient values between dependant and independent variables

Independent variable	Coefficient between dependant variable -- liver disease (r)	Square of the coefficient value (r^2)	Coefficient of determination (R) %
Blood transfusion	0.621	0.3854	38.564
Unprotectecd sex	0.347	0.1204	12.04
Untreated water	0.265	0.0702	7.02
Unsterile body piercing instruments	0.694	0.4816	48.16
AFB_1 contaminated maize grain	0.449	0.2016	20.16
AFB_1 contaminated maize flour	0.560	0.3136	31.36

Note: Correlation test was significant at p = 0.05 (2 –tailed test).

4.13 The Z statistic value for hypothesis testing

The total hospital admissions due to HBV was 46.99% (n = 133), out of total sample (N=283), but 2.47% (n = 7), had serological evidence of HBV and AFB_1 as shown by presence of serum HBsAg and AFB_1 lysine albumin adducts. Thus, the case subjects admitted to various health centers with evidence of HBsAg, and hence with HBV was 49.46% (n = 140). Those samples testing positive for HBsAg as evidence of exposure to HBV were 14.84% (n = 42), but 9.2% (n = 26) out of a control sample (N= 283), had evidence of exposure to both HBV and aflatoxinB_1 (AFB_1). The subjects with evidence of exposure to both disease factors but did not have any active liver disease was therefore 24.0% (n=68). For test of hypothesis, Z statistic was calculated from table values below (Table 4.36).

Table 4.36: Subjects testing positive for HBV in both case and control cohorts

cohort	**HBV**	**Frequency**	**Mean** (Iu/mL)	**Sd**
	(+ve)	(%)	(10^3)	(10^3)
Case	n_1= 140	49.46	3.4810	2.681
Control	n_2= 68	24.0	0.34713	0.2913

Note: sd = standard deviation

To determine Z values for comparison with Zc = 1.96,

$$z = \frac{\overline{X}_1 - \overline{X}_2}{\sqrt{n_1\underline{\delta_1^2} + n_2\,\underline{\delta_2^2}}} \times \sqrt{\frac{1}{1} \times \frac{1}{}}$$

where Z was the value in the normal curve, δ is the standard deviation, n_1 was the sample size in case cohort, n_2 was sample size in control sample, δ_1^2 and δ_2^2 were the standard deviation from mean levels of HBsAg in both case and control groups.

From table 4.39, the values of various parameters where, $n_1 = 140$, $n_2 = 68$

$\delta_1^2 = 7.182$; $\delta_2^2 = 0.08485$; $X_1 = 3.4810 \times 10^3$; $X_2 = 0.34713 \times 10^3$ hence

substituting, $Z = \dfrac{(3.4810 - 0.34713) \times 10^3}{\sqrt{4.6819} \times \sqrt{1/140 + 1/68}}$

and then

simplifying further, the above equation translated to the equation below;

$$Z = \frac{3.1339}{\sqrt{4.6819}\ \sqrt{0.0218}}$$

$Z = \dfrac{3.1339}{0.3197}$ and hence, Z= 9.802

Z= 9.802, and Zc= 1.96.

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMEDATIONS

5.1 Aspertate amino transfarase in case and controls and the difference in mean levels per cohort

Since the overall mean aspertate amino transfarase (AST), in cases was 154.86 Iu/mL with a range of 55.6 Iu/mL to 344.5 Iu/mL in all the 19 health centers, the implication was that 100% (N=283) of all serum samples within the centers had AST values above the normal mean of 40 Iu/mL.

In contrast the overall mean for AST in the control cohort for all the centers was 31.31 Iu/mL, with a range of 19.10 Iu/mL to 101.16 Iu/mL which was far below the case range AST levels. Out of the total control samples examined, only 10.53% (n=2), of blood samples from one (1), of the health facilities had a mean AST value above 40 Iu/mL, while 89.47% (n = 17), of the blood samples from eighteen (18), health centers had AST values below 40 Iu/mL, which is the normal values for non-liver condition patients.

These findings are in agreement with those by Mariana *et al,* (2016), who had suggested that AST normal range for non-patients vary but in general are less or equal to 40 Iu/mL, while AST mean values greater than 40 Iu/mL, suggested liver injury including injury due to acute viral hepatitis, Ischemic injury, toxin injury, medicinal or auto immune hepatitis liver injury (Johnson, 1999).

According to Robert *et al,* (2010) hepatitis B virus, is non cytopathic unlike in hepatitis C and cirrhosis, where hepatocytes apoptosis is a characteristic of the disease (Hall & Cash, 2012). Thus, liver injury in viral hepatitis B infection is thought to be immunologically mediated and the inflammation results in leakage of AST and ALT enzymes into circulation (Heidelbaugh *et al,* 2006).

Even then, the severity and prognosis of liver diseases in HBV infection may not always correlate well with the levels of AST in blood circulation.

Furthermore, elevated AST values in 10.53% (n=2) of control cohort sample in one of the centers could have been due to production of AST enzymes by the patients due to muscle inflammation or a defect in clearance of this enzyme from circulation. This observation was in agreement with a study by Johnson, (1999), on markers of hepato cellular injury in liver disease.

Chronic liver inflammation in this study was defined as any HBV inflammation where HBsAg persisted for a period of 6 months or more (Keefe *et al.,* 2004). In this study therefore, AST mean values in case cohort was higher with a mean of 154 Iu/mL, way above the normal mean value of 40 Iu/mL for non-HBV cases or normal subjects. Again, this agrees with the studies by Johnson, (1999), which found that AST and ALT levels in circulation tend to be higher in patients with chronic liver inflammation or necrosis, than those without chronic or any liver injury.

5.1.1 Alanine amino transferase in case and controls and the difference in mean levels per cohort

Alanine amino transferase (ALT) is an enzyme primarily found in the liver and to some small extent the kidney of human body. This enzyme was originally referred to as glutamic pyruvic transaminase (SGPT). It is the enzyme necessary for breaking glucose into energy. About 99% of this enzyme is found in the liver cells (Ishiguro *et al.,* 1991). Alanine transferase, levels in the blood stream increases with liver damage and hence could be a good predictor for liver disease and also could be of use in monitoring liver damage in such condition (Hoofnagle *et al.,* 2013). Liver damage may be occasioned by alcohol, HBV, HCV, or higher consumption of vitamin A. Various studies have shown that damaged liver cells will always release stored ALT enzyme in the blood stream in liver disease there in increasing ALT levels (Hall & Cash, 2012).

In contrast aspertine amino transferase can be produced from other body organs including the human muscle during inflammation (polymyositis) or during conditions including myocarcdial infarction (Johnson, 1999).

In the study, individual patient mean alanine amino transferase (ALT) level in case cohort was 180.13 Iu/mL (CI; 169.32 to 190.94 Iu/mL) at 95% confidence level with a range of 1.2 Iu/mL to 444.5 Iu/mL, while the overall median mean level per centre was 172.02 Iu/mL.

In contrast, for the control cohort distribution and ALT levels, the overall individual patient mean was 27.11 Iu/mL (CI; 25.71 to 28.51 Iu/mL) at 95% confidence level, with a range of 8.20 Iu/mL to 73.50 Iu/mL and a median mean for the health centers of 27.77 Iu/mL. Comparing the two median means between the case and control subject groups therefore, points to a disease burden among the cases since elevated ALT above 40 Iu/mL is one of the biomarkers of liver disease (Pacifico *et al.*, 2013). Indeed, in this study the difference registered between the two ALT median means was statistically significant at 95 % confidence level ($p \leq 0.05$).

In a similar study in the United States, Rubl *et al*, (2012) found that alanine amino transferase (ALT) body activity was an important screening, diagnostic and monitoring parameter for liver disease and that the cut off for 95% specificity was an ALT level equal to 44 Iu/mL (64% sensitivity) for men and 32 Iu/mL (59% sensitivity) for women. The disease burden in this study was also confirmed by the fact that those hospitalized had already been diagnosed with liver disease by the clinicians using other differential methods including elevated AST levels, De ritis ratio of less than one (ratio< 1), HBsAg and AFB_1 lysine albumen adducts in blood samples.

5.1.2 Hepatitis B surface antigen levels and impact on liver disease

The presence of hepatitis surface antigen (HBsAg) in serum remains an important factor in the diagnosis and biogenesis of liver disease when the etiological agent is hepatitis B virus (Liaw, 2011). This coupled with alanine amino transferase (ALT) which is a liver enzyme more specifically produced only in the liver and hence a more specific indicator of hepatocellular damage could be a useful tool in diagnosis and control of this disease. Thus, newer methods for quantification of HBsAg in untreated and treated patients in chronic liver disease will be a better public health strategy for control of this disease.

In this study, quantification of HBsAg in blood sample (N = 283), showed 49.47% (n = 140), had various levels of HBsAg in the patient (case), cohort with a range of 500 Iu/mL to 9800 Iu/mL and a mean of 3481 Iu/mL.

This contrasted sharply with the quantified levels of HBsAg in the non-patient (controls) cohort which had an HBsAg range of 150 Iu/mL to 990 Iu/mL, with a mean of 506 Iu/mL in this study. Further, the control patient cohort had an overall 24% (n = 68), of subjects which had evidence of HBsAg in serum samples.

This suggest that the HBsAg cut off levels for those with active liver disease and those deemed inactive carriers for HBV are different for different genotypes of hepatitis B virus (HBV). Studies by Jaro Szewicz *et al.*, (2010), Braneto *et al.*, (2010) and Hoofinagle *et al.*, (2013), have proposed cut off levels for HBsAg and ALT that when used concurrently can accurately identify active and inactive patients with liver disease. Using the cut off range of 1000 Iu/mL – 2000 Iu/mL for HBsAg and that of ALT for up to 44 Iu/mL, active and inactive carrier of the HBV can be identified with much higher degree of accuracy, almost comparable to the suggestion made in similar studies by Brunecto *et al.*,(2010) where an HBsAg cut off levels of 1 x 10^3 Iu/mL and 2 x 10^3 Iu/mL for HBV DNA was suggested as a diagnostic method to reliably identify patients with active and inactive disease, when used concurrently with an accuracy of 94% to100% (Liaw *et al.*, 2011).

The low levels of HBsAg in 24% (n = 68) of the patients in control cohort under this study suggested HBsAg decline after initial infection associated probably with higher HBsAg sero clearance. It could also mean improved immune control and hence stronger viral suppression as suggested by Chan *et al.*, (2010). Alternatively, it could mean that these persons where at the initial stages of the HBV infection and that the levels where suggestive of initial HBsAg sero conversion when even the ALT levels were normal. It could suggest that the sixty-eight (68) control patients in this study where HBV carriers hence the need for study of HBV using PCR to show DNA cut off levels which can define inactive HBsAg level carrier state. An HBV – DNA cut off level should be able to show zero HBV- DNA in serum, when HBsAg are still in serum at some level. Inactive HBsAg carriers may suggest inactive HBV carrier state

hence a biological reservoir for the hepatitis B virus which may later cause a liver infection (Chan *et al.,* 2010).

Indeed, a study by Brunetto *et al.,* (2010) and Martinot *et al.,* (2010) on inactive HBsAg carriers showed that in comparison, those with subsequent HBsAg sero clearance had a higher HBsAg decline, than those who remained HBsAg sero positive (0.28 – 0.29 Vs 0.054 – 0.058 log 10 Iu/mL/ year). This means that more of those with higher HBsAg sero clearance rates tend to recover fully from HBV infection than those with a lower rate of HBsAg sero clearance. This therefore is more of an individual patient immune response factor more ingrained in their DNA than an environmental factor (Martinot *et al.,* 2010).

5.1.3 Aflatoxin B_1 lysine albumin adducts levels and control of liver diseases

In the study, the number of patients admitted to various health centers and whose serum samples had evidence of aflatoxin B_1 lysine serum adducts at various levels out of the total sample(N=566), were 25.97% (n = 147) for cases and 15.54% (n = 88) for controls. The mean being 42.19 pg/mg and 13.15 pg/mg for cases and controls respectively, however 5.83% (n=33) out of total combined sample (N=566) had evidence of co infection with HBV in this particular study. For the 2.47% (n= 7) which was the Case co infection sub cohort, the levels of both ALT and AST as predictors of liver damage was high with means of 123.44 Iu/mL for ALT and 233.1 Iu/mL for AST respectively, while for Case non co infected subject mean levels were 180.13 Iu/mL and 154.86/mL for ALT and AST respectively. The higher mean AST level for those co infected suggests a synergistic effect to the liver disease burden among those co infected with HBV. This finding is supported by other similar studies which have shown that aflatoxin B_1 dietary exposure increases the risk of hepato cellular carcinoma in addition to having synergistic effect to liver disease where a patient is co-infected with HBV (Chui, *et al.,* 2018).

In the control cohort, 31.09% (n = 88) of the serum samples had low levels of AFB_1 lysine albumen adducts with a mean of 13.15pg/mg indicating low level dietary exposure hence low key aflatoxicosis. The low mean for this control cohort of 13.15pg/mg, was also an indicator that the exposure level for the case cohort to

aflatoxin B_1 contaminated foodstuff was higher than the control group since the patients come from the same geographical locality. It is also instrumental that the stable food for this region of Kenya is maize grain and products delivered from maize grain including maize flour.

This finding was collaborated by the fact that the mean AFB_1 levels determined in the area of study for maize grain was 11.14 ppb and for maize flour was 16.06 ppb for the case household food samples while the control households had 5.029 ppb as mean level for maize grain and 7.60 ppb as mean level for maize flour in the same study, indicating a higher dietary exposure for AFB_1 among the general population in this region. Furthermore, for both Case and Control subject household maize grain and flour samples, flour samples had a higher AFB_1 mean levels than the grain samples even when both were stored in same conditions. This is due to the fact that grain flour has more starch exposed to humid air which promotes the growth of *A.parasiticus* which in turn produces aflatoxin B1 as a fungal metabolite.

These findings are supported well by other studies in United Kingdom which found that using AFB_1 lysine albumin adducts as a biomarker serves to estimate dietary AFB_1 intake and that for AFB_1 albumen adducts, there is a linear relationship between AFB_1 intake and AFB_1 lysine albumin adducts on population basis (Turner *et al.,* 1998).

In this study, 2.47% (n = 7), in the case cohort had serum samples showing both HBsAg and AFB_1-lysine albumin adducts at various levels while for the control cohort 9% (n = 26) had evidence of HBsAg and AFB_1 lysine albumin adducts at various lower levels. The synergistic effect for both disease factors on severity of liver disease was indicated by a higher ALT value in this cohort with a mean of 180.13 Iu/mL for the cases and 27.11 Iu/mL for the control cohort. Furthermore, a similar study in Ghana which examined the temporal variations in AFB_1 lysine albumin adduct levels in HIV positive persons and food consumptions suggested a synergistic effect among etiologic agents for liver disease (Jolly *et al*., 2015).

5.1.4 Significance of De ritis ratio and use in disease diagnosis

In this study De ritis ratio was determined by using the AST and ALT values as biochemical parameters quantified from the blood samples collected from patients sampled for the study. For the case cohort, the mean AST/ALT ratio (De ritis ratio), was 0.9866, with a median mean ratio of 0.9681 while in controls the mean AST/ALT ratio (De ritis ratio), was 1.120, with a median mean of 1.112 in addition, for the case cohort, the values of AST and ALT were highly elevated with means of 154.86 Iu/mL and 180.13 Iu/mL respectively, while for controls the means where 31.31 Iu/mL for AST and 27.109 Iu/mL for ALT, comparatively in this case-control study. Studies from other investigators have shown that a De ritis ratio greater, equal to or less than one (ratio $\geq$ or $\leq$ 1), when ALT and AST levels are elevated is indicative of liver conditions attributed to various etiological agents. This therefore means that the majority of liver disease cases in the two counties could be attributed to hepatitis B virus (HBV) or hepatoxicity due to AFB_1 but not alcoholic liver disease(cirrhosis) or non-alcoholic fatty liver diseases (NAFLD) (Peter *et al.*, 2016). Furthermore, in this study, the case cohort had 69.6% (n=197), of samples showing a De ritis ratio mean of less than one (ratio < 1) and 30.4% (n=86) of the same cohort presenting a De ritis ratio of greater than one (ratio > 1), indicating the possibility of other etiological factors of liver disease in this study including AFB_1 and also other forms of liver diseases including cirrhosis; non alcoholic fatty liver disease (NAFLD), alcoholic liver disease (ALD) or hepatitis A or C. This fact is corroborated by other findings by Deb *et al*, (2016), which found that De ritis ratio elevated levels of greater than one (ratio >1.0) was highly indicative of alcoholic herpetic injury and also non-alcoholic fatty liver disease (NAFLD). Similarly, studies by Park *et al.*, (2004) found that elevated AST/ALT ratio (De ritis ratio), greater than one (ratio >1), could predict progressive fibrosis and liver cirrhosis, which supports this study view that, 30.4% (n=86), of the patient's liver condition were caused by other etiological agents, other than HBV or AFB_1 contaminated foodstuffs.

In this study again, some patients had 10 times the ALT and AST level limits for normal persons, but the De ritis ratio was less than one (ratio<1). This could have meant or suggested a co-infection with several etiological agents, hence synergistic

effect by the causal agents for the liver condition including viral hepatitis B, hepatitis A, hepatitis C and aflatoxins B_1 since the study area was known for aflatoxin contaminated food stuffs hence frequent aflatoxicosis outbreaks including that of the year 2004 in eastern Kenya (Muthomi *et al.*, 2009; Mwihia *et al.*, 2008).

This also agrees with the findings of a study by Park *et al.*, (2004), who found that AST/ALT ratios less than one (ratio<1), when both ALT and AST are elevated above 40 Iu/mL was a strong indicator (predictor), of chronic viral hepatic infection including hepatitis B and C.

Furthermore, since in controls 91.8% (n=260) had a mean AST/ALT ratio (De ritis ratio), greater than one (ratio >1), it pointed to the fact that, majority of control subjects were having higher AST values than ALT values. This is a fact supported by many studies which indicates that even though AST stays less in circulation because it has a shorter half (½) life of about 17 hours than ALT with estimated 47 hours more, other organs in the body produce AST in addition to the liver, including the body muscle structures and the mitochondria if a patient is alcoholic (Xu *et al*., 2015).

5.1.5 The significance and relationship of correlation coefficient "r"and the coefficient of determination.

The coefficient of determination " r^2" denoted in this study as" R", explained in absolute terms how any change in dependent variable (liver disease), was affected by a change in any independent variable (King'oriah, 2004). The Pearson correlation coefficient calculations showed that there was a significant positive correlation between the independent variable and the dependent variable (liver disease). The independent variables in this study having been blood transfusion, body piercing instruments, AFB_1 contaminated maize grain and flour, unprotected sex and use of untreated water. For the study area, the indication was that, 48.1% of non causal absolute effect on liver disease was due to unsterile body instruments and 38.5% of the same effect was due to blood transfusion. The non causal contributory effect of AFB_1 contaminated maize grain and flour to liver disease was lower than that of blood transfusion at 31.4% and 20.1% respectively. This was unexpected since the

study area was an aflatoxicosis zone (Mutegi *et al.,* 2018). The lowest non causal contributory effect of 12.04% and 7% to liver disease was due to unprotected sex and use of untreated river water respectively, indicating that fewer respondents in the study used untreated water.

5.1.6 Sero prevalence of HBV among population of the study area

It is explained here that, the sero prevalence of the HBV among the subjects of the study area, was determined as the ratio of those samples from case subjects who had confirmed liver disease and had evidence of exposure to HBV due to sero presence of HBsAg, (n = 140), to the total population sample (both cases and controls) in the study which was randomly selected (N = 566). This was a ratio of 0.2473, which was a disease prevalence of sixteen percent (24.73%), in the two counties of lower eastern Kenya.

The calculated sero prevalence in the study is a good estimate of the disease prevalence in this area because according to Jaaskelainen *et al*, (2018) an idea also held by Schmidt and Colman, (2008), when controls were obtained concurrently with cases, they were a representative of exposure experience of the population from which cases were drawn. This study therefore, suggests that, 24.73% of the population at any one time had liver disease due to exposure to HBV, a virus causing liver damage and that those residing at the study area were at a certain risk of acquiring the disease.

5.1.7 Sero prevalence of aflatoxinB_1 as AFB_1 lysine albumin adducts among study subjects

The prevalence of liver disease due to sero presence of AFB_1 lysine albumin adducts, in subject blood samples was determined as a ratio of the number of patients with liver disease due to AFB_1 toxicity whose blood samples had evidence of exposure (n = 147), to the randomly selected total population, both cases and controls in the sample (N = 566) as per studies by Schmidt & Colman (2008) and therefore the prevalence was determined as 0.2597 (25.97%) in this study (Rothman, 2012).

Furthermore, in case subjects population (N = 283), 51.94% (n = 147) of blood samples had evidence of AFB_1 lysine albumin adducts. This included those with mixed infection with HBV. The overall range was 15.5pg/mg to 135 pg /mg with an overall mean of 42.19 pg/mg of albumin, while in controls (N=283), 31.09% (n = 88), of samples had an overall AFB_1 lysine albumin adducts range of 3.5 pg/mg to 60.5 pg/mg with a mean of 13.65 pg/mg of albumin. Comparatively therefore, the study further suggests that both Case and Control subjects in this study were highly exposed to dietary AFB_1 in area of residency but those in control cohorts were more tolerant to toxic effects of dietary aflatoxin B_1 (*A. flatus* toxin*)* and that all those residents at the area of study were at high risk of exposure.

It is also evident that the sero prevalence determined in the study was a good estimate of the disease prevalence currently because the controls were obtained concurrently with cases and therefore was a representative of exposure experience of the population from which the Cases were drawn from (Schmidt & Colman, 2008).

5.1.8 Prevalence of liver disease due to a combined sero presence of AFB_1 and HBV

The prevalence of liver disease due to sero presence of both HBsAg an indicator of HBV presence and AFB_1 was determined as a ratio of those case subjects with confirmed physical presence of liver disease and evidence of exposure to both HBV and AFB_1 (n = 7), to the total population (cases and controls), in the randomly selected sample (N = 566). This ratio was 0.0124, which was a disease prevalence of ten percent (1.24%).

Among the case subjects with liver disease due to sero presence of HBV and AFB_1 toxicity, 2.47% (n=7) had high levels of greater than 34 Iu/mL for ALT and AST, suggesting that infection with HBV and exposure to dietary AFB_1 had synergistic effect on liver damage among those patients who had the disease. Further, the study suggests that 1.24% of the population between ages of 12 to 90 years in lower eastern Kenya, were likely to suffer direct illness due to exposure to both HBV and dietary AFB_1 which have an additive or synergistic effect on liver damage. Since the additive effect of the two disease factors has higher mortality index, this could have

meant that the population with such mixed etiologies for liver disease was low at time of study. This meant that the liver disease prevalence of 1.24% (0.0124) though a good estimate for the relatively lower chances of acquiring this condition by the two factors, could have been much higher than observed. For residents of this region in lower eastern Kenya, the relative risk of acquiring liver disease due to the two disease factors may have been higher (Dallal, 2012; Schmidt & Colman, 2008).

5.1.9 The relative risks of liver disease among subjects with HBV, AFB_1 and HBV-AFB_1 combined sero positivity

In a case exposure study, when the controls are obtained concurrently with cases and if they are representative of the exposure experience of the population from which the cases were drawn, and if the Odds ratio is less or equal to 10%, the odds ratio (OD) is equivalent to the relative risk (RR) of acquiring a disease (Dallal, 2012; Schmidt & Colman, 2008; Robert *et al*, 2012). In this study, the odds ratio (OD), was the probability of acquiring a liver disease to the probability of not acquiring the liver disease and was combarable to the relative risk (RR) since the disease prevalence was low (Schmidt & Colman, 2008; Dallal, 2012). This disease prevalence included 24.73% for HBV, 25.97% for AFB_1 toxicity and 1.24% for HBV-AFB_1 combined together.

For this study therefore, it is suggested that following the low disease prevalence scenario (Dallal, 2012), and since the odds ratio (OR) was determined as 1.152 for AFB_1 toxicity, 1.097 for infection with both HBV and AFB_1 and 1.043 for HBV infection, then it was comparable to the relative risk (RR) of contracting the liver disease due to HBV which was 1.022, that due to AFB_1 toxicity which was 1.073, and that due to combined effect of HBV and AFB_1 toxicity which was 1.048.

5.1.10 The association between liver disease and AFB_1 contaminated household maize grain and flour

Since the Pearson correlation coefficient (r) between AFB_1 contaminated maize grain and flour was found to be 0.449 and 0.560 respectively (p=0.05), then the aflatoxin B_1 contaminated maize flour was more strongly associated with liver disease than

AFB_1 contaminated maize grain. It can be suggested that statistically and in absolute terms, AFB_1 contaminated flour contributed 56.0 % of dietary AFB_1 exposure rate among subjects who use maize grain and flour as stable food in lower eastern Kenya, while maize grain contributed 44.9% of AFB_1 dietary exposure rate. Aflatoxin B_1 induced liver disease rates would therefore follow the same pattern due to direct toxicity of dietary aflatoxin B_1 ingested with contaminated food. It is suggested that there was evidence of a strong association between the dietary AFB_1 exposure and liver disease due to ingestion of aflatoxin B_1 contaminated food and that the association in this case was causal.

Furthermore, once maize grain is ground to flour, the fungus especially that of genus *A. flatus* gains easy access due to removal of protective grain cover, humidity, increased surface area and increased storage temperatures (Chauhan *et al.,* 2016). This study therefore suggests that a mass of maize flour is likely to have higher AFB_1 contents than an equivalent mass of maize grain, stored at the same conditions.

5.1.11 A comparison between control participants mean household AFB_1 levels for both maize grain and flour samples

All the maize grain and flour samples (n = 19), associated with control subjects recorded higher mean aflatoxin B_1 levels for maize flour samples than control household maize grain samples.

Comparatively aflatoxin B_1 overall mean for control subject household flour samples at 7.26 ppb was greater than the overall mean for control subject household grain samples at 4.93 ppb for all the health centers for the control group and so was the median for house hold maize grain (4.79 ppb) and flour (7.30 ppb), for the two cohorts respectively. These results agree with other studies by Gathumbi *et al*., (2001) which found that once maize outer coating was removed as in flour milling process or in *"Muthokoi"* making as in some traditional food processing methods, then AFB_1 was more easily formed especially by the two types of molds namely *Aspergillus flatus* and *Aspergillus parasiticus* than the normal whole maize grain.

5.1.12 The association between non HBVand AFB_1 factors and liver disease in the study

The linear regression analysis of data between dependent variable (liver disease), and the independent variables including blood transfusion, unprotected sex, untreated water, unsterile body piercing instruments and liver disease yielded a strong positive correlation (r) levels including 0.094, 0.621, 0.347, and 0.265; respectively.

From this linear regression modeling between the suspected contributory factors and liver disease, it can be suggested that there was a non causal association between liver disease and the variables. A correlation coefficient of greater than five (0.5) is considered high, hence the correlation between liver disease and body piercing instruments was the highest (r = 0.694), that of blood transfusion (r = 0.621), that unprotected sex (r = 0.347), and that of untreated water (r = 0.265), which was the lowest measure of association between a variable and liver disease in this study.

Further, blood transfusion activities suspected to lead to HBV transmission had a relatively lower correlation (r = 0.621), hence lower level of association than that of body piercing instruments (r = 0.694). There is evidence that the strong association was an indication of a possible transmission of disease-causing agents through those variables including unsterile body piercing instruments, blood transfusion, untreated drinking water, and unprotected sex while the correlation between dietary AFB_1 exposure and liver disease was a measure of association and an indication of direct liver toxicity (Mutegi *et al.,* 2018).

5.1.13 The Assocition between aflatoxin B_1 and AFB_1 lysine albumin adducts levels

In this study, higher aflatoxin B_1 content in household foodstuffs was associated with a remarkable higher value of AFB_1 lysine albumin adducts in serum samples of those admitted to various health centers with liver conditions. Out of the total sample population under study, including case and control serum sample (N = 566), 45.05% (n = 255), of the sample was positive for AFB_1 lysine albumin adducts at various levels ranging from 3.5 pg/mg in controls to 135 pg/mg in cases.

A follow up of the patient's dietary habits through household maize grain and flour sample analysis in this study showed 41.16% (n = 233), in both case and control household maize and flour samples had AFB_1 levels exceeding 10 ppb, while 29.7% (n = 168) of maize grain samples from both case and control households in all associated health centers had AFB_1 levels above 10ppb, the codex allimentarius commission allowable dietary limit. The rest of the grain and flour household samples in both case and control cohorts had AFB_1 content less than 10 ppb with a range of 0 ppb to 48.30 ppb. There was a positive correlation coefficient between the household dietary grain and flour AFB_1 levels, the case subject serum AFB_1 lysine albumin adducts levels and liver disease (r= 0.560) at a level of significance of 0.05 (p = 0.05). This discussion points to a higher AFB_1 dietary exposure for both patients in case and control cohorts in the region under study.

This observation agrees with other studies by Muthomi *et al,* (2009) and Muhia *et al,* (2008) which all points to the fact that, subjects in this region were exposed to higher levels of aflatoxins from dietary maize grain and flour and that according to studies by WHO (2018) and Raad *et al.,* (2009), exposure to dietary AFB_1 and other mycotoxins, end up in the liver and the circulatory system, including AFB_1 lysine albumin adducts and may be highly toxic to the human body, at times causing acute jaundice and liver failure (WHO, 2018).

5.1.14 Distribution and differences in sero prevalence of AFB_1 and HBV among study participants

A Critical analysis had shown that, this case exposure study had an overall 51.94% (n = 147) of the case subject samples (N=283), showing AFB_1 lysine albumin adducts ranging from 15.5 pg/mg to 135 pg/mg with a mean of 42.19 pg/mg while the controls had an overall 31.09% (n = 88) of subject samples with a range of 3.5 pg/mg to 60.5 pg/mg and a mean of 13.15 pg/mg albumin. This included subject samples with combined evidence of HBsAg and AFB_1 toxicity in both case and controls.

Comparatively therefore, it can be suggested that the Case subjects household maize grain and flour samples had remarkably high levels of aflatoxinB_1 than the controls

and this explained the higher case subjects AFB_1 lysine albumin adducts levels. It is therefore suggested that, although case subject AFB_1 lysine adducts means were higher than the controls, overall, 37.98% (n=215) of all subject serum samples had AFB_1 lysine albumin adduct levels above 4.0 pg/mg with a range of 4.3 pg/mg to 135 pg/mg. It is observed that, this correlated well with the observed AFB_1 sero prevalence of 25.97 % in the study area of lower eastern Kenya.

Since in this study, the case and controls were matched by age, residency and blood was also collected within same season to avoid variations in hematological parameters of interest and other temporal variations, then it can be concluded that the data confirms the relatively higher AFB_1 dietary exposure to the population and also links this exposure to increased incidences of liver toxicity (aflatoxicosis) in the study area.

The level of liver damage due to HBV was estimated by the quantified levels of HBsAg for the case and control subjects. For the case subjects, 51.94% (n = 147), of samples had the HBsAg range of 500 to 9800 Iu/ml with a mean of 3481 Iu/mL while for the controls, 14.84% (n = 42) of the samples had a lower range at 150 Iu/mL to 990 Iu/mL, with a mean of 506.3 Iu/mL. Furthermore, the case subject ALT levels ranged from 55.40 to 444.50 Iu/mL with a mean of 180.332 Iu/mL, while for controls subjects, ALT values ranged from 8.20 to 73.50 Iu/mL, with a mean of 27.11 I u/mL. Again, in this study, 99.64% (n = 282), of Case subject samples had ALT levels above 30 Iu/mL while for controls 99.65% (n = 282) had ALT levels below 30 Iu/mL. Similar trend was observed for AST levels on case and control subject samples. This was also corraborated by the observed sero prevalence of HBV of 24.73% in the same study. It is therefore suggested that, over 99% of all HBV associated liver disease Case subjects had elevated ALT and AST levels due to tissue and liver cell damage.

On pairing case AST, ALT and residency, there was a significant association between case AST serum levels and the health centers to which the subjects were admitted and from whom subject blood samples were drawn ($\chi^2_{(0.05,\ 18df)} = 70.536$, p=0.05), while a significant association was noted between the health centers and the

case ALT serum levels in the same study ($\chi^2_{(0.05,\ 18df)}$ = 109.124, p=0.05). This was against an observed chi squire (χ^2= 28.678; p= 0.05), which was way below the determined value. These findings suggest that there was a high probability of acquiring liver disease by residents at study area due to high prevalence of deitary AFB_1 and HBV within the population.

Further, in this study, 24% (n =68), of the control subject samples had confirmed low levels of HBsAg. Since the samples were drawn from clinically confirmed non disease subjects, it is suggested that these subjects were either healthy carriers of the HBV virus and hence resistant by some immunological mechanism or had fully recovered from the disease.

Comparatively, the AFB_1 sero prevalence of 24.73% was higher than HBV sero prevalence of 23.49% among the subject's resident in this area. It is therefore suggested that, the residents of lower eastern Kenya were more likely to suffer from liver damage due to AFB_1 toxicity (aflatoxicosis), than liver disease due to hepatitis B virus (HBV) and that there were more subjects suffering from liver disease due to dietary AFB_1 exposure than those exposed to HBV alone.

5.1.15 The significance of hypothesis testing in the study.

Since the serum sample was randomly selected, it then followed a normal distribution and thus the hypothesis test statistics "Z" calculated was compared with "Z" critical (Zc) at 95% confidence level (Kothari *et al.,* 2014).

According to King'oriah, (2004), and Devore *et al,* (1967), if the calculated "Z" statistic was greater than Zc, where Zc = 1.96, an hypothesis as stated was to be rejected, but if the "Z" calculated statistic was less than Zc, then the hypothesis was accepted as was stated. This could also be stated, in an equation thus, ($-1.96 \geq Z \geq 1.96$) so that if the equation was correct then the null hypothesis (Ho) was to be rejected, and if ($-1.96 \leq Z \leq 1.96$), then the null hypothesis (Ho) was accepted for a two tailed test.

Since the population mean (μ_1) of those suffering from liver disease (HBV case), and those who were not (μ_2) but showed evidence of low-key HBV exposure (Controls) was not known, the study used the sample means of case and control HBsAg levels ($\bar{X}_1$ and $\bar{X}_2$), as evidence of HBV infection and exposure respectively to test the hypothesis that, "There was no significant difference between the disease prevalence of HBV between cases with active liver disease and the controls.'' Since Z was calculated as 9.802 while Zc was 1.96 it followed that Z > Zc, and therefore the null hypothesis (Ho), that "there is no significant difference in HBV disease burden between Case subjects with active liver disease and controls was rejected" and the alternative hypothesis (Hα) that "there was a significant difference in prevalence of active liver disease between those with HBV and those without" was accepted (Kothari *et al.,* 2014).

The prevalence of liver disease due to a combined sero prevalence of HBV and AFB_1 was estimated at 10%, while that due to sero prevalence of HBV and AFB_1 separately was determined as 16.07% and 17.84% respectively. In comparison therefore, there was a difference of 6.07% between sero prevalence of HBV and sero prevalence due to a compination of both HBV and AFB_1, while that due to sero prevalence of HBV and AFB_1 separately was 7.84%. The null hypothesis (Ho) that there was no difference in prevalence of liver disease between those with compined sero presence of HBV and AFB_1 and those without was rejected and the alternative hypothesis (Hα) that there is difference in sero prevalence accepted.

The Odds of contracting HBV and AFB_1 toxicity in the study area was estimated at 1.043 and 1.152 respectivly. According to Robert *et al,* (2012) and Schmidt & Colman, (2008), when the prevalence of a disease was low, then the odds ratio (OD) was approximated to be equal to the relative risk (RR) of contracting the disease. Since the relative risk of contracting the HBV and AFB_1 was determined as 1.022 (95%, CI; 0.806 to 1.2052), $p \leq 0.05$ and 1.073 (95%, CI; 0.910 to 1.265), $p \leq 0.05$ respectively. The null hypothesis (Ho) that, there was no significant risk of liver disease among those exposed to HBV and AFB_1 was therefore rejected while the alternative hypothesis (Hα) that, there was a significant risk of liver disease among those exposed was accepted.

A linear regression analysis of data between liver disease as a dependent variable and dietary AFB_1 exposure to maize grain and flour as independent variables, indicated that there was a strong and significant correlation (r), of 0.449 and 0.560 respectively (p=0.05). Further, the odds and relative risks of contracting aflatoxicosis in the study area due to dietary AFB_1 exposure was estimated as 1.152 and 1.073 respectively while the sero prevalence was at 25.97% in this study. The null hypothesis (Ho) that AFB_1 exposure was not a major cause of liver disease was therefore rejected and the alternative hypothesis (Hα) that, dietary AFB_1 exposure was a mojor contributory factor to liver disease in the study area was accepted.

5.2 Conclusions

1. In this study, all the Case subjects had elevated levels of both ALT and AST when compared with the controls. For cases, mean ALT levels were remarkably higher than mean AST levels, while for controls mean ALT levels were lower than mean AST levels. It is therefore evident that ALT levels are generally lower than AST levels where one of the causal factors for liver disease is HBV or AFB_1 toxins or a combination of both aetiologic agents.
2. Given the fact that for liver diseases, the liver enzymes are elevated, while suppressed in non disease subjects (Controls), it is suggested that AST/ ALT ratio (De ritis ratio) can be used as a diagnostic tool for liver disease in addition to other liver disease diagnostic algorithms, including laboratory tests for elevated AST and ALT levels.
3. According to studies by Jaaskelen *et al*, (2018) and also Schmidt *et al*, (2008), the HBV sero prevalence of 24.73% and AFB_1 sero prevalence of 25.97% determined in this study indicated the exposure experience of the population from where the Cases were drawn. This was therefore suggestive of a population highly exposed to both HBV and dietary aflatoxin B_1, both of which were etiological agents for liver disease.
4. Even though the study determined sero prevalence of 1.24% due to combined etiology of AFB_1 and HBV for liver disease as a fair estimate for lower eastern Kenya compared to national prevalence of between 5-8% of HBV

alone (Kathleen *et al*., 2016), it is suggested that the prevalence due to a mixed etiology could have been higher than determined since the factors have synergistic or additive effect to liver disease and hence higher mortality rates indicating lower disease cases at any given period of time.

5. The study also determined that there was a higher relative risk of acquiring liver disease due to dietary AFB_1 and HBV infection separately than a mixed infection of both of HBV and AFB_1 together in the two counties of lower eastern Kenya, even though the two etiological factors had additive effect to the liver disease burden.
6. It was found that, there was a higher magnitude of association between dietary AFB_1 exposure and liver disease and also a non causal association between non-HBV and AFB_1 factors and liver disease including blood transfusion, unprotected sex and untreated water.
7. Comparatively, the study suggests that both Case and Control subjects where exposed to dietary AFB_1 toxins but those in control cohort were more tolerant to toxic effects of dietary aflatoxin B_1 than those in case cohorts, since AFB_1 sero positivity in controls had not resulted into physical disease by the time the subjects were enrolled to this study.

5.3 Recommedations

i. This study's findings on prevalence of liver disease due to HBV and the relative risks of acquiring the same by the residents justifies a vaccination campaign by both the County government and the Ministry of health to immunize the resident population against HBV. It has been demonstrated by various studies that immunization is an effective tool of prevention of hepatitis B viral infections (CDC, 2013).
ii. The finding that the current aflatoxicosis prevalence rate was 25.97 % in the study area is an indication that dietary AFB_1 exposure to the residents of the study area was a major contributor to liver disease in lower eastern Kenya. It is suggested that the Ministry of Agriculture, Kenya and the County governments of Kitui and Makueni do initiate a campaign to sensatise and train farmers on proper drying and storage methods for maize grain and

flour in lower eastern Kenya. This will minimize the infestation of maize grain and flour with moulds especially that of the genus *A. flatus* which produce AFB_1 in maize grain and flour under humid and hot conditions.

iii. This study determined that some study subjects in the control groups had low levels of HBsAg yet they did not have active liver disease. It is recommended that MOH and KEMRI undertake a study among the residents, which could link the amount of serum HBV DNA to HBsAg levels in both cases and controls so as to determine whether those controls having HBsAg levels had actual HBV DNA as health carriers and also determine the mean cut off levels for this biomarker for both cases and controls in lower eastern Kenya.

iv. This was the first study done in lower eastern Kenya to link the synergistic or additive effect of HBV infection and the higher dietary AFB_1 exposure among the resident population to current liver disease prevalence. It is suggested that the Ministry of health undertake to provide the current anti HBV treatment to residents of Makueni and Kitui counties as a matter of priority in addition to other forms of standard care for HBV infection as a better treatment method to those already infected.

REFERENCES

Andre, F. (2000). Hepatitis B epidemiology in Asia, the Middle East and Africa. *Vaccine supplement*. 1:20-22.

Anaedobe, C.G., Fowatade, A., Omuruyi, C., Bakare, R.(2015). Prevalence, sociodemographic features and risk factors of hepatitis B virus infection among pregnant women in south western Nigeria. *Pan African Medical Journal*. *20*, 406

Arkell, E.I. (2015). The world most carcinogenic toxin was discovered via turkey plague. *Food science.* Retreived from http://iog.gizmodo.com/ the-world-most-carcinogenic-toxin-was-discovered-via-t-1700581670.

Askun, T. (2018). Fusarium: Pathogenicity, infections, disease, mycotoxins and management, *1*(3-15).

Ataei, B., Alavian, M.S., Fard, F.S., Rabiei, A.A., Safaei, A., Rabiei, A., Ataei, M. (2019). A Case – Control study of risk factors for hepatitis B infection: A regional report among Isfahanian adults. *Journal of Research in Medical Sciences*. 24:22

AOAC, (1999). AOAC official method 990.34, in *Official methods of analysis of AOAC international 1999,* 16th Edition, *49*(16), 13-14.

Ayala, A., Munoz, M.F., Arguelles, S. (2014). Lipid peroxidation: production, metabolism and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. *Oxidadative medicine and Cellular longevity*, 2, 5-10

Agro food & veterinary diagnostics organisation (AVD). (2013). Mycotoxins Handbook: Euroclone kits for mycotoxins detection: Retrieved from http://www.euroclonegroup.it/allegati/prodotti/flyermycoen/3288/pdf

Bao, L., Trucksess M. W., White, K. D. (2010). Determination of Aflatoxins B_1, B_2, G_1 and G_2 in olive oil, peanut oil and sesame oil. *Journal of AOAC international, 93*(3), 936 – 942.

Bell, S.J., Nguyen, T. (2009). "The management of hepatitis B infection." *Australian presser, 23*(4), 99-104.

Bedard, L., Massey, T.E. (2006). AflatoxinB1—induced DNA damage and its repair. *Cancer letters, 241*, 174--183

Benkerroum, N. (2019). Retrospective and prospective look at aflatoxin research and development from a practical standpoint: An introduction. *Journal of environmental public health. 16,* 3033

Brooks, M.G. (2002). Sexually acquired hepatitis. *Sexually transmitted infections. 78,* 235--240

Bowyer, S. M., Sim, J. K. M., Webber, L.M. (2011). Current laboratory diagnosis of hepatitis B virus infection including 8 years of retrospective laboratory data. *Hepatitis B virus, CME*, 29(5): 210--213

Buddeberg, F., Schimmer B., Spahn, D. (2008). Transfusion-transmissible infections and transfusion-related immunomodulation. *Best Practice & Research. Clinical Anaesthesiology 22*(3), 503–17.

CAC, (2008). Effects on public health of an increase of the levels for aflatoxin total from 4μg/kg to 10μg/kg for tree nuts other than almonds, hazel nuts and pistachios – a statement from CONTAM. *Codex committee for food additives and contaminants* (CCFAC), *2,* 15 – 25.

CAST, (1989). Mycotoxins: Economics and health risks. *Task force report No.116 Ames,* IOWA, *1,* 5-8.

CBS, (1999). Population census of 1999: Ministry of planning and National development, Nairobi Kenya, 2, 120 – 125.

Chan, H. L., Wonq, G, L., Chin, A.M., Yu, K.K., Chan, H.Y., Sunq, J.J., Wonq, V.W. (2009). Hepatitis B virus genotype C is associated with more severe liver fibrosis than genotype B. *Clinical gastroenterelogy hepatology, 7*(12), 1361-1366

Chauhan, N.M., Washe, A.P., Minota, T. (2016). Fungal infection and aflatoxin contamination in maize collected from Gedeo, Ethiopia. *Springerplus* 5(1): 753

Chauhan, A., Adams, D. H., Watson, P. S., Lalor, P.F. (2016). Platelets: No longer bystanders in liver disease. *Hepatology. 64*(5), 1774--1784

CDC, (2012). Epidemiology and prevention of preventable diseases. The Pink Book: Courses textbook- 12th edition, *9*, 216--240

CDC, (2011). Hepatitis B FAQS for the public. Retrieved from http://www.cdc.gov/hepatitis/B/bFAQ. htm#trans

CDC, (2004). Outbreak of aflatoxins poisoning in Eastern and Central provinces, Kenya, January–July 2004. *Morbidity Mortality Weekly Report, 53,* 790 –

792.

CDC. (2001). Hepatitis B virus: A comprehensive strategy for eliminating transmission in the USA, through universal child hood vaccination: Immunization practices committee. *Morbidity and mortality weekly report,1991*, 40, 1-19

Chan, H. L., Wong, V. W., Wong, G. L., Tse, C. H., Chan, H.Y., Sung, J. J. (2010): A Longitudinal study on the natural history of serum hepatitis B surface antigen changes in chronic Hepatitis B. *Journal of hepatology. 52,* 1232-1241

Charan, J., Biswas, T. (2013). How to calculate sample size for different study designs in medical research. *Indian journal of psychological medicine. 35*(2):121--126

Chu, Y. J., Yang, H. I., Wu, H. C., Lee, M. H., Liu, J., Wang, L. Y., Lu, S. N., Jen L. C., You, S. L., Santella, R. M. (2018). Aflatoxin's exposure increases the risk of hepatocellular carcinoma associated with hepatic C virus infection or alcohol consumption. *European journal of cancer*, 94, 37 – 46.

Coffin, C. S., Mulrooney-Cousins, P.M., Vanmarle, G., Roberts, J.P., Michalak, T.I., Terrault, N. (2011). "Hepatitis B virus (HBV) quasi species in hepatic and-extrahepatic viral reservoirs in liver transplant recipients on prophylactic therapy". *Liver Transpl*, *17*(8), 955–62

Coppock, R.W., Christian, R.G., Jacobson, B.J. (2018). Aflatoxins. Veterinary toxicology, 3rd ed: Gupta R.C edition, Academic press, Cambridge 983—994

Coughlin, S.S. (2006). Ethical issues in epidemiological research and public health practice. *Emerging themes in epidemiology*, *3*, 16

Danuba, F, G., Tatiama, M., Daisson, J, T., Roger, A.V., Janaina, L.N., Fabian, S.T., Leon L.S. (2013). Prevalence and factors associated with, hepatitis B virus infection among senior citizens in a sourthern Brazillian city. *Hepatitis Monthly 2013*, *13*(5), e7874.

Denner, J., Pischke, S., Steimman, E., Blurnel, J., Glebe, D.(2019). Why blood donations should be tested for hepatitis E Virus. *BMC infectious disease*, *19*, 541

Deguchi, M., Yamashita, N., Kagita, A., Watani, Y., Tsuchida. (2004). Quantification of hepatitis B surface antigen by an automated chemiluminiscencent of microparticle immuno assay. *Journal of virology methods Feb. 2004*; *115*(2), 217 – 222.

Deveci, O., Sezgin, E. (2005). Aflatoxin M_1 levels in Skimmed milk powders produced in Turkey. *Journal of food and drug analysis*, *2*, 139 – 142.

Devore, J., Peck, R. (1986). Statistics: The exploration and Analysis of data. West publishing company. New York, *8*, 302—348.

Dexheimer, T.S., Kozekova, A., Rizzo, C.J., Stone, M.P., Pommier, Y.(2008). Modulation of topoisomerase I--mediated DNA cleavage and the induction of DNA topoisomerase I cross links by crotonaldehyde--derived DNA adducts. *Nucleic acid Research, 12*, 4128--4136

Deb, N., Lahon, D., Sharma, P. (2016): A Study on probable correlation of AST/ALT (De ritis ratio), with the advancement of underlying severity of alcoholic liver disease in the North East Indian population. *Asia pacific journal of health sciences*. *3*(2), 5-10

Doi, K., Uetsuka, K.(2014). Mechanism of mycotoxin—induced dermal toxicology and tumorigenesis through oxidative stress pathways. *Journal of toxicology and pathology*, *27*, 1--10

European Center for Disease Control (ECDC). (2010). Hepatitis B and C in the EU neighborhood: Prevalence, Burden of disease and Screening policies; *Literature Review, Stockholm, 2*, 50-55

European Food Safety Authority (EFSA). (2013): Aflatoxins in food. Retrieved from http://www.efsa.europa.eu/en/topics/topic/aflatoxins.htm

El- Amir, Y.O., Yousef, M.S., Yahia, D. (2012). Assessment of DNA damaging effect of aflatoxin B_1 expressed by single gel electrophoresis in liver of Spraque-Dawley rats. *International journal of agro veterinary and medical science*, *6*(6), 466-472

Elizabeth, M. (2010). *Concise medical dictionary* (8^{th} ed.). Oxford University Press, *6*,120-832

Evans, M.D., Dizdaroglu, M., Cooke, M.S. (2004). Oxidative damage and disease: Induction repair and significance. *Review in mutation research*, *21*, 1--61

Fairley, C., Read, T. (2012). Vaccination against sexually transmitted infections. *Current Opinion in Infectious Diseases, 25*(1), 66–72

Federal Drug Administration (2009). Adulterated food: Federal food, drug and cosmetic act, chapter (iv). *Definitions and standards for food,* section 402 (a) (1). Washington USA, *15*, 678-690

Fisher, A., Laing, J. E., Townsend. (1998). Hand book for family planning research Operation research design, 2nd edition population council N Y, 3, 97-103

Fouad, M.A., Ruan, D., El-Senousy, Chen, W., Jiang, S., Zheng, C. (2019). Harmful effects and control strategies of aflatoxin B1 produced by *Aspergillus flatus* and *Aspergillus parasiticus* strains in poultry: *Review of toxins (2019), 11*, 176

Fugard, A.J. B., Potts, H.W.W. (2015). Supporting thinking on sample size for thematic analyses: A quantitative tool. *International journal of social research methodology, 18*(6), 669--684

Gan, S.I., Devlin, S.M., Scott-Douglss, N.W., Burak, K.W. (2005). "Lamivudine for the treatment of membranous glomerulopathy secondary to chronic hepatitis B infection". *Canadian journal of gastroenterology*, *19*(10), 625-629.

Gathumbi, J.K., Usleber, E., Martlbauver, E. (2001). Production of ultra sensitive antibodies against aflatoxin B_1. *Letters in Applied Microbiology, 32*, 347-351.

Gentile, F., Arcaro, A., Pizzimenti, S., Dega, M., Centrangolo, G.P., Dianzani, C., Lepore, A., Graf, M., Annes, P.R.J., Barrera, G. (2017). DNA damage by lipid peroxidation products. Implications in cancer, inflammation and auto immunity. *Aims genetology*; *4*. 103--137

Gibb, H., Devleesschauwer, B., Bolger, P.M., Wu, F., Ezendam, J., Cliff, J., Zeilmaker, M., Verger, P., Pitt, J., Bainnes, J. (2010). World health organization estimates of the global and regionaldisease burden of four food borne chemical toxins: *A data synthesis*, 4, 1393

Glebe, D., Urban, S. (2007). Viral and cellular determinants involved in hepa- DNA-viral entry". *World Journal of Gastroenterology. 13*(1), 22–38

Golthardt, D., Riediger, C., Weis, K.H., Encke, J., Schemmer, P., Schmidt, J., Sauer, P. (2007). Fulminant hepatic failure, etiology and indications for liver transplantation. *Nephrology dialysis & transplantation*, *22*(8), 85-88

Hall, P., Cash, J. (2012). What is the real function of liver function test? *Uster medical society, 81*(1), 30--36

Hassan, G., Lloyd, B.B. (1995). *Aspergillus flatus* and *Aspergillus parasiticus*. Aflatoxigenic fungi of concern in foods and feeds. *A review journal of food protection, 58,* 1395 – 1404

Henry, S.H., Bosch, F.X., Bower, J.C. (2002). Aflatoxin hepatitis and world-wide liver cancer risks. *Advances in experimental medical Biology, 504*, 229—233

Heidelbaugh, J.J., Bruderly, M. (2006). Cirrhosis and chronic liver failure Part 1: Diagnosis and Evaluation. *American family physician,* 74(5), 758---820

Hoofnagle, J. H., Van Natta M. L., Kleiner, D. E., Clark, J. M., Kowdley, K. V., Loomba R. (2013): Vitamin E and Changes in serum alanine aminotransferase levels in patients with non – alcoholic steato hepatitis. *Aliment Pharmacology therapeutics, 38*(2), 134 – 143

Ignatov, A.V., Bondarenko, K. A., Makorov, A.V. (2017). Non bulky lesions in human DNA: The ways of formation, repair and replication. *Acta—Naturae*, *9*, 12--26

Ishigaro, M., Takio, K., Suzuki, M., Oyama, R., Matzuzawa, T., Titanin, K. (1991). Complete amino acid sequence of human liver cytosolic alamine amino transferase (GPT) determined by a combination of conventional and mass spectral methods. *Journal of Biochemistry*. *30*(43), 1045-7.

Jaaskelainen, I. H., Harberg, L., Schyman, T., Jarvinen, A. (2018). A potential benefit from infectious disease specialist and a stationery Ward in rational antibiotic therapy of complicated Skin and skin structure infections. *Journal of Infectious disease (London), 50*(2), 107--116

Johnson, D. E. (1999): Special Considerations in interpreting liver function tests. *American Family Physician 59*(8), 2223-2230.

Jolly P. E., Akinyemiju T. F., Jha, M., Aban I., Forero A.G., Joseph D. (2015), Temporal variation and association of aflatoxin B_1 albumin-adduct levels with socio – economic and food consumption factors in HIV positive adults. *Journal of toxins 7,* 5129-5140.

Kalra, A., Yetikul, E., Wehrle, C. J., Turna, F. (2021). Liver physiology *1*, 5 - 15

Kangethe E. (2011). Situation Analysis: Improving food safety in the maize value in the Kenya. *A report for FAO, 1*, 16-19.

Karayiannis, P., Thomas, H., Mahy, B., Regenmortel, M. (2009). *Desk Encyclopedia of Human and Medical Virology*. Boston: Academic Press. *5*, 110-115.

Kathleen, N.L., Andrea, A. K., Eyasu, H.T. (2016). The prevalence of hepatitis B virus infection in Kenya, 2007. The American journal of tropical medicine and hygiene, *95*(2), 348--353

Keller, N.P., Butchko, R.A.E., Sarr, B., Phillips, T.D. (1994). A visual pattern of mycotoxin production in maize kernels by *Aspergillus* spp. *Phytopathology, 84*, 483 – 488.

Kelly, J.D., Eaton, D.L., Gyengerich, F.P., Coulombe, R. A. (1997). Aflatoxin activation in human lung: *Toxicology and Applied pharmacology, 144*, 88—95

Klavana, M., Bren, U. (2019). Aflatoxin B1- Formamido pyrimidine DNA adducts: relationships between structure, free energies and melting temperatures. *Molecules, 24*, 150

Klaunig, J.E., Kamendulis, L.M., Hocevar, B.A. (2009). Oxidative stress and oxidative damage in carcinogenesis. *Toxicological pathology 38,* 96--109

Kenya National Bureu of Statistics (2019). Kenya, 2019 population and housing census highlights, *11*, 2:1--12

Kothari E, R., Garg, G. (2014). Research Methodology: methods and techniques. New age international publishers. New Delhi, *10*, 182—197.

King'oriah, G. (2004). Faundamentals of Applied Statistics. Jomo Kenyatta foundations. Nairobi, *9*, 196 — 197.

Keefe, E. B., Dietrich D. T., Hans S. H. (2004): A treatment algorithm for the management of chronic hepatitis B virus infection in the United States. *Clinical Gastroenterology and Hepatology 2*, 87-106.

Kew, M.C. (2003). Synergistic interactions between aflatoxins B1 and hepatitis B virus in hepato carcinogenesis. *Liver international, 23*, 405-409

Lala, V., Goyal, A., Bansal, P., Minter, D, A. (2021). Liver functions and tests. 1: 3 - 20

Lawley, R. (2013). Aflatoxins: The science of safe food: *Food safety watches*. Retrieved from http://www.foodsafetywatch.org/factsheet/aflatoxins.

Leiskau, C., Baumann, U. (2017). Stucture, Functions and repair of the liver *1*, 1--20

Lewis, L., Onsongo, M., Njapau, H., Schurz – Rogers, H., Luber, G., Kierzak, S. (2005). Aflatoxin contamination of commercial maize products during an outbreak of acute aflatoxicosis in eastern and central Kenya. *Environmental health perspective, 113*, 1763 – 1767.

Li, F.Q., Li, Y.W., Luo, X.Y., Wang, Y.R., Luo, X.Y. (2009). Natural occurrence of aflatoxin in Chinese peanut butter and sesame paste *Journal of agricultural and food chemistry*, *57*(9), 3519-3524.

Liaw, Y. F., Brunetto, M. R., Hadziyannis, S. (2010). The natural history of chronic HBV infection and geographical difference. *Antiviral therapy*, *15*, 25 – 33

Liu, U., Wu, F. (2010). Global burden of aflatoxin- Induced hepatocellular carcinoma: A risk assessmement. *Environmental health perspective 118*, 818-824

Lok, A., McMahon, B., (2007). Chronic hepatitis B infections. *Hepatology*, *45*(2), 507–39

Lazo, M., Clerk, J. (2016): Liver Functions. *John Hopkins Diabetes Guide 1*(1-5).

Liaw, Y. F. (2011): Clinical utility of hepatitis B surface antigen, quantification in patients with chronic hepatitis B: A review. *Journal of hepatology. 53*, 2121--2159.

Martel, C., Maucort-Boulch, D., Plummer, M., Franceschi, S.(2015). World wide relative contribution of hepatitis B and C virus in hepatocellullar carcinoma. *Hepatology 62*(4), 1190—1200.

Marchese, S., Polo, A., Ariano, A., Velotto, S., Constantine, S., Severino, L. (2018). Aflatoxin B1 and M1: Biological properties and their involvement in cancer development. *Toxins 10*, 214

Marin, D.E., Tarrany, I. (2012). Overview on aflatoxins and Oxidative stress. *Toxins Review* 31, 32—43

Marzad, J., Devriendt, B., Baert, K., Cox, E. (2014). AflatoxinB1 interferes with the antigen presenting capacity of porcine dendritic cells. *Toxicology invitro*, *28*, 531--537

McGlynn, K. A., London, W.T. (2005). Epidemiology and natural history of hepatocellular carcinoma: best practices. *Resident clinical Gastroenterology, 19*, 3—23

Mckeen, C., Tang, L., Billian, M., Theodokakis, C.W., Kendal, R.J. (2006). Comparative acute and compinative toxicity of aflatoxin B_1 and T-2 toxin in animals and immortalized human cell lines. *Journal of applied toxicology*, *26*, 139--147

Min. of Finance and Planning, Kenya (MF&P). (2009). Second report on poverty in Kenya, Vol. I: Incidence and depths of poverty in Kenya. 2:30-35

Michell, N.J., Bowers, E., Hurburgh, C., Wu, F. (2016). Potential economic losses to the USA, corn industry from aflatoxin contamination. *Food additives and contaminants: PartA, Chem Anal control Expo risk assess. 33*(3), 540--550

Mutegi, C.K., Cotty, P.J., Bandyopadhyay, R. (2018). Prevalence and mitigation of aflatoxins in Kenya (1960- to date). *World Mycotoxin Journal*, *11*(3), 341—357

Muchiri, I., Okoth, F.A., Ngaira, J., Tuei, S. (2012). Seroprevalence of HAV, HBV, HCV and HEV, among acute hepatitis patients at Kenyatta National hospital in Nairobi, Kenya. *East African medical Journal*, *89*, 199--201

Muthomi, J. W., Njenga, L. N., Gathumbi, J. K., Chemining'wa, G. W. (2009): The Occurrence of aflatoxins in maize and distribution of mycotoxin – producing fungi in Eastern Kenya. *Journal of plant pathology*, *8*, 113-119.

Muhia, J. T., Straetmans, M., Ibrahim, A., Njau, J., Muhanje, O., Gurach, A., Gikundi, S., Mutonga, D., Tetteh, C., Likimani, S., Breiman, R. F., Njenga, K., Lewis, L. (2008). Aflatoxin levels in locally grown maize from Makueni District in Kenya. *East african medical journal*. Vol. *85*(7), 312 -316

Mutungi, C., Lamuka, P., Arimi, S., Gathumbi, J., Onyango, C. (2008). The fate of aflatoxins during processing of maize into *Muthokoi*, a traditional Kenyan food. *Food control, 19,* 714--721

Mohzard, J., Malvandi, A.M., Alipour, M., Hosseinkhani, S. (2017). Enviromentally relevant level of aflatoxin B1 elicits toxic pro- inflammatory response in murine CNS—derived cells. *Toxicology letters, 279*, 96—106

Nardin, R., Autorino, G.L., Issel, C.J., Cook, R.F., Ricci, I., Frontoso, F., Sciduma, M.T. (2017). Evaluation of six Serological ELISA kits available in Italy as screening tests for equine infectious anaemia surveillance *Veterinary research 13*, 105

Nasreddine, L., Hillan, C., Bartosk., Massin, C. (2014): Dietary exposure to aflatoxins, Ochratoxin A and deoxynivalenol from a total diet study in an adult urban Lebanese population. *Journal of food and chemical toxicology, 73,* 35-43.

Pacifico, L., Ferraro, F., Bonci, E., Anamia, C., Romaggioli, S., Chiesa, C. (2013): Upper limit of aminotransferase*: "Quo Vadis?"*. *Clinical Chin. Acta. 422*, 29-39.

Peter, R., Martin P., Reinhard R., Franz H., Armin G., Phillip E., Marianne B., Thomas G. (2016). The AST/ALT ratio (De-ritis ratio): A novel marker for critical implied Ischemia in peripheral arterial occlusive disease patients. *Medicine. 95*(24), Pe 3843.

Park, S. Y., Kang K. H., Park, J. H., Lee J. H., Cho, C. M., Talk, W. Y. (2014). Clinical efficiency of AST/ALT ratio and platelet counts as predictors of degree of fibrosis in HBV infected patients without clinically evident liver cirrhosis. *Korean Journal of Gastroenterology*, 43, 246-51.

Pearce, N. (1983). What does the Odds ratio estimate in a Case- Control study mean? *International Journal of Epidemiology, 22*(6):, 1189-1193.

Peersman, G.(2014).Overview: Data collection and analysis methods in impact evaluation. *Methodological briefs in impact evaluation, 10*(1), 2—10.

O'Neill, R.T., Walsh, P., Coen, N., Fortune, C., Regan, D. (2012). Introduction of an auto dilution for architect HB_SAg quantitative assay. *Association for the study of the liver* –Taipei Conference, *2012* (1), 1-30.

Rahil, E. C., Everhert E. J. (2012): Upper limits of normal for alanine aminotrasferase activity in the United States Population. *Journal of Hepatology. 55*(2), 447-454.

Raad, F., Samuel, N., Ezri, Y., Farah, R., Igor, V., Husssein, A., Rubinshtein, O., Assy, N. (2009). Acute aflatoxicosis resulting in Rhabdomyolysis. *Gastroenterology research, 2*(1), 48-50

Ramalho, L.N.Z., Porta, L.D., Rosim, R.E., Pella, T., Augusto, D.M., Oliveira, C.F. (2018). Aflatoxin B1 residue in human livers and their relationships with markers of hepatic carcinogenesis in Sao Paulo, Brazil. *Toxicology reports, 5*, 777-784

Ran, D. B., Stevens, G.A. Theaker. J., Witten born, J.S., Wiersma, S.T. (2012). The Global pattern burden of hepatitis E virus genotypes 1 and 2 in 2005. *Hepatology*. *55*(4)

Rastogi, S., Dogra, R.K.S., Khama, S.K., Das, M. (2006). Skin tumorigenic potential of aflatoxin B1 in mice. *Food chemistry & toxicology*, *44*, 670--677

Redd, J.T., Baumbach, J., Kohn, W., Williams, I. (2009). Patient to patient transmission of hepatitis B virus associated with Oral surgery. *Journal of infectious diseases, 195*(9), 1311-1314.

Robeck, J.D. (2011). Administration of blood components. *AABB technical manual,* (17^{th} ed.), *21*, 801-814

Robert, H.F., Suzanne, W. F., Grant, S.F. (2012). The odds ratio: An estimate of Relative Risk. *Clinical epidemiology: The essentials.* (5th ed.), *6*, 530-640

Romero, A., Anes, S., Castellano, V., Martinez-Larranago, M.R., Anadon, A., Martinez, M.A. (2016). Mycotoxins modify the barrier function of Caco-2 cells through differential gene expression of specific claudin isoforms: Protective effect of illite mineral clay. *Toxicology*, *353*, 21--33

Rothman, J. K. (2012). Epidemiology: An introduction; Measuring of disease occurance and Causal effects. Oxford University Press, *4*, 53--66

Rural 21, (2013). No chance for aflatoxins. *International Journal for rural development*; Retrieved from http:www.rural21.com/English/news/details/article/no-chance-for-aflatoxin-0000656

Robert, S. O. (2010): The Epidemiology of Hepatitis B. *Cleveland Clinic Centre for Continuing Education 1*, (1-8).

Rushing, B.R., Selim, M.I. (2017). Structure and Oxidation of Pyrrole adducts formed between aflatoxin B2a and biological amines. *Chem. Res.Toxicology, 30*, 1275--1285

Schessellman, J.J. (1982). Sample size determination in epidemiologic studies. *American journal of epidemiology, 115*, 752-758.

Sears, J. (1992). Anatomy and Physiology of the liver *1*, 1--10

Sitia, G., Iannacone, M., Ruggeri, Z., Guidotti, L. (2007). HBV pathogenesis in animal models: recent advances on the role of platelets. *Journal of Hepatology*, *46*(4), 719–26.

Smith, J.E., Solomon, G.L., Lewis, C.W., Anderson, J.G. (1994). Mycotoxins in human nutrition and health. *European commission publication, 10*, 670 - 700.

Sherman, K. E. (1991): Alanine aminotransferase in clinical practice: A review. *Archives of Internal Medicine. 151*(2), 260-265.

Spangenberg, H. C., Thianine R., Blum, H. E. (2006): Serum markers of hepatocellular Carcinoma. *Seminal Liver Disease 26*, 385-390.

Stickel, F., Dartz, C., Hampe, J., Ballaler, R. (2017). Pathophysiology and management of alcoholic liver disease: update 2016. *Gut and liver*, 2: 173 - !88

Szumilas, M. (2010). Explaining Odds ratios; *Journal of Canadian Academy of Adolescent psychiatry. 19*(3), 227—22

Tavakol, M., Dennick, R. (2011). Making sense of Cronbach alpha. *International Journal of Medical Education. 2*, 53—54

Terrault, N., Roche, B., Samuel, D. (2005). Management of the hepatitis B virus in the liver transplantation setting: A European and an American perspective. *Liver Transplants. 11*(7), 716–32.

Troiano, R., Reuter, W.M. (2007). Rapid quantification of Aflatoxins B_1 , B_2 , G_1, G_2 in corn by HPLC with Kobra cell® derivatization and fluorescence detection. *Perkin Elmer, 1*,1—3

Turner, P.C., (2013). The molecular epidemiology of chronic aflatoxin driven impaired child growth. *Scientifica 2013*, 121

Uslusoy, H.S., Nak, S.G., Gulten, M. (2011). Non invasive predictors for liver fibrosis in patients with nonalcoholic steatohepatitis. *World journal of hepatology, 3*(8), 219-227

Vanclay, F., Bainnes, J.T., Taylor, C.N. (2013). Principles for ethical research involving humans: *Ethical professional practice in impact assessment and project approval, 31*(4), 243--253

Verma, R. (2017). Aflatoxins cause DNA damage. *International journal of human genetics, 4*, 231—236

Ventola, C.L. (2016). Immunizations in the United States: recomedations, Barriers, and Measures to improve compliance. *Pharmacy and Therapeutics*, *41*(7): 426—436

Vosough, M., Bayat, M., Salami, A. (2010). Matrix- free analysis of aflatoxins in pistachio nuts using parallel factor modeling of liquid chromatography diode – array detection data. *Analytica chrimica acta*, *663*(1), 11 – 18

Xu, Q., Higgins, T., Cembrowski, G.S. (2015). Limiting the testing of AST: A diagnostically non-specific enzyme. *American journal of clinical pathology*. *144*, 423--426

Waslay, A., Kruszon –Moran D., Kuhnert, W., Simard, E.P., Finelli, L., Mc Quillan, G. (2010). The Prevalence of hepatitis B virus infection in the United States in the era of vaccination. *Journal of Infectious Diseases. 202*(2), 192-201

Wang, J., Zhao, G., Chen, J., Xia, J., Wang, S., Huang, G., Chen, F., Shi, J., Yang, Y., Wei, Y. (2019). Association of hepatitis B infection to high-risk complications in total joint arthroplasty. *BMC Masculoskeletal Disorders*. *20*, 163

Wang, F., Zuo, Z., Chen, K., Gao, C., Yang, Z., Zhao, S., Li, J., Song, H., Peng, X., Fang, J. (2018). Histopathological injuries, ultrastructural changes and depressed TLR expression in the small intestine of broiler chicken with aflatoxin B1. *Toxins*, *10*, 131

Wang, W., Srivastava., Sudhir. (2002). "Serological markers", In Lester Breslow. *Encyclopedia of public health N.Y. 4*, 1088-1090

Wild, C. D., Gong, Y, Y. (2010). Mycotoxins and humans' disease: A largely ignored global health issue. *Carcinogenesis, 31*, 71-82

Wild, C.P., Miller, J.D., Groopman, D. (2015). Effects of Aflatoxins on aflatoxicosis and Liver cancer: Mycotoxins control in low—and middle- income countries. *IARC working group reports No., 9*(3), 155

William, J. H., Phillips, T.D., Jolly P. E., Stiles, J. K., Jolly C. M., Aggarwal, D. (2004). "Human aflatoxicosis in developing countries: A review of toxicology exposure, potential health consequences and interventions" *American journal of clinical nutrition*, *80*(s), 1106 – 22

WHO. (2010). WHO guidelines on drawing blood: best practices in Phlebotomy. *NLB classification: WB 381*, *4*, 25-29

WHO. (2017). Evaluation of certain contaminants in food: Eight--third report of the joint FAO/WHO expert committee on food additives Geneva, Switzerland *2*, 150—153

WHO. (2018): Aflatoxins: Risk assessments on aflatoxins undertaken by the joint FAO/WHO, expert committee on food additives. *JECFA*

Yan, H., Zhong, G., Xu, G., He, W., Jing, Z., Gao, Z., Huang, Y. (2012). Sodium taurocholate co transporting polypeptide is a functional receptor for human hepatitis B and D virus. *ELife, 1*, e00049.

Yard, E. E., John, H. D., Lauren, E.l., Michael, E. R., Ekaterina, M. P., Adrea, A. K., Joel, M. M., Rebecca, B., Shanaaz, K. S. (2013): Human Aflatoxin exposure in Kenya, 2007: A cross sectional study. *Journal of food additives and contaminants, 30*(4), 1321-1331).

Yin, L.K., Tong, K.W. (2009). Elevated ALT and AST in an asymptomatic person: What the primary care doctors should do. *4*(3), 98-102

Yin, H., Jiang, M., Peng, X., Cui, H., Zhou, Y., He, M., Zuo, Z., Ouyang, P., Fan, J., Fang, J. (2016). The molecular mechanism of G_2M cell cycle arrest, induced by AFB1 in the Jejunum. *Oncotarget, 7*, 35592-3560

APPENDICES

Appendix I: Study approval from KEMRI Ssc Committee

APPENDIX I: Study Approval From KEMRI Ssc Commitee

KENYA MEDICAL RESEARCH INSTITUTE

P.O. Box 54840-00200, NAIROBI, Kenya
Tel (254) (020) 2722541, 2713349, 0722-205901, 0733-400003; Fax: (254) (020) 2720030
E-mail: director@kemri.org info@kemri.org Website:www.kemri.org

KEMRI/SSC/103476 13th January, 2015

Pius Mutisya

Thro'
Director, CPHR
NAIROBI

Forwarded with compliments
15/01/2015

REF: SSC No. 2988 (Revised) – The Prevalence of Liver Disease among Sero-positive HBV and AFB_1 Subjects and the Factors Associated with the Disease in Kitui and Makueni Counties, Kenya

Thank you for your letter dated 20th December, 2014 responding to the comments raised by the KEMRI SSC.

I am pleased to inform you that your proposal now has formal scientific approval from SSC.

The SSC however, advises that work on the proposed study can only start after ERC approval.

Sammy Njenga, PhD
SECRETARY, SSC

Appendix II: Study Approval from KEMRI Scientific and Etthics Review Unit (SERU)

APPENDIX II: Study Approval From KEMRI Scientific and Ethics Review Unit (SERU)

KENYA MEDICAL RESEARCH INSTITUTE

P.O. Box 54840-00200, NAIROBI, Kenya
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KEMRI/RES/7/3/1 **May 8, 2015**

TO: **PIUS MUTISYA KIMANI,**
PRINCIPAL INVESTIGATOR

THROUGH: **DR. CHARLES MBAKAYA,**
THE DIRECTOR, CPHR,
NAIROBI

Forwarded 15/05/2015

Dear Sir,

RE: **SSC PROTOCOL NO. 2988 (*RESUBMISSION2 OF INITIAL SUBMISSION*): THE PREVALENCE OF LIVER DISEASE AMONG SERO-POSITIVE HBV AND AFB$_1$ SUBJECT AND THE FACTORS ASSOCIATED WITH THE DISEASE IN KITUI AND MAKUENI COUNTIES, KENYA.**

Reference is made to your undated letter. KEMRI/Scientific and Ethics Review Unit (SERU) acknowledges receipt of the revised protocol on 30th April 2015.

This is to inform you that the Committee notes that the issues raised at the 236th meeting of the KEMRI/Ethics Review Committee held on 17th February 2015 have been adequately addressed.

Consequently, the study is granted approval for implementation effective this **8th May, 2015** for a period of one year. Please note that authorization to conduct this study will automatically expire on **7th May, 2016.** If you plan to continue data collection or analysis beyond this date, please submit an application for continuation approval to SERU by **26th March, 2016.**

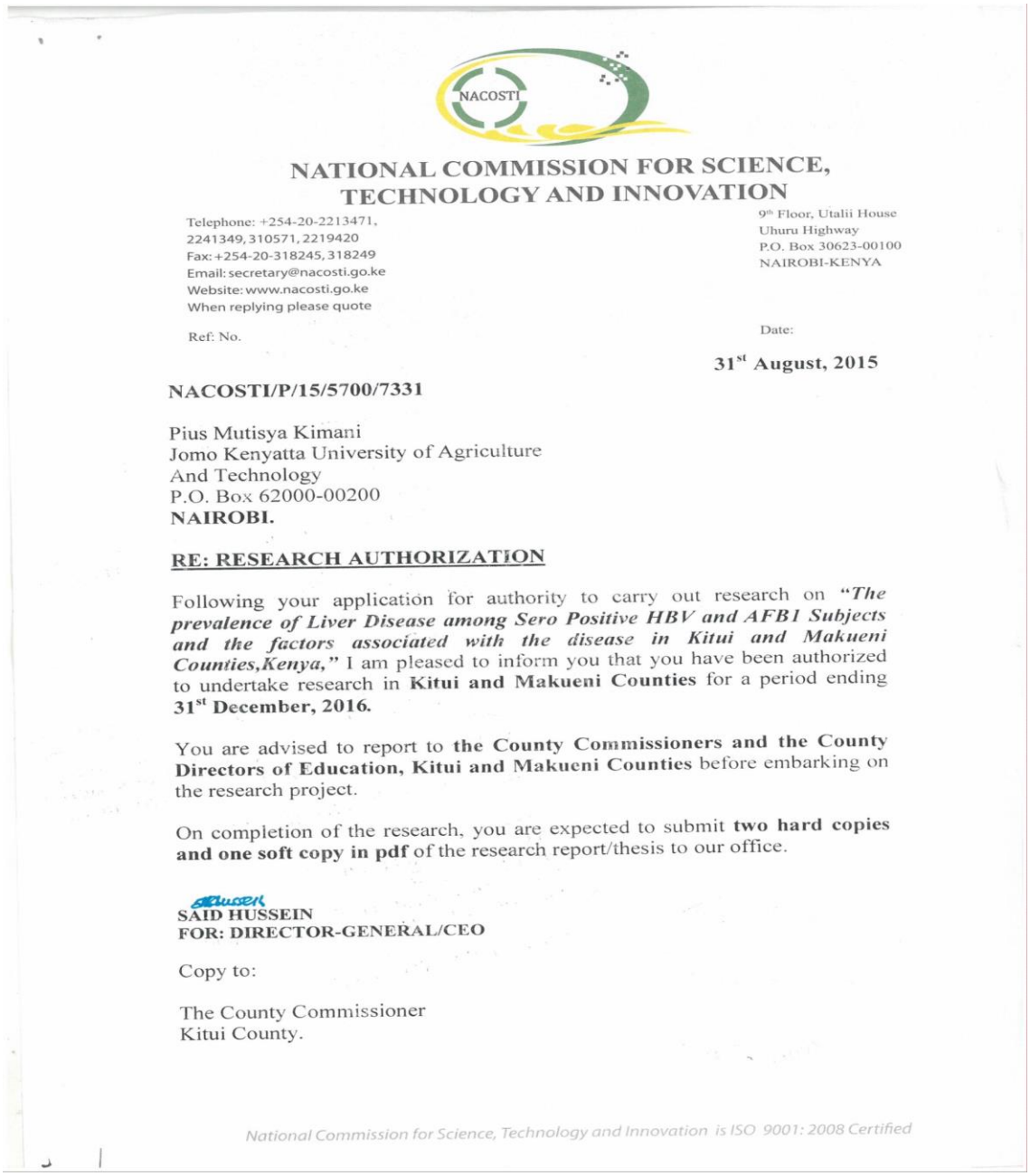
You are required to submit any proposed changes to this study to the SERU for review and the changes should not be initiated until written approval from SERU is received. Please note that any unanticipated problems resulting from the implementation of this study should be brought to the attention of SERU and you should advise SERU when the study is completed or discontinued.

You may embark on the study.

Yours faithfully,

PROF. ELIZABETH BUKUSI,
ACTING HEAD,
KEMRI/SCIENTIFIC AND ETHICS REVIEW UNIT

Appendix III: Ethical Approval from NACOST



NACOSTI

NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY AND INNOVATION

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Ref: No.

Date:

31st August, 2015

NACOSTI/P/15/5700/7331

Pius Mutisya Kimani
Jomo Kenyatta University of Agriculture
And Technology
P.O. Box 62000-00200
NAIROBI.

RE: RESEARCH AUTHORIZATION

Following your application for authority to carry out research on ***"The prevalence of Liver Disease among Sero Positive HBV and AFB1 Subjects and the factors associated with the disease in Kitui and Makueni Counties,Kenya,"*** I am pleased to inform you that you have been authorized to undertake research in **Kitui and Makueni Counties** for a period ending **31st December, 2016.**

You are advised to report to **the County Commissioners and the County Directors of Education, Kitui and Makueni Counties** before embarking on the research project.

On completion of the research, you are expected to submit **two hard copies and one soft copy in pdf** of the research report/thesis to our office.

SAID HUSSEIN
FOR: DIRECTOR-GENERAL/CEO

Copy to:

The County Commissioner
Kitui County.

National Commission for Science, Technology and Innovation is ISO 9001: 2008 Certified

Appendix IV: Informed Consent Document for Human Subject

Jomo Kenyatta University Of Agriculture And Technology In Collaboration With Institute Of Tropical Medicine And Infectious Diseases

P.O. Box 62000-00200, Nairobi.

"The prevalence of liver disease among sero positive HBV and AFB_1, subjects and the factors associated with the disease in Kitui and Makueni Counties, Kenya".

Investigators

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Prof. Fred Wamunyokoli (investigator)

Director External Studies

Jomo Kenyatta University of Science and technology

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Prof. James K Gathumbi

Kabete Animal Pathology Labs.

University of Nairobi

Mobile: 0722- 434001

Purpose of study

Liver diseases arise due to malfunction of the liver. This occurs due to several reasons including but not limited to Hepatitis B virus infection and Aflatoxin poisoning arising from consumption of mouldy grains and chronic alcoholism. Infection with Hepatitis B Virus and concurrent consumption of mouldy grains lead to rapid development of liver disease. The purpose of this study is to find out the prevalence of liver diseases in Kitui and Makueni Counties as a result of Hepatitis B infection and aflatoxin poisoning in the population. This will provide information that will assist in prevention of liver diseases in future.

Participation

Your participation in this study is voluntary and you are free to reject a request to participate without giving any reason. Make sure you read and understand what is contained in this document before agreeing to participate. You are encouraged to ask questions in areas that you do not understand. Parents must give an informed consent that their children participate in the study before they are included. A child who does not assent to participate in the study will not be included even if the parent has consented to the same. The consenting documentation will be done before blood sample is obtained from the participant.

Study procedure

Having read, understood and accepted to participate in this study then the following will be requested from you:- 4 millilitres of blood drawn from cubital vein within

the cubital area of the forearm, 0.25kg each of household maize grain and flour samples, will be collected from you. By using a questionnaire, the investigator will ask you questions concerning your age, gender, where you obtain white maize grain and flour from, mode of storage and other life style questions including whether you take alcohol in any form. You are free not to answer any question and you may decide not to answer all the questions. You will not be required to give reasons as to why. The exercise will be conducted once and there will be no follow up.

Risks and discomforts

During and immediately after drawing of the blood you will feel pain at the punctured point. The area where blood was drawn may swell and/or you may take several minutes before you stop bleeding. You may faint and at times though rarely you may get infection through the site of puncture. To ensure your safety, blood will be drawn by a trained technician/phlebotomist who has been allowed to practice. A clinician will be requested to assist and manage localised phlebitis and pain if it occurs. All equipment used will be sterilized and needles and syringes once used will not be re-used. In addition, this exercise will be done in a hospital laboratory and the highest care will be taken to ensure your safety even though you will be inconvenienced slightly.

Benefits

There is no material or financial benefits for participating in this study. However, the findings of this research will benefit the entire community as it will help improve general public health and prevent occurrence of hepatitis B virus infection and aflatoxicosis which are often suspected to be the major causes of liver disease.

Costs

You will not spend any money while participating in this study. The 0.25kg each of maize grain and flour samples requested from your household as part of this investigation will be compensated for at market rates of Sh100.00 per the two 0.25kg samples or you may voluntarily donate for this investigation only.

Confidentiality

Your name will not be recorded and the blood, maize grain and flour sample collected will have no marks or labels (identifiers) that can enable anyone trace from whom it was collected. Only codes will be used as labels. The data will also be coded and stored electronically with an appropriate password for security. Filled questioners will be kept in a metal safe with restricted access to authorised persons only. The blood sample will only be used for this study and no other person will be allowed to handle or use it.

Enquiries

If you need further explanation or you have questions concerning your participation in this study you can contact me through any of the following ways

Pius K Mutisya (Principal investigator),

P. O. Box 40326-00100

Nairobi

Email: pkmutisya58@gmail.com

Telephone: 0713781785

If you would like to know more about your rights as a participant in this study you can contact the Ethical Review Committee based at Kenya Medical Research Institute (KEMRI) Nairobi by sending your enquiries to:

The Secretary, KEMRI, **Ethics Review Committee, P.O. Box 54580-00200, Nairobi; Telephone numbers: 0717 719477, 020-2722541, 0722 205901, 0733 400003; Email address: seru@kemri.org**

Questionnaire

You will be issued with a questionnaire that will enable gathering of more information that will help me investigate occurrence of liver diseases in human subjects. All the answers will be treated as confidential and will not be used for any other purpose except for this study. If you wish to know the results of the test please

register your contacts with the following:

Pius K Mutisya (Principal investigator

Po box 40326-00100

Nairobi, Kenya.

Mobile 0713 781 785.

PATIENT CONSENT STATEMENT

I have read the information in this form / the information in this form has been read to me in the presence of a witness. I have discussed the contents of this form with the interviewer in a language that I fully understand. I had a chance to ask questions and my questions were all answered to my satisfaction.

I agree to participate in the study [***tick (✓) one]***: **Yes ( ) No ( )**

I agree to donate blood sample in support of the study [***tick (✓) one]***: **Yes ( ) No ( )**

Name: _____

Signature: _____ or Mark (Thumbprint) : _____**Date: ___/____/____**

Witness Name:_____

Witness Signature: _____**Date: ___/____/_____**

Name of research assistant taking consent: _____

Signature of the research assistant taking consent: _____**Date: ___/____/____**

Appendix V: Informed assent form for children 10--17 Yrs

Title: "The prevalence of liver disease among sero positive HBV and AFB_1 subjects and the factors associated with the disease in Kitui and Makueni counties, Kenya."

Institutions and Investigators:

Pius k Mutisya (Principal Investigator)

Min of educ. Science and technology

Po Box 40326-00100

Nairobi

Mobile: 0715 781785. e mail; pkmutisya58@gmail.com

Prof. Yeri Kombe (investigator)

Director

Kenya medical research institute

Mobile: 0734 257 864

Prof. Charles Mbakaya (investigator)

Director

CPHR

Kenya medical research institute

Mobile: 0722 846964

Prof. Fred Wamunyokoli (investigator)

Director External studies

Jomo Kenyatta University of science and technology

Mobile: 0721 801065

Prof. James Gathumbi (investigator)

Kabete Veterinary Pathology labs

University of Nairobi

Mobile: 0722 434 001

Why are we doing this study?

Liver diseases arise due to malfunction of the liver. This occurs due to several reasons including but not limited to Hepatitis B virus infection and Aflatoxin poisoning arising from consumption of mouldy grains and chronic alcoholism. Infection with Hepatitis B Virus and concurrent consumption of mouldy grains lead to rapid development the disease.

The purpose of this study is to find out the prevalence of liver diseases in Kitui and Makueni Counties as a result of Hepatitis B infection and aflatoxin poisoning in the population. This will provide information that will assist in prevention of liver diseases in future.

Why am I being asked to participate?

Your participation in this study is voluntary and you are free to reject a request to participate without giving any reason. Make sure you read and understand what is contained in this document before agreeing to participate. Your parent must give an informed consent that you can participate in the study before you are included. A

child who does not assent to participate in the study will not be included even if the parent has consented to the same. The consenting documentation will be done before any blood sample is obtained from you.

What if I have questions?

In this study you will be free to ask questions that you may have at any time. If you do not understand anything for any reason, you can talk to me again or even ask your parent to call me anytime.

If I am in the Study what happens to me?

Having read, understood and accepted to participate in this study then the following will be requested from you:- 4 millilitres of blood drawn from cubital vein within the cubital area of the forearm, 0.25kg each of household maize grain and flour samples, will be collected from your homestead. By using a questionnaire, the investigator will ask you questions concerning your age, gender, where you obtain white maize grain and flour from, mode of storage and other life style questions including whether you take alcohol in any form. You are free not to answer any question and you may decide not to answer all the questions. You will not be required to give reasons as to why. The exercise will be conducted once and there will be no follow up.

Will I be hurt if I am in the study?

During and immediately after drawing of the blood you will experience minimal pain at the punctured point. You may develop anxiety because of some questions .The area where blood was drawn may swell and/or you may take several minutes before you stop bleeding. You may faint and at times though rarely you may get infection through the site of puncture. To ensure your safety, blood will be drawn by a trained technician/phlebotomist who has been allowed to practice. A doctor will be requested to assist and manage localised phlebitis and pain if it occurs. All equipment used will be sterilized and needles and syringes once used will not be re-used. In addition this exercise will be done in a hospital laboratory and the highest

care will be taken to ensure your safety even though you will be inconvenienced slightly.

Will the study be of any benefit to me?

There is no material or financial benefits for participating in this study. However, the findings of this research will benefit the entire community as it will help improve general public health and prevent occurrence of hepatitis B virus infection and aflatoxicosis which are often suspected to be the major causes of liver disease.

Will my parents incur any cost?

Your parent will not spend any money while you participate in this study. The 0.25kg each of maize grain and flour samples requested from your parent household as part of this investigation will be compensated for at market rates of Sh100.00 per the two 0.25kg samples or it may voluntarily be donated for this investigation.

How Confidential is this study?

Your name will not be recorded and the blood, maize grain and flour sample collected will have no marks or labels (identifiers) that can enable anyone trace from whom it was collected. Only codes will be used as labels. The data will also be coded and stored electronically with an appropriate password for security. Filled questionnaires will be kept in a metal safe with restricted access to authorised persons only. The blood sample will only be used for this study and no other person will be allowed to handle or use it.

Do I have to be in this study?

You do not have to be in this study if you do not want to be. It is purely voluntary and you can opt out at any time including when the study begins. Since we are discussing the study with your parents, you can talk to them also.

Enquiries

If you need further explanation or you have questions concerning your participation

in this study you can contact me through any of the following ways

Pius K Mutisya (Principal investigator),

P. O. Box 40326-00100

Nairobi

Email: pkmutisya58@gmail.com

Telephone: 0713781785

Questionnaire

You will be issued with a questionnaire that will enable gathering of more information that will help me investigate occurrence of liver diseases in human subjects. All the answers will be treated as confidential and will not be used for any other purpose except for this study. If you wish to know the results of the test please register your contacts. If you have any questions regarding your rights as concerns this study as a study participant or you want to know more about this study, you may enquire using the address below:

The Secretary, KEMRI Ethics Review Committee, P O Box 54840-00200, Nairobi; Telephone numbers : 0717 719477, 020-2722541, 0722 205901, 0733 400003; Email address: seru@kemri.org

PATIENT ASSENT/ CONSENT STATEMENT (between 10 to 17yrs)

I have read the information in this form / the information in this form has been read to me in the presence of a witness. I have discussed the contents of this form with the interviewer in a language that I fully understand. I had a chance to ask questions and my questions were all answered to my satisfaction.

I agree to participate in the study [***tick (✓) one]:*** **Yes ( ) No ( )**

I agree to donate blood sample in support of the study [***tick (✓)*** **one]: Yes ( ) No ( )**

Name: _____

Signature: _____ or Mark (Thumbprint) : _____**Date: ___/____/____**

Witness Name: _____

Witness Signature: _____ **Date: ___/____/____**

Name of Study assistant taking consent: _____

Signature of the Study assistant taking consent: _____**Date: ___/____/____**

Principal investigators (PI) name_____

PI signature_____**Date____/____/___**

Appendix VI: Laboratory determination of AST levels (Uv- Kinetic Tc-Matrix Method®)

Introduction

In hepatitis and other forms of liver disease associated with hepatic necrosis, both AST and ALT are elevated. Elevated levels of serum AST activity are also observed in infectious mononucleosis, muscular dystrophy, dermatogisitis and in other forms of muscle and liver injury.

This method is UV-Kinetic; TC-Matrix method® (Teco diagnostics, USA) based on the rate of NADH oxidation in a coupled malic dehydrogenase reaction as shown below: -

Reaction principle

Aspartate Aminotransferase3

AST

2-Oxoglutarate + L-aspartate ⟷ L-glutamate + oxaloacetate

MDH

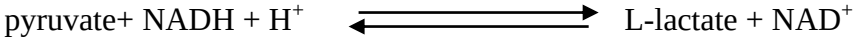
Oxaloacetate + NADH + H^+ ⟷ L-malate + NAD^+

Alanine Aminotransferase

ALT

2-oxoglutarate + L-alanine ⟷ L- glutamate + pyruvate

LDH



Reagent stability and preparation

The reagents in the kit are ready for use and are usually stable after opening for 14 days.

The AST reagents are stable, even after opening, up to the stated expiry date when stored and protected from light at 2°C to 8°C.

Specimen and Procedure

The test will be performed on serum or plasma. For serum, blood will be drawn to a tube which will not contain anti coagulant and allowed to clot. The serum will then be separated from the clot. If by any chance the assays are not completed within 8 hours, then serum and plasma will be stored at 2°C to 8°C and later than that, the samples will be frozen at -15°C to -20°C. Care shall be taken so that frozen samples shall only be thawed once. Ammonium Heparin or EDTA, shall be the anti coagulants of choice for the plasma in this procedure.

Precautions: -

Since all specimen are potentially infectious, they shall be handled with appropriate precautions and practices in accordance with Biosafety level 2 as recommended by USA NIH manual on Biosafety in microbiology and Biomedical laboratories, and in accordance with National or local regulations related to the safety of blood materials.

Analysis: -

Some 200ul of sample blood plasma and a buffer of 1000ul will be pipetted into the TC matrix systems. To this 250ul of substrate will be added; The TC matrix system® automatically proportions the appropriate sample and reagents volumes into the cuvette. The system monitors the change in absorbance at 340 nanometres. This change in absorbance is directly proportional to the activity of asparte amino transference in the sample and is used by the TC matrix system to calculate and

express asparte aminotransfarence levels in IU/mL. It has been shown that AST analyzed by AST reagents on TC matrix system is linear from 5 to 400 IU/mL.

The absorbance and AST levels are read at 1-, 2- and 3-minutes interval

Appendix VII: Questionnaire for Patients

Part A: Back ground Information

Please answer all the questions in this Questionnaire to the best of your ability. Write and Tick in the appropriate boxes where indicated.

1. What is the name of your County?.....
2. What is the name of your District?.....
3. What is the name of your Location?.....
4. What is the name of your Village?.....
5. How old are you?.....

1 ( ) Between 12- 17 years

2 ( ) Between 18-35 years

3 ( ) Between 36-53 years

4 ( ) Between 54-71 years

5 ( ) Between 71-88 year

6 ( ) Don't know

6. What is your gender 1 ( ) Male

2 ( ) Female 3 ( ) Non of the above

7. Marital Status if any 1 ( ) Married 3 ( ) single

2 ( ) Divorced 4 ( ) widowed 5 ( ) separated

8. In your own opinion, how many times have you visited this hospital with the complaint you currently have before admission?

1 ( ) None at all

2 ( ) Once only

3 ( ) twice only

4 ( ) More than 3 times

5 ( ) Cannot remember

9. Do you drink beer or wine or any traditional liquor like “Muratina or Kaluvu”?

1 ( ) Yes 2 ( ) No

10. If yes what kind of beer? 1 ( ) muratina 2 ( ) Busaa 3 ( ) chang’aa

4 ( ) Tusker 5 ( ) wines 6 ( ) whisky

11. How often do you take the beer in a week? 1 ( ) once 2 ( ) twice 3 ( ) thrice

4 ( ) four times 5 ( ) five times

6 ( ) daily

12. How many beer bottles do you take per day? 1 ( ) One 2 ( ) two 3 ( ) four

4 ( ) five 5 ( ) over five

13. How long have you been taking beer? 1 ( ) two years 2 ( ) three years

5 ( ) four years 6 ( ) over five years

14. How frequent do you use white maize as food per week?

1 ( ) once 2 ( ) twice 3 ( ) thrice 4 ( ) four times

5 ( ) five times 6 ( ) six 7 ( ) seven times

15. Where do you obtain white maize grain from?

1 ( ) Own farm

2 ( ) Bought from others

3 ( ) Bought from markets

4 ( ) Donated relief food

16. Where do you obtain white maize flour from?

1 ( ) Own maize grain

2 ( ) Buy from others

3 ( ) Buy from markets

4 ( ) Donated relief food

17. How do you store your “dry” harvested Maize?

1 ( ) In sisal gunny bags

2 ( ) In plastic gunny bags

3 ( ) In open traditional stores (“Ikumbi”).

4 ( ) On the floor of mud houses.

5 ( ) Others

18. How do you store your maize flour?

1 ( ) In sisal gunny bags

2 ( ) In plastic gunny bags

3 ( ) In plastic container

4 ( ) In metallic containers

5 ( ) In Paper bags

6 ( ) Never do it 7 ( ) others

Section B.

19. Where do you get your drinking and cooking water from?

1 ( ) Bore holes

2 ( ) Open dams

3 ( ) Seasonal streams

4 ( ) Sand wells in streams

5 ( ) Treated tap water

6 ( ) Harvested tank water 7 ( ) others

20. Do you have any type of toilet/latrines at home?

1 ( ) Yes

2 ( ) No

21. If yes, what type of toilets? 1 ( ) pit type

2 ( ) flash/septic tank

3 ( ) bucket type

4 ( ) others

22. If no, where do you go for ‘calls of nature’?

1 ( ) bush

2 ( ) others

23. Have you ever received donated blood in a blood transfusion exercise anywhere?

1 ( ) Yes

2 ( ) No

24. If yes, how many times have you received the donated blood?

1 ( ) once

2 ( ) twice 3 ( ) thrice

4 ( ) four times 5 ( ) many times

6 ( ) don’t know

25. Have you ever donated blood for any reason?

1 ( ) Yes 2 ( ) No

3 ( ) Don’t know 4 ( ) Cannot remember

26. Do you use injection needles and syringes for any purpose?

1 ( ) Yes

2 ( ) No

27. If yes, do you always sterilize any injection syringe and needles before use?

1 ( ) Always

2 ( ) Most of the time

3 ( ) Rarely

4 ( ) Not at all

28. Do you use condoms for sexual encounters?

1 ( ) Yes

2 ( ) No

3 ( ) Not sure

29. If yes, how often do you use them?

1 ( ) always

2 ( ) most of the time

3 ( ) Rarely

30. If yes, how often have you experienced condom failure?

1 ( ) always

2 ( ) most of the time

3 ( ) Rarely

4 ( ) not at all

31. Part C: Blood transfusion

To what extend do you agree with the following statements?

Please tick at the appropriate box corresponding to the ratings below:-

1. Never (N) 2. Rarely (R) 3. Uncertain (U) 4. Often (O) 5. Always (A)

	Questions	**1. N**	**2. R**	**3. U**	**4. O**	**5. A**
(i)	Have you ever received blood in any blood transfusion procedure?					
(ii)	How frequent have you undergone the blood transfusion procedure if any?					
(iii)	Do the clinicians explain the safety of the procedure before you consent to it?					
(iv)	Did you/Do you experience unusual health episode including fever, yellow skin, dark urine, nausea, vomiting yellowing of the eyes and unexplained lack of appetite especially between 3 to 54 weeks after blood transfusion?					
(v)	Have you ever handled blood/blood samples of another person in the course of your work at any time?					

32. PART D: Unprotected sex

Indicate your level of agreement with the following statement by ticking at the appropriate box corresponding to the ratings below; -

1. Never (N) 2. Rarely (R) 3. Uncertain (U) 4. Often (O) 5. Always (A)

	Questions	1. N	2. R	3. U	4. O	5. A
(i)	Do you engage in sexual intercourse?					
(ii)	If so, how regularly does this happen?					
(iii)	Do you use condoms (male or female) for protection during sex?					
(iv)	How often do you use the condoms?					
(v)	Have you ever had more than one sexual partner in a given period of time?					
(vi)	Do they use condoms as a method of protection?					
(vii)	If yes, how often do they use condoms with you?					

33. PART E: Untreated River Water

Indicate your level of agreement with the following statements by ticking at the appropriate box under the ratings below;

1. Never (N) 2. Rarely (R) 3. Uncertain (U) 4. Often (O) 5. Always (A)

	Questions	1. N	2. R	3. U	4. O	5. A
(i)	Do you use pit/septic tank toilets at home?					
(ii)	Do you have piped water for domestic use, including for drinking?					
(iii)	Do you use untreated Bore hole water for drinking?					
(iv)	Do you use river or stream water for domestic use including drinking?					
(v)	Do you treat your water by any means					

	including chlorination, boiling, or sieving before any domestic use, including drinking?					
(vi)	How often do you undertake the treatment of water?					

34. PART F: Unsterile body piercing instruments

Indicate your level of agreement with the following statements by ticking at the appropriate box corresponding to the ratings below: -

1. Never (N) 2. Rarely (R) 3. Uncertain (U) 4. Often (O) 5. Always (A)

	Questions	**1. N**	**2. R**	**3. U**	**4. O**	**5. A**
(i)	Do you use syringes and needles for injecting medicines or other drugs to yourself?					
(ii)	Do you sterilize needles and syringes by boiling or other means before use?					
(iii)	Are these needles shared with any other person?					
(iv)	Have you ever shared personal items including tooth brush, razors or nail clippers with others?					
(v)	If yes, in (iii) and (iv) are these instruments sterilized before use?					
(vi)	Do you ever use ear piercing instruments?					
(vii)	If yes, are they sterilized or do you sterilize them?					
(viii)	Have you been injured by a sharp or blunt object?					
(ix)	Did you seek a medical treatment for it?					

	Questions	1. N	2. R	3. U	4. O	5. A
(i)	Do you use maize grain or maize flour as food at home?					
(ii)	How frequent do you use maize grain or its products as food?					
(iii)	Do you grow the white maize you use for food at home?					
(iv)	Do you store your maize in sisal gunny bags all the time?					
(v)	Do you store your maize at least 3 feet from the ground?					
(vi)	Do you re-dry your maize even after harvest before storage?					
(vii)	Do you use polished maize grain (muthokoi) for food?					
(viii)	How often do you use commercial or relief donated maize as food?					

35. PART G: AFB_1 contaminated maize grain

Indicate your level of agreement with the following statement by ticking at the appropriate box corresponding to the ratings below: -

1. Never (N) 2. Rarely (R) 3. Uncertain (U) 4. Often (O) 5. Always (A)

36. PART H: AFB_1 contaminated maize flour

Indicate your level of agreement with the following statements by ticking at the appropriate box corresponding to the ratings below: -

1. Never (N) 2. Rarely (R) 3. Uncertain (U) 4. Often (O) 5. Always (A)

	Questions	**1. N**	**2. R**	**3. U**	**4. O**	**5. A**
(i)	How frequent do you use white maize flour to prepare food at home?					
(ii)	Do you use local "posho" mills to mill white maize grain to flour from the maize harvested from your home store?					
(iii)	Do you store your maize flour in plastic paper bags?					
(iv)	Have you ever noticed a change of taste of your flour after storage for between 1 to 2 weeks?					
(v)	Do you store your white maize flour at about 3 feet from the ground?					
(vi)	Do you store your maize flour in sisal gunny bags?					
(vii)	How often do you use commercial or donated relief flour as food at home?					
(viii)	Have you ever used local brew made from white maize flour milled at your area?					

37. PART I: Liver disease as a dependent variable

Indicate your level of agreement with the following statement by ticking at the appropriate box corresponding to the ratings below:-

1. Never (N) 2. Rarely (R) 3. Uncertain (U) 4. Often (O) 5. Always (A)

	Questions	**1. N**	**2. R**	**3. U**	**4. O**	**5. A**
(i)	Have you ever experienced yellowing of the eyes or skin accompanied by fever and passing of dark urine approximately 2 to 3 weeks after a meal of white maize grown at home?					
(ii)	Have you ever experienced yellowing of the eyes or skin accompanied by fever and passing of dark urine approximately 2 to 3 weeks after a meal of "ugali" or porridge made of white maize flour milled locally?					
(iii)	Have you ever got the following physical symptoms including yellowing of the skin eyes and passing of dark urine accompanied by fever 3 to 54 weeks after unprotected sex?					
(iv)	Have you ever experienced yellowing of the eyes or skin accompanied by fever and passing of dark urine approximately 2 to 54 weeks after drinking untreated dam, river, or stream water?					
(v)	Did you get any of the following physical symptoms including, yellowing of the skin yellowing of eyes; dark urine fever fatigue and loss of 3 to 54 weeks after receiving blood in a blood transfusion exercise?					
(vi)	Have you ever experienced the following symptoms including yellowing of the skin, eyes or passing of dark urine, fever, fatigue, and loss of appetite 3 to 54 weeks after sharing body piercing instruments or					

	shaving razors?					
(vii)	Have you ever experienced symptoms including yellowing of the skin, eyes, passing of dark urine, fever, fatigue, and loss of appetite 3 to 54 wks after consuming a local brew made from maize flour or other maize by products?					

37 (i), Determination of correlation (r) and coefficient of determination (R^2), between risk factors (contributory factors), and liver disease.

The factors (determinants) associated with liver disease are the Independent variables (x) while the liver disease is the dependent variable (y).

If a correlation exist, then $y = a + bx_i$ (x_1, x_2, x_k) and $r = \frac{\Sigma (xy/n) - \Sigma(x/n) \cdot \Sigma(y/n)}{(^{\delta}x \cdot ^{\delta}y)}$

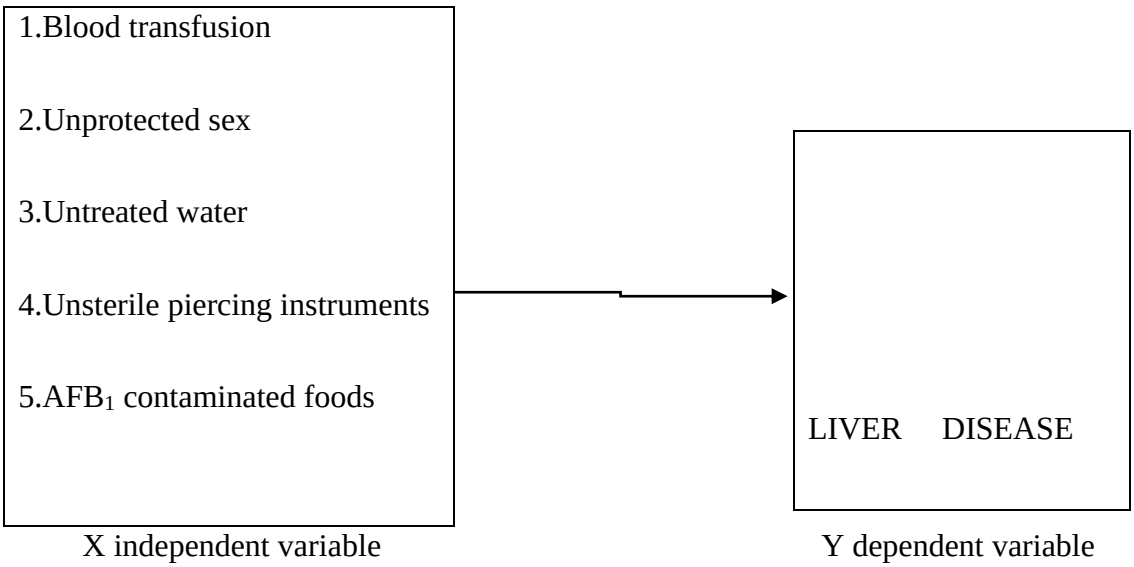
Where, $^{\delta}x$ and $^{\delta}y$ are the standard deviations of data in x and y respectively (King'oriah *et al*., 2004).

The coefficient of determination (R^2) is such that $R^2 = r^2$ in this study. This is the value in real terms (%) of the dependent variables (liver disease) which can be attributed to certain Independent variable including **blood transfusion**, **unprotected sex, untreated water, unsterile body piercing instruments, and AFB_1 contaminated grain and flour**. A table for determination of **mean** for blood transfusion is given below (**Table I**).

The mean values in Likert scale for contributory factors, (Independent variables) and the mean values for liver disease shall be entered into an SPSS programme version

18.0 for **multivariate regression analysis** for values of r and R^2 (**Table II**). The values of r shall be significant at p = 0.05 in a two tailed test.

A theoratical framework of this relationship is as follows:-



X independent variable Y dependent variable

Table I

	Blood Transfusion			**Likert Scale**		
		Mean	**Standard Deviation**	**Minimum**	**Maximum**	**N**
(i)	Have you ever received blood in any blood transfusion exercise?					
(ii)	How frequent have you undertaken blood transfusion exercise if any?					
(iii)	Do the clinicians explain the safety of the procedure before you consent to it?					
(iv)	Did you experience unusual health episode including fever, yellow skin, dark urine, nausea, vomiting yellow eyes and lack of appetite 3 to 54 weeks after blood transfusion?					
(v)	Have you ever handled blood samples of another person in the course of your work at any time?					
	Overall mean values for blood transfusion	=				

Table II

	Independent variable																	
Dependant Variable	Blood transfusion			AFB_1 Bearing flour			Unprotected Sex			Untreated Water			Unsterile Piercing instruments			AFB_1 Bearing Maize grain		
Liver disease Data values	r	R^2	%	r	R^2	%	r	R^2	%	r	R^2	%	r	R^2	%	r	R^2	%
N																		

The values of r shall be significant at p=0.05 in a 2 tailed test.

Appendix VIII: Laboratory Determination of alanine aminotransferase (ALT)

Introduction

The enzyme alanine aminotransferase (ALT), reacts primarily with L-alanine and l-glutamate, but will also use aminobutyrate, ornithine or aspartate instead of alanine. For example,

2 – oxoglutarate + L-ananine ⟶ glutamate + pyruvate.

The liver is an important site for this metabolic reaction. Increased levels of ALT are associated with hepatitis and other liver disorders associated with liver necrosis as in cirrhosis, carcinoma and obstructive jaundice. Elevated levels occur in circulatory failure and extensive trauma values may go up to 30U/L.

Principle of Test

In the presence of alanine, pyruvate is formed by the action of ALT. This is in turn converted to lactate by enzyme lactate dehydrogenase (LDH) in the presence of NADH; which is simultaneously converted to NAD and this is monitored at 340nm. Since the action of MDH is inhibited by increasing lactate concentration, which actually favours the reverse reaction and formation of pyruvate the assay is monitored by following the initial rate of NAD formation over 3 minutes.

Method:-

This analysis will use Libra® S21/S22 system (Biochrom Ltd. UK), which is an automatic ALT analyser. One (1) ml of the reagent (Randox labs, UK), is pipetted into two (2) disposable cuvettes. Each 1ml of the reagent contains 1.25U/mL of LDH; 0,018mmol/mL of NADH, 15mmol/mL of 2-oxoglutarate; 0.5mmol/L of L-alanine and 1000mmol/L Tris buffer of pH 7.5. A sample of 0.1ml is then added to the reagent in one cuvette warmed to 37ºC, for sensitivity and mixed well.

A blank determination before run is necessary for reference values. The preparation of the reagent is the same except that instead of the sample, 0.1 ml of pure water is

added, warmed to 37ºC, mixed, and run in the Libra® S21/S22 analyser for a duration of 4 minutes at a wavelength of 340nm. A factor 1.746 is entered in the analyser to change readings directly to IU/mL, then recorded.

The mixer of samples and reagent above, are then inserted to the analyser, and analyser for between 1 to 4 minutes. The absorbance rate are read but converted to IU/mL units by a factor of 1.746, then automatically printed out.

Appendix IX: Data capture form for aflatoxinB_1 contamination in household white maize grain and flour samples for case and controls

Sample no.	County of origin	Health center	Maize AFB_1 levels(ppb)	Flour AFB_1 levels(ppb)	Age	Remarks

Appendix X: Data capture form for ALT, AST, AFB_{1}- albumin adducts and HBsAg test results for study subjects

Case/control Code	Age(yrs)	AST (IU/mL)	ALT (IU/mL)	AFB_1 albumin adduct (pg/mL)	HBsAg (Iu/mL)