THE ROLE OF AFLATOXIN B_1 IN THE ETIOLOGY OF LIVER CIRRHOSIS IN EASTERN ETHIOPIA

ABRAHAM NIGUSSIE MEKURIA (BPHARM, MSC.)

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The role of aflatoxin B_1 in the etiology of liver cirrhosis in Eastern Ethiopia

Abraham Nigussie Mekuria (BPharm, MSc.)

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This is to certify that the dissertation prepared by Abraham Nigussie Mekuria, entitled: ***The Role of Aflatoxin B_1 in the etiology of liver cirrhosis in Eastern Ethiopia"***, *and* submitted in fulfillment of the requirements for the Degree of Doctor of Philosophy (Pharmacology) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

Name: Abraham Nigussie Mekuria Signature: _____ Date 28/2/2024

Singed by the Examining Committee:

External Examiner: Prof. Marthe De Boevre (PhD) Signature_____Date_____

Internal Examiner: Dr. Solomon Mequanenete (PhD) Signature_____ Date 29/02/24

Main Advisor: Prof. Tefera Abula (PhD) Signature_____ Date 5/03/24

Co-Advisors: Prof. Ephrem Engidawork (PhD)、 Signature_____ Date 29/02/24

Prof. Yun Yun Gong (PhD) Signature_____ Date 28/2/2024

Dr. Teshome Nedi (PhD) Signature_____ Date 1/3/24

Chairperson: Dr. Wondmagegn Tamiru (PhD) Signature_____ Date _____

Head, Department of Pharmacology & Clinical Pharmacy: _____

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LIST OF SCIENTIFIC PAPERS

This dissertation is based on the following published and unpublished works:

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2. Mekuria AN, Xia L, Ahmed TA, Bishaw S, Teklemariam Z, Nedi T, Abula T, Engidawork E, Gong YY. Contribution of Aflatoxin B_1 Exposure to Liver Cirrhosis in Eastern Ethiopia: A Case-control Study. *Int J Gen Med. 2023*; 16:3543-3553. https://doi.org/10.2147/IJGM.S425992

3. Mekuria AN, Seyoum T, Alemayehu DH, Abebe M, Nedi T, Abula T, Gong YY, Engidawork E. Copy number variation in the *GSTM1* and *GSTT1* genes and the risk of liver cirrhosis in Eastern Ethiopia. *Appl Clin Genet*. 2023; 16: 171-179. https://doi.org/10.2147/TACG.S435852

ABSTRACT

The Role of Aflatoxin B_1 in the Etiology of Liver Cirrhosis in Eastern Ethiopia

Abraham Nigussie Mekuria (B.Pharm, MSc)

Addis Ababa University, Addis Ababa Ethiopia

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Background: Liver cirrhosis is deemed to be a major cause of morbidity and mortality in Eastern Ethiopia; however, few studies have been conducted to identify its possible etiologies. For instance, in a hospital-based cross-sectional study, no definite etiologies were found in more than half of liver cirrhosis patients, suggesting a yet unidentified cause of this disease in this area. Meanwhile, as indicated in several studies, Eastern Ethiopia is a region with a diet particularly high in aflatoxin (AFB_1). AFB_1, a very toxic compound with sufficient evidence of carcinogenicity in humans, has been implicated as an emerging cause of liver cirrhosis.

Objective: This study evaluated the role of AFB_1 in the etiology of liver cirrhosis among patients attending Hiwot Fana Comprehensive Specialized University Hospital (HFCSUH), Eastern Ethiopia, in 2020-2021.

Methods: A consecutive sample of 127 adult patients diagnosed with liver cirrhosis from internal medicine unit of HFCSUH were enrolled as cases, and 253 adults without any evidence of liver disease were enrolled from the same unit of the hospital as controls. Demographic, dietary, and lifestyle characteristics of the participants were collected using a structured questionnaire. Clinical data were abstracted on standardized reporting forms. Blood sample were collected and tested for hepatic enzymes, hepatitis C virus antibody, hepatitis B surface antigen, and AFB_1-albumin adduct. Gluthatione S transferase M1 and T1 (*GSTM1* and *GSTT1)* genes copy number variations were also determined by TaqMan™ Assay. SPSS version 26.0 was used to compute the statistical analysis.

Results: The etiology of liver cirrhosis was known in only 23% of patients. Sorghum consumption as a staple food was significantly associated with liver cirrhosis of unknown etiology. AFB_1-albumin (AF-alb) detection rate was higher ($p<0.05$) in cases (75%) than in controls (64%) and with a median (interquartile range [IQR]) level of 11 pg/mg (5.5-25) and 7.0 pg/mg (4.3-20.5), respectively ($p<0.05$). Exposure to high levels of AF-alb (adjusted odds ratio (AOR) = 2.0; 95% CI: 1.1–3.7) were significantly ($p<0.05$) associated with liver cirrhosis. The frequencies of null genotypes for *GSTM1* and *GSTT1* were 0.39 (95% CI: 0.32, 0.46) and 0.32 (95% CI: 0.26, 0.39), respectively. The risk of liver cirrhosis was shown to be reduced ($p<0.05$) in *GSTT1* carriers compared to those with *GSTT1* null genotypes (AOR = 0.47; 95% CI: 0.25, 0.86. Furthermore, the risk of liver cirrhosis was significantly lower in those with one copy of *GSTT1* (AOR = 0.48; 95% CI: 0.25, 0.91) and a two or more combined copy of *GSTT1* and *GSTM1* genes (AOR = 0.38; 95% CI: 0.16, 0.91).

Conclusions: Sorghum consumption was identified as a potential source of exposure in the current investigation, which implicated AFB_1 exposure as a possible etiology of hepatic cirrhosis in Eastern Ethiopia with a high prevalence of cryptogenic cases. *GSTT1* and *GSTM1* genotypes also played a role in the risk of liver cirrhosis. To decrease the disease burden in this part of Ethiopia, plausible aflatoxin control strategies should be implemented.

Key words: Liver cirrhosis; Aflatoxins; Hepatitis B Virus; *glutathione S-transferase*; Ethiopia

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ABBREVIATIONS/ACRONYMS

AFBO	AFB_1-exo-8,9-epoxide
AFs	Aflatoxins
AF-alb	Aflatoxin B_1-albumin
AFAR	AFB_1-aldehyde reductase
ALT	Alanine transaminase
AOR	Adjusted odds ratio
AST	Aspartate transaminase
BMI	Body mass index
CNV	Copy number variation
CYP	Cytochrome P450
EAC	East Africa Community
ELISA	Enzyme-linked immunosorbent assay
mEH	microsomal epoxide hydrolase
FFQ	Food frequency questionnaire
EFDA	Ethiopian Food Drug Authority
GST	Glutathione S-transferase
HBsAg	Hepatitis B surface antigen
HCC	Hepatocellular carcinoma
HFCSUH	Hiwot Fana Comprehensive Specialized University Hospital
IARC	International Agency for Research in Cancer
IL	Interleukin
IQR	Inter quartile range
IRB	Institutional Review Board
LOD	Lower limit of detection
NF-κB	Nuclear Factor kappa B
PCR	Polymerase chain reaction
ROS	Reactive oxygen species
TNF-α	Tissue Necrosis Factor alpha

1. INTRODUCTION

1.1. Background

Aflatoxins (AFs), first described in the early 1960s, are secondary fungal metabolites produced mainly by *Aspergillus flavus* Link, *A. parasiticus* Speare, and *A. nomius* Kurtzman (Benkerroum, 2020; Pickova *et al*., 2021). Moreover, other fungal species, including *A. minisclerotigenes*, *A. ochraceoroseus*, *A. arachidicola*, *A. bombycis*, *A. tamarii*, *A. pseudotamarii*, *A. rambellii*, and *Emericellam venezuelensis,* have been identified as producing AFs (Carvajal-Campos *et al.,* 2017; Nleya *et al.,* 2020). These fungi are extremely abundant in tropical and subtropical areas, particularly in sub-Saharan Africa, Southeast Asia, and South American countries, where they thrive in the warm, humid conditions that promote their growth (Benkerroum, 2020; Mohammed *et al*., 2021; Abrehame *et al*., 2023). AFs include about 20 chemically related furanocoumarins, of which AFB_1, AFB_2, AFG_1, and AFG_2 are known to be the most prevalent AFs that naturally contaminate foods (Bräse *et al*., 2009). Besides, their hydroxy metabolites, AFM_1 and AFM_2, could appear in milk and milk products when lactating domestic animals consume feed contaminated with AFs (Sumon *et al*., 2021; Zentai *et al*., 2023).

AFB_1 is a highly carcinogenic substance that has been categorized under Group 1 due to substantial evidence of carcinogenicity in humans by the International Agency for Research on Cancer (IARC) (IARC, 2012). This potent mutagen and carcinogen is known to be a well-established cause of hepatocellular carcinoma (HCC). Furthermore, Liu *et al*. (2012) demonstrated in a meta-analysis that AFB_1 multiplicatively interacts with Hepatitis B virus (HBV) to produce HCC in high-risk areas; lowering AFB_1 exposure reduces HCC cases by roughly 23% in high-risk areas. In this regard, Liu and Wu (2010) reported that over 4.5 billion people, mostly in developing nations, are in danger of chronic exposure to AFB_1 from contaminated foods. Accordingly, AFB_1 plays an etiologic role in 5-28% of all HCC cases globally (Liu and Wu

(2010). Moreover, emerging reports have linked AFB_1 with liver cirrhosis, which shares several common causative factors with HCC, including chronic infection with HBV and/or Hepatitis C virus (HCV), excess alcohol intake, and non-alcoholic steatohepatitis/fatty liver disease (Kuniholm *et al*., 2008; Chu *et al.*, 2017; Alvarez *et al*., 2020a). Liver cirrhosis, known to be a well-established risk factor for HCC, represents a late stage of liver fibrosis that is progressive and characterized by the formation of regenerating nodules and hepatic architecture deformation (Zuñiga-Aguilar and Ramírez-Fernández, 2022).

The primary route of AFB_1 exposure is through direct consumption of contaminated food (Alameri *et al.*, 2023). AFB_1 exposure can begin in utero through trans-placental exposure and continue throughout the life course (Gong *et al*., 2016; Smith *et al*., 2022). Once absorbed, AFB_1 undergoes metabolic activation mainly by cytochrome P450 (CYP) 3A4, followed by CYP3A5 and CYP1A2, to form reactive and electrophilic AFB_1-exo-8,9-epoxide (AFBO), which can result in the formation of DNA adducts at the N7 position of Guanine, as reviewed by Lootens *et al*. (2023). In order to prevent the formation of DNA adducts, detoxification of this highly genotoxic intermediate (AFBO) was deemed critical. As shown in Figure 1, the three potential mechanisms of detoxification for AFBO are conjugation with glutathione, hydrolysis to dihydrodiol, and reduction to dialcohol by the enzymes Glutathione S-Transferase (GST), microsomal epoxide hydrolase (mEH), and AFB_1-aldehyde reductase (AFAR), respectively (Murcia and Diaz, 2021; Wang *et al*., 2022; Lootens *et al*., 2023). Conjugation of AFBO with gluthatione by GST enzymes is considered a major detoxification mechanism in animals (Lootens *et al*., 2023).

It is possible that genetic variations in metabolizing enzymes may impact an individual's susceptibility to the toxic or carcinogenic effects of AFB_1. This is because the rate and extent of AFB_1's metabolic activation and detoxification can vary based on the activity of these enzymes (Eaton *et al*., 2001; Song *et al.*,

2012). In this regard, the GST genes, coding the principal enzyme involved in the detoxification of activated AFB_1, are highly polymorphic (Wang *et al*., 2022; Lootens *et al*., 2023).

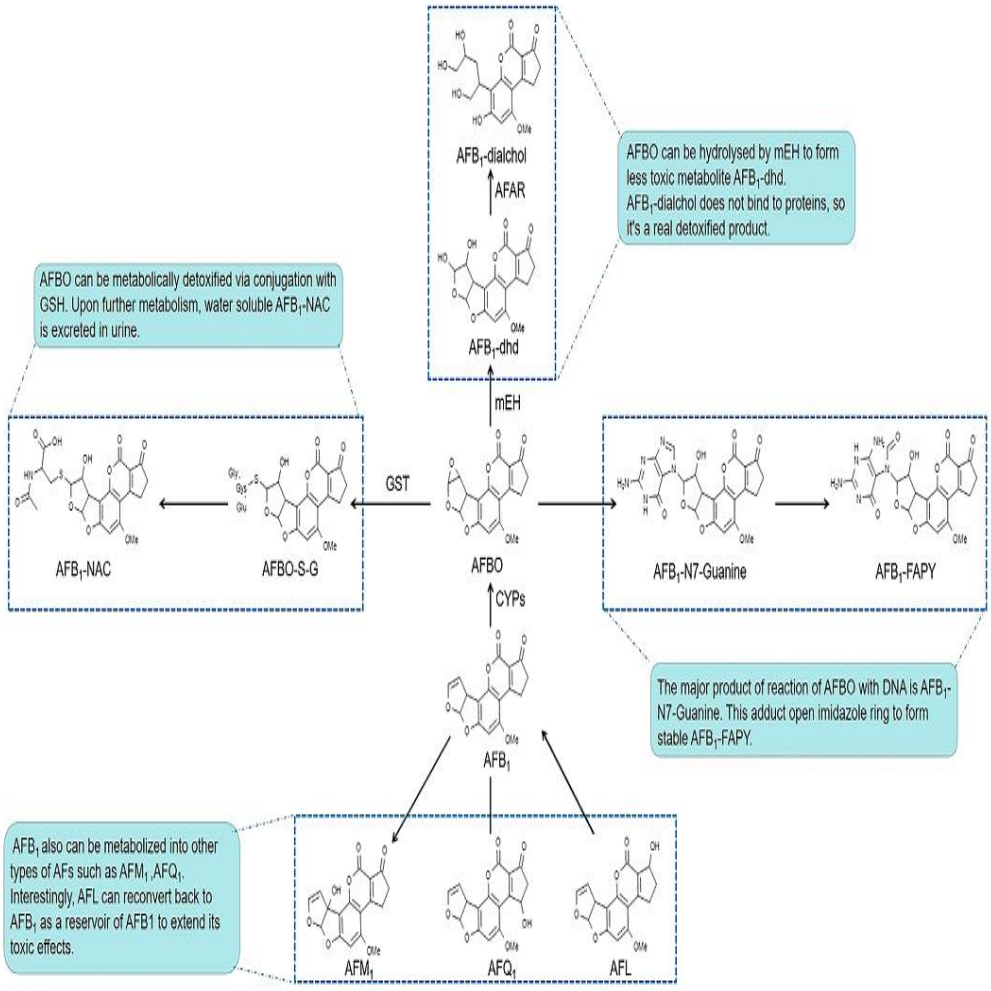

Figure 1: The bioactivation and related detoxification pathways of aflatoxin B_1 (AFB_1).

AFAR, aflatoxin aldehyde reductase; AFBO-S-G, AFB_1-exo-8,9-epoxide-gluthatione conjugate; AFB_1-FAPY, AFB_1-formamidopyrimidine; AFB_1-NAC, AFB_1-N-acetyl cysteine; CYP, Cytochrome P450, GSH, gluthatione; GST, glutathione *S*-transferase; mEH, microsomal epoxide hydrolase (Hua *et al*., 2021).

Human-soluble GSTs are encoded by 16 genes, which are further grouped into 7 classes based on sequence similarity: alpha (*GSTA*), mu (*GSTM*), pi (*GSTP*), theta (*GSTT*), omega (*GSTO*), sigma (*GSTS*), and zeta (*GSTZ*) (Mannervik *et al*., 2005; Mazari *et al*., 2023). A variety of polymorphisms have been

discovered within each GST genes, primarily due to single nucleotide alterations that result in the occurrence of various allelic forms (Polimanti *et al*., 2011). However, copy number variations (CNVs) due to insertion deletion polymorphism have been shown in the two GST enzymes known to be critical in AFB1 detoxification, namely, GSTM1 and GSTT1, expressed from the *GSTM1* and *GSTT1* genes, respectively (Rose-Zerilli *et al*., 2009; Nørskov *et al*., 2009). Hence, null genotypes (homozygous deletion) of these genes lead to enzyme deficiency and may, consequently, impair detoxification of AFB_1 (Sun *et al*., 2001; Long *et al*., 2005).

Exposure assessment is a critical part of epidemiological studies for identifying the effect of AFB_1 on human health and its level of public health burden. In this aspect, a lack of repair of the DNA adducts caused by AFBO may cause DNA mutations, specifically G–to-T transversions. According to studies, the TP53 tumor suppressor gene, specifically at codon 249 (AGG to AGT, Arginine to Serine, R249S), has been identified as a hotspot for AFB_1-related mutations (Besaratinia *et al*., 2009; Chittmittrapap *et al*., 2013). While mostly generated in liver cells, AFB_1-DNA adducts are also detected in peripheral blood white blood cells (Long *et al*., 2006; Long *et al*., 2015). Likewise, studies reported a significant ecological concordance between AFB_1 exposure, chronic infection with HBV, and the occurrence of R249S in patients with HCC (Kuniholm et al., 2008; Qin-Qin *et al*., 2019; Alvarez *et al*., 2020b). Therefore, the detection of plasma 249serTP53, which may be less sensitive, would be indicative of longer-term exposure to AFB_1 with lower susceptibility to plasma alterations related to illness (Kuniholm *et al*., 2008).

In addition to codon 249serTP53 mutation, there are other biomarkers of exposure in biological fluids such as blood and urine for assessing AFB_1 exposure in humans as they are the tools that integrate exposures from various routes (Routledge *et al*., 2014). For instance, AFB_1-N7-guanine adducts are removed via the urine (Nasir *et al*., 2021); AFM_1, the hydroxylated metabolic

product of AFB_1, is detected in breast milk, and measuring both metabolites in the above-mentioned body fluids reflects exposure over the previous one day (Eshete *et al*., 2021). However, the AFB_1-albumin (AF-alb) adduct, measured in blood, is thought to reflect cumulative exposures over 2–3 months due to the prolonged *in vivo* half-life of albumin in contrast to breast milk or urinary metabolic products (Vivian *et al*., 2018). A competitive enzyme-linked immunosorbent assay (ELISA) has been employed to detect the AF-alb adduct in the blood, and the level is expressed as pg AF-alb per mg albumin (Wild *et al*., 1990). The validity of this assay has been well documented in several population-based studies, and the AF-alb level exhibits a good correlation with AFB_1 intake from food staples (Routledge *et al*., 2014; Xu *et al*., 2018; Kroker-Lobos *et al*., 2019).

In Eastern Ethiopia, there is a high burden of AFB_1 in food staples that are commonly produced and consumed by the people, as well as a significant burden of cryptogenic cases of liver cirrhosis, according to reports from local health personnel and epidemiological studies (Ashenafi *et al*., 2017; Orlien *et al*., 2018a). But there is no study that has investigated the contribution of AFB_1 exposure to the disease, particularly in this area with a high prevalence of cryptogenic cases of liver cirrhosis as well as at the national level in Ethiopia. Hence, the study aimed at evaluating the role of AFB_1 in the etiology of liver cirrhosis in Eastern Ethiopia.

1.2. Statement of the problem

There is no region of the world that is free from the AFB_1 problem; however, global diet variation may lead to heterogeneity in AFB_1-induced disease burden (Liu and Wu, 2010). In addition, the tight food and feed quality regulation programs implemented in developed nations significantly decrease the AFB_1 burden in those countries (Meneely *et al*., 2023). However, this is not the case in Africa and, to a lesser extent, Asia. Africa is the most AFB_1-vulnerable region in the world, in addition to the absence of control of

mycotoxins on the continent due to the typical hot, humid climate, reliance on foods ideal for AFB_1 contamination, and other reasons (Benkerroum, 2020; Alameri *et al*., 2023; Abrehame *et al*., 2023). Furthermore, according to sources, sub-Saharan Africa is highly contaminated with AFB_1, with levels occasionally exceeding the internationally recommended maximum limits; there are different limits set by different authorities (Xu *et al*., 2018; Meijer *et al*., 2021). Regionally, the East African Community (EAC) standardized and established regulatory limits of 10 µg/kg for total AFs (B_1, B_2, G_1, and G_2) and AFB_1 content from the total as 5 µg/kg in all foods except milk and a limit of 0.5 µg/kg for AFM_1 in milk (Boni *et al*., 2021).

In Ethiopia, the problem of AFB_1 contamination in food commodities is much more concerning than commonly imagined (Yenasew, 2019; Mamo *et al*., 2020). Several reports have been made on AFB_1 contamination of grains, including sorghum, maize, wheat, teff, legumes, and groundnuts collected from stores and market areas (Ayalew *et al*., 2006; Ayalew, 2010; Chala *et al*., 2013; Chala *et al*., 2014; Chauhan *et al*., 2016; Taye *et al*., 2016; Assefa, 2018; Mohammed *et al*., 2022a). However, according to several reports Eastern Ethiopia is an area with a diet particularly high in AFB_1. According to previous studies, major staple and cash crops such as sorghum, maize, and groundnuts have been known to be heavily contaminated with AFB_1 (Ayalew *et al*., 2006; Ayalew, 2010; Mohammed *et al*., 2022a). Accordingly, AFB_1 analysis conducted on fresh or stored sorghum samples as well as sorghum injera samples collected from this area showed an AFB_1 concentration ranging from 10 to 53.3 µg/kg (Yenasew, 2019; Marie, 2017). Furthermore, studies reported by Chala *et al*. (2013) and Taye *et al*. (2016) showed that total AFs levels in groundnuts samples ranged between 15 µg/kg and 11,900 µg/kg. Despite this heavy contamination of dietary products by AFB_1 in food staples, there is no previous study that investigated the role of exposure to AFB_1 as a cause of liver cirrhosis using biological markers, generally in Ethiopia and specifically in Eastern Ethiopia with a high burden of cryptogenic cases of liver cirrhosis.

To this end, this study aimed at elucidating the role of AFB_1 in the etiology of liver cirrhosis in Eastern Ethiopia.

1.3. Objectives

1.3.1. General objective

The aim of this study was to evaluate the role of AFB_1 in the etiology of liver cirrhosis among patients attending Hiwot Fana Comprehensive Specialized University Hospital (HFCSUH), Harar, Eastern Ethiopia from January 1st 2020 to July 31st 2021.

1.3.2. Specific objectives

- To assess predictors of liver cirrhosis of unknown etiology among liver cirrhosis patients attending HFCSUH, Harar, Eastern Ethiopia;
- To determine the association between AFB_1 exposure and liver cirrhosis among patients attending HFCSUH, Harar, Eastern Ethiopia;
- To investigate the association between polymorphisim in AFB_1 metabolizing enzymes and liver cirrhosis among patients attending HFCSUH, Harar, Eastern Ethiopia.

1.4. Research questions

- What are the predictors of liver cirrhosis of unknown etiology in Eastern Ethiopia?
- Is AFB_1 involved in the etiology of liver cirrhosis in Eastern Ethiopia?
- Is there any association between polymorphisim in AFB_1 metabolizing enzymes and liver cirrhosis in Eastern Ethiopia?

1.5. Significance of the study

The data generated from this study provides crucial information on main etiologic factors of liver cirrhosis. In addition, the finding of this study helps in

identifying the role of AFB_1 and associated genetic factors with liver cirrhosis in Eastern Ethiopia. The data will be available to stakeholders in the health sectors, including Oromiya, Harari, Dire Dawa and Somali Reginal Health Bureaus, besides to the Ministry of Health of Ethiopia and the Ethiopian Food Drug Authourity (EFDA), to develop a plan and action that aimed at reducing the number of liver cirrhosis cases in the region. The finding of the current study could also be used by Ministry of Agriculture and its stakeholders to device a plan in controlling AFB_1 contamination in agricultural commodites. The outcome of this study could also help food processing firms of the area and the country to take measures in production of foods free from AFB_1. The result of this study also serves as a basis for setting AFB_1 regulatory limits in the country. Furthermore, there are few epidemiological studies that reported the effect of AFB_1 alone and in association with HBV infection on the development of liver cirrhosis. Thus, this study findings can contribute to expand the knowledge about the detrimental impact of AFB_1 on liver cirrhosis. Moreover, this study established baseline information for other similar researches, conducted in the future.

2. LITERATURE REVIEW

2.1. Exposures to liver cirrhosis

Numerous etiological factors, the prevalence of which varies geographically, have been linked to liver cirrhosis (Liu and Chen, 2022). Accordingly, alcoholic liver disease, HCV infection, and fatty liver disease are the major causes in western countries, whereas chronic HBV infection is the principal etiologic agent in Africa and Asia (Huang *et al*., 2023). Other less common etiologic factors of liver cirrhosis include autoimmune liver disease, Wilson's disease, haemochromatosis and alpha1-antitrypsin deficiency (Pellicelli, 2023).

Despite considerable advancement in our knowledge of the causes of liver cirrhosis, instances worth addressing are cryptogenic, i.e., liver cirrhosis with an unknown cause (Mokdad *et al*., 2014). This is notably true in sub-Saharan Africa, where the etiology is unknown in 31% of the cases, according to the global burden of disease report by Mokdad *et al*. (2014). Consistent with this, a systematic review and meta-analysis revealed alcohol intake, HBV, and HCV infections as the three most common etiologies of liver cirrhosis in Ethiopia (Tesfaye *et al*., 2021). However, the etiology of 45% of the cases remains unknown, ranging from 26% in Jimma (Western Ethiopia) to 55% in Harar (Eastern Ethiopia). In this regard, histopathological studies revealed that these patients had toxic liver damage in Eastern Ethiopia (Orlien *et al*., 2018a).

In addition, Orlien *et al*. (2018b) carried out a hospital-based case-control study in Eastern Ethiopia with the aim of examining the relationship between consumption of khat and hepatic cirrhosis using 150 adult patients of hepatic cirrhosis and 300 healthy individuals. According to this study, there was a strong association between the use of khat and the risk of developing hepatic cirrhosis (OR = 2.6; 95% CI: 1.6, 4.6). However, the study did not account for exposure to dichlorodiphenyltrichloroethane (DDT) and other possible hepatotoxic pesticides through chronic intake of khat leaves. Furthermore, the study did not consider exposure to environmental contaminants such as AFB_1,

despite the fact that its burden is high in the region's dietary staple (Yenasew, 2019; Mamo *et al*., 2020).

2.2. Aflatoxin B_1 exposure and liver cirrhosis

Liver cirrhosis is known to be a predecessor of human HCC, as it arises in a cirrhotic background in majority of the cases (Zanotti *et al*., 2022). In addition, the etiologic factors for HCC have been well studied. HCC is linked to AFB1 exposure in addition to the aforementioned hepatic cirrhosis-causing variables (Liu *et al*., 2012). Although the pathogenesis of hepatic cirrhosis and HCC are distinct, the significant overlap in causal etiologies between liver cirrhosis and HCC suggests that AFB_1 exposure, known to be an established cause of HCC, may also be related to liver cirrhosis (Mekuria *et al*., 2020). Accordingly, emerging studies linked chronic exposure to AFB_1 with increased risk of liver cirrhosis (Kuniholm *et al*., 2008; Chu *et al*., 2017; Alvarez *et al*., 2020a).

A case-control study done by Kuniholm *et al*. (2008) on 97 liver cirrhosis cases and 397 individuals without any liver disease as a control in the Gambia reported that increasing lifetime groundnut intake (a surrogate for AFB_1 consumption) was strongly associated with the risk of liver cirrhosis. Besides, a positive marker of the 249SerTP53 mutation was also markedly associated with liver cirrhosis after adjustment for HBV and HCV exposure, with an OR of 3.8 (95% CI: 1.5, 9.6). The authors also reported evidence of a synergestic interaction between exposure to AFB_1 and HBV that significantly increased the risk of liver cirrhosis (Kuniholm *et al*., 2008).

Additionally, a case-control study nested in a large community-based cohort study has been done by Chu *et al*. (2017) in Taiwan that aimed at examining the influence of AFB_1 exposure on the development of HCC and hepatic cirrhosis in chronic HBV carriers. In this study, serum AF-alb adduct levels were assessed in cases with liver cirrhosis (232), HCC (262), and controls (577) using ELISA. Among all chronic HBV carriers, the time interval between study entry and diagnosis of liver cirrhosis and HCC was significantly

shorter in participants with high (>21.5 fmol/mg) AF-alb adducts than in those with low (<21.5 fmol/mg) or undetectable levels. Moreover, there was a marked dose-response relationship between AF-alb adduct and the risk of HCC in patients with iver cirrhosis. The author's conclusion is that exposure to AFB_1 increases the risk of liver cirrhosis and HCC in a dose-response fashion in patients with chronic HBV infection (Chu *et al*., 2017).

Moreover, a case-control study done in Guatemala among 100 liver cirrhosis cases and 200 controls reported a marked positive relation between AFB_1 exposure and hepatic cirrhosis. In the study, the median AF-alb adduct concentration was substantially higher in cases (11.4 pg/mg) than in controls (5.1 pg/mg). Moreover, higher serum concentrations were linked with hepatic cirrhosis (Alvarez *et al*., 2020a). The potential toxic effects of AFB_1 on the liver are connected to AFBO, which is produced by CYP enzymes, and the subsequent generation of reactive oxygen species (ROS). This in turn results in oxidative stress, inflammation, and apoptosis (Mekuria *et al*., 2020).

AFB_1 exposure disturbs the equilibrium of oxidants and antioxidants in the liver, resulting in damage to biomolecules such as DNA, proteins, and lipids (Sui *et al*., 2022). This was supported by studies that proved the role of antioxidants in minimizing the risk of hepatotoxicity caused by AFB_1 exposure (Guo *et al*., 2022; Dai *et al*., 2022; Cheng *et al*., 2023). Also, ROS buildup causes apoptosis by lowering mitochondrial membrane potential and activating cytochrome C, which is known to affect antiapoptotic Bcl2 and proapoptotic Bax expression, followed by stimulation of caspase 9 and caspase 3, leading to cell death (Dai *et al*., 2022; Cheng *et al*., 2023). Moreover, studies demonstrated that oxidative stress has a crucial role in the activation of the nuclear factor kappa B (NF-κB) signaling pathway, known to play a critical role in inflammation (Lingappan, 2018; Mahdiani *et al*., 2022). In support of this, several *in vivo* studies revealed that AFB_1 induced inflammatory responses and liver damage via upregulation of the NF-κB pathway that leads to the generation of inflammatory cytokines, including tissue necrosis factor

alpha (TNF-α), interleukin 1 beta and six (IL-1β and IL-6) (Ma *et al*., 2021; Guo *et al*., 2022; Cheng *et al*., 2023). Furthermore, the anti-inflammatory *IL-10* gene, which is associated with liver function, was markedly downregulated following exposure to AFB_1 (Sui *et al*., 2022). Hence, these changes in inflammatory factor expression levels in liver tissue suggest that AFB_1 exposure induces inflammatory damage to the liver. Still, further reports are necessary before any conclusions can be drawn about the mechanisms of AFB_1-induced liver cirrhosis. This is an area that merits more attention, given the global burden of liver cirrhosis and the prospect of an increase in the spread of AFB_1-producing fungus in previously inhospitable regions, including Europe, as a result of climate change (Leggieri *et al*., 2021).

2.3. Genetic polymorphisim in AFB_1 metabolising enzymes and liver cirrhosis

The activity of enzymes in the xenobiotic detoxification system is thought to be the primary determinant of hepatocyte sensitivity to environmental exposure, including AFB_1 (Gu and Manautou, 2012). The genes that encode the principal AFB_1 detoxifying enzymes, *GSTM1* and *GSTT1,* differ greatly among populations. According to studies, the null genotype of *GSTM1* was more prevalent in Asians, Caucasians, and Arabs compared to Africans, with a frequency of 0.23 to 0.66, 0.45 to 0.57, 0.50 to 0.63, and 0.11 to 0.44, respectively (Alshagga *et al*., 2011; Piacentini *et al*., 2011; Salem *et al*., 2011; Mansour *et al*., 2015; Nakanishi *et al*., 2022). However, the null genotype of *GSTT1* was reported to be more common in Asians, Africans, and Arabs in comparison to Caucasians, with a frequency of 0.20 to 0.47, 0.20 to 0.37, and 0.14 to 0.28, respectively (Alshagga *et al*., 2011; Piacentini *et al*., 2011; Salem *et al*., 2011; Mansour *et al*., 2015; Nakanishi *et al*., 2022).

On the other hand, double null genotypes of both *GSTM1* and *GSTT1* were observed at a frequency of 0.27, 0.21, and 0.15 among Asians, Arabs, and Africans, respectively (Guo *et al*., 2008; Salem *et al*., 2011; Kiendrebeogo *et*

al., 2019). Moreover, the present assays can precisely determine the *GSTM1* and *GSTT1* copies, and either of the gene duplications has been detected at a significantly higher percentage among Caribbean populations originated from Africa (3.4%) compared to Caucasians (0.2%) (Nørskov *et al*., 2011; Emeville *et al*., 2014). In this regard, a correlation has been reported between gene copy number and enzymatic activity (Sprenger *et al*., 2000; Girault *et al*., 2005). Hence, investigation of the exact copies of the genes would best describe any association with the risk of disease.

Functionally defective GST genes have been linked to the pathogenesis of a variety of diseases (McIlwain *et al*., 2006). Genetic variations in *GSTM1* and *GSTT1* have been shown to enhance human sensitivity to hepatitis of various etiologies and liver cirrhosis (Baclig *et al*., 2012; Ibrahim *et al*., 2016; Kapahtia *et al*., 2018). Early studies have also reported that polymorphisms in *GSTM1* and *GSTT1* are possible factors in individual vulnerability to AFB_1-induced HCC (McGlynn *et al*., 1995; Chen *et al*., 1996). Moreover, a study conducted in Taiwan aimed at investigating the effect of variations in *GSTM1* and *GSTT1* genes on AFB_1-induced HCC in patients with chronic HBV infections reported that the effect of AFB_1 exposure was increased among *GSTT1* null genotypes (OR = 3.7; 95% CI: 1.5, 9.3) (Sun *et al*., 2001). Besides, the interaction between AF-alb adduct levels and *GSTT1* genotypes was also significant (p<0.05) (Sun *et al*., 2001).

In a study that compared 216 incident HCC cases with 408 frequency-matched controls, the *GSTM1*-null genotype (OR = 2.45; 95% CI: 1.2, 5.0) was strongly related to HCC. In the same study, the risk for HCC was most prominent among those with the highest level of groundnut consumption with the *GSTM1* null genotype (OR = 4.7; 95% CI: 1.5, 15.1) (Kirk *et al*., 2005). Taken together, both null allels of *GSTT1* and *GSTM1* are linked with increased risk of AFB_1 related HCC. However, there role in AFB_1 induced liver cirrhosis have not yet been investigatedAdditionally, investigations conducted so far on HCC have not been able to distinguish between carriers of the functional

alleles of *GSTM1* and *GSTT1* who are heterozygous from those who are homozygous. The classification of such genotypes as carriers or null indicates, i.e., one or more copies vs. zero copies, that might not accurately represent the underlying genetic risk (Emeville *et al*., 2014). Determining the precise CNVs of the genes is therefore necessary to further elucidate the potential interaction between these genes and the risk of liver cirrhosis.

3. MATERIALS AND METHODS

3.1. Study setting and design

A case-control study was carried out between January 1, 2020 and July 31, 2021, at HFCSUH, in Harar, Eastern Ethiopia. Nearly, six million people who residing in Harari, and Somali Regional States, Eastern Oromia, and Dire Dawa City Administration are served by the hospital, which is affiliated with Haramaya University. The hospital has a bed capacity of 238 and provides service to more than 154,196 patients annually (Mohammed *et al*., 2022b). The hospital has various units, through which it provides a number of general and specialized clinical services. One of the units is the Internal medicine unit, which has a bed capacity of 33 and admits on average 5 patients per day. The unit comprises more than 35 medical staff including medical specialists, residents, and nurses.

3.2. Study population and eligibility

From all clients who visited the hospital's Internal Medicine unit during the study period, patients diagnosed with liver cirrhosis by abdominal imaging were identified as cases. Control patients were those who attended the same unit but did not have a history of liver disease or any clinical indications for it. In cases, patients aged ≥18 years old and having a score of ≥7 based on an ultrasound-based cirrhosis scale were included, whilst those with evidence of space-occupying hepatic focal lesions, and unable or unwilling to provide informed consent were excluded. For controls, patients aged ≥18 years old and having ultrasound scores of ≤4 were included, while those with elevated liver function tests (ALT or AST above 40 IU/L) and unable or unwilling to provide informed consent were excluded.

3.3. Sample size determination

Sample size was calculated using Epi-info 7 software for unmatched case-control studies. Sample size estimation was performed using double population proportions formula based on the inclusion of 2 controls per 1 case, and the conventional type I error of 5%, power of 80%, and assuming minimum OR detected was 2.0 and 50% of AFB_1 exposure in the control groups. The minimum total sample size required considering 10% for methodological non-contingency was 368 (123 cases and 245 controls).

3.4. Sampling techinique and procedure

Cases were recruited using consecutive sampling technique, i.e. those patients who met the criteria of inclusion were enrolled until the required sample size was achieved during the study period. Regarding controls selection, individuals without suspected liver disease were recruited from the same department of the hospital using simple random sampling techniques based on the list of individuals triaged to the department as a sampling frame. Ratios of controls to cases were 2:1.

3.5. Data collection and managment

3.5.1. Data collection Instrument

A structured questionnaire developed based on the literature was employed to collect data on sociodemographic characteristics, alcohol consumption, khat chewing, tobacco usage habits, body mass index (BMI), and dietary history, including peanut intake and staple foods. Moreover, food consumption in the past year was collected in a food frequency questionnaire (FFQ) (Appendix D). The questionnaire's original English form was translated by multilingual experts first into the indigenous languages (Afaan-Oromo and Amharic) and then back into English to ensure consistency.

3.5.2. Clinical examination

The attending physician and radiologist did clinical and ultrasound examinations, respectively. Abdominal ultrasonography was performed using a mobile digital color Doppler ultrasound machine (Model: SSI-8000, SonoScape Co. Ltd.) in accordance with a predefined standard (Appendix E). A cirrhosis scale developed by Lin *et al*. (1993) and validated by Shen *et al*. (2006) using ultrasonography was used to diagnose liver cirrhosis. The components of the scoring system are the intrahepatic vascular type (score 1-3), liver parenchyma (score 1-3), liver surface (score 1-3), and spleen size index (score 1-2; 1 for a spleen size index of 20 cm^2 and 2 for a bigger spleen size index). A score of 7 out of 11 on this scale shows the existence of liver cirrhosis, with a normal liver receiving a maximum score of 4.

3.6. Blood sample collection and analysis

A blood sample was drawn via venipuncture using the BD Vacutainer® blood collection system (Becton Dickinson, NJ, USA), and collected in a serum separator tube (3-5 mL) and two EDTA tubes (3 mL and 2 mL). The serum was then used for liver function tests (LFTs), Hepatitis B surface antigen (HBsAg), HCV antibody tests, and serum creatinine tests. The blood collected on the first EDTA tube was used for platelet count (2 mL) and the remaining whole blood was stored at -80°C for genetic studies. Meanwhile, the 3 mL blood collected in the 2^{nd} EDTA tube was centrifuged at 1100-1300 g for 10 min at 4 °C and the plasma was separated for AF-alb analysis. The whole blood and plasma were stored at -80°C using a cryotubes until transport to Armauer Hansen Research Institute (AHRI), Addis Ababa, Ethiopia and the University of Leeds, United Kingdom, respectivelly. A sufficient amount of dry ice was used during the transport and repeated freezing and thawing was also avoided.

3.6.1. Clinical laboratory tests

For screening HBV and HCV, an advanced-quality one-step HBsAg test kit (InTec Products, Inc.) and a one-step HCV test kit (Guangzhou Wondfo Biotech Co. Ltd.) were used, respectively. Clinical chemistry parameters, including aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), serum bilirubin (total and direct), albumin, and serum creatinine tests, were measured using a fully automated and closed clinical chemistry analyzer instrument (Roche/Hitachi Cobas C-311, Roche Diagnostics GmbH, Mannheim, Germany). In addition, platlet counts were conducted using a DxH 800 hematology analyzer (Beckman Coulter, Germany). Abnormal laboratory findings are defined as test results outside of the following ranges: ALT (8–40 IU/L), AST (14–40 IU/L), ALP (48–164 IU/L), albumin (35–52 g/L), bilirubin direct (0.4–8.8 μmol/L), bilirubin total (3–38 μmol/L), creatinine (47–109 μmol/L), platelet count (126–438 × 10^9/L) (Karita *et al*., 2009).

3.6.2. Aflatoxin B_1-albumin (AF-alb) adduct analysis

Plasma levels of AF-alb were measured using an established ELISA method in the mycotoxin laboratory at the School of Food Science and Nutrition, University of Leeds. The methods include albumin extraction, enzyme digestion, Sep-Pak C18 column purification, and finally quantification of the adduct using a competitive ELISA method as previously described (Chapot and Wild, 1991). Samples were measured in duplicate on two separate days and measurements with a coefficient of variations of less than 25% were taken. For quality control, each batch of plasma samples was examined together with three positive and one negative control sample. Additionally, 10% of the entire sample was randomly chosen, and the entire procedure was repeated to guarantee the test's repeatability. The limit of detection (LOD) was 3 pg AFB_1-lysine equivalents per mg of albumin (pg/mg).

3.6.3. DNA extraction

PureLink™ Genomic DNA Mini Kit (Invitrogen) was used to isolate genomic DNA based on the company's recommendation. NanoDrop 2000 spectrophotometers (ThermoFisher Scientific, USA) were used to determine the quality and quantity of extracted DNA. Besides, agarose gel electrophoresis with the help of Gel Doc (Bio-Rad) was run on 10% of the samples to make sure the DNA eluted was of good quality. Prior to CNV analysis, the extracted genomic DNA samples were kept at -20°C.

3.6.4. *GSTM1* and *GSTT1* copy number analysis

Bio-Rad CFX96 Touch Real-Time System were used for *GSTM1* and *GSTT1* copy number analysis. The final reaction mixture (10 uL) consisted of FAM-labeled 20x TaqMan^{TM} Copy Number determination kit (AB Applied Biosystems) for *GSTM1* (Hs02595872_cn) or *GSTT1* (Hs01731033_cn), VIC-labeled 20X TaqMan^{TM} Reference determination kit (AB Applied Biosystems: 4403326), TaqPath^{TM} ProAmp^{TM} Master Mix (Applied Biosystems), nuclease-free water and 10 ng genomic DNA template. Thermocycling was initiated at 95°C for 10 min, ensued by 40 cycles of 15 sec of denaturation at 95°C, and 60-sec annealing-extension at 60°C.

Each target assay was performed in the same PCR as RNase P to assure quality of the analysis. The amplification of RNaseP and GST genes in the same sample adjusted DNA concentration variations between samples and guaranteed that no false-negative GST*0/0 genotypes were resulted due to PCR or pipetting error or insufficent DNA concentrations in the original sample. The genotyping was performed blind to the sample's case or control status. The samples were run in duplicate. Besides, each run contained a no-template control to preclude any contamination. Copy number estimation was done by the ΔCt method as follows: CT target gene= $2x2^{-\Delta\Delta CT}$, where ΔΔCT= ΔCtGST sample – ΔCtRNase P and ΔCt was the average Ct value of 2 repeated measurements of the sample. The CT value was the relative copy

number of *GSTM1* and *GSTT1* genes and RNase P of internal reference control (Zhang *et al*., 2023).

3.7. Statistical analysis

In this study, data analysis were carried out using Statistical Package for Social Sciences (SPSS) version 26.0. When it came to categorical variables, percentages were utilized, and all continuous variables were considered to have a non-normal distribution and were expressed as the median (inter quartile range (IQR)). To examine differences in the characteristics between the groups of interest, the Mann-Whitney U or Kruskal Wallis test was used for continuous variables, and $\chi 2$ or Fisher exact tests for categorical variables. The Hosmer and Lemeshow test was used to assess model fitness to the data, and the variance inflation factor was used to explore multi-collinearity. Both biologically significant variables, such as age and gender, as well as variables with a *p*-value < 0.25 in the univariate analysis, were fitted to the logistic regression model in papers I and II. In the final logistic regression model, odds ratios (ORs) and 95% confidence intervals (CIs) were computed.

In paper III, logistic regression was also used to estimate the association of *GSTM1* and *GSTT1* copy numbers with the risk of liver cirrhosis. ORs were computed for *GSTM1* and *GSTT1* genotypes after adjustment for age and gender, comparing groups defined by the gene copy number coded as a categorical variable (0, 1, or $\geq$2 copies), null, and carriers (1, 2, or more copies). A linear regression model was used to examine the association between *GSTM1* and *GSTT1* CNVs and blood chemistry parameters. The log-transferred data were utilized for the analysis since the Shapiro-Wilk test indicated that the clinical chemistry data had a non-normal distribution. The regression coefficient (β) along with the CI were estimated after adjustment for age and gender. In the study the level of significance was set at $p < 0.05$.

3.8. Operational definitions

Alcohol consumption: The current and past history of alcohol intake was collected and quantified in grams. A daily alcohol intake of more than 30 grams for males and more than 20 grams for females for a minimum of six months was considered alcohol abuse (Hagström, 2017). The average alcohol concentration by volume of common alcoholic drinks found in Ethiopia including Tella, Areke, wine, hard liquor, and beer were 6.4%, 11.5%, 37.2%, 11%, 40%, and 5%, respectively (Negash and Demissie, 2021) and used for calculating consumption using the following formula:

Average Daily intake (gm) = Alcohol conc by volume * 0.78 * volume consumed (mL)/100

Where, 0.78 is the specific gravity of alcohol

Khat use: The amount of khat, a herb chewed for its amphetamine-like effect, consumed was measured in grams using a visual analog scale (Appendix D). During a typical session, one chews 100–300 g of fresh khat leaves. Hence, khat use was referred to as having used a minimum of 200 g of khat daily for at least a year (Orlien *et al*., 2018a).

Tobacco use: Data regarding tobacco use in the past and present was gathered, and ciggarete and pipe smoking were defined as having smoked at least 100 cigarettes and 50 pipes, respectively, in one's lifetime. The definition of shisha smoking was once a week for a minimum of a year. The use of smokeless tobacco was defined as chewing tobacco for at least a year. Thus, tobacco user was referred to as having used any of the above-listed tobacco products (Heikkinen *et al*., 2008).

Body mass index (BMI): It was expressed in terms of kg/m2 and calculated as the body mass (weight) divided by the square of the body height, and then

classified as underweight (<18.5), normal (18.5–24.9), overweight (25.0–29.9), and obese (≥30.0) (Hidese *et al*., 2022).

Peanut consumption: Participants were asked about how often they took peanuts in the past year. The selected responses were: "(1) Never", "(2) seldom", "(3)1–3 times per month", "(4) 1-2 times per week", "(5) 3–4 times per week", "(6) once a day". We then reclassify the frequency of peanut intake into 3 groups: the "non-intake group (previous group 1)", the "intermittent intake group (groups 2–5)", and the "frequent intake group (groups 6)". As a result, the non-intake, intermittent intake, and regular intake groups were created from subjects who ate peanuts infrequently, 0.25 to 4 times per week, and at least once per day, respectively (Jung *et al*., 2020).

AF-alb adduct: The cutoff for low (<8.6 pg/mg) and high (≥8.6 pg/mg) amounts of AF-alb adduct was set at 8.6 (4.7-21.9) pg/mg of albumin, which was the median (IQR) detectable adduct levels among the study population.

Etiologic classification of liver cirrhosis patients: After screening patients for the presence of the principal etiologies of liver cirrhosis in Ethiopia, namely, HBV, HCV, and alcohol abuse, those patients whose etiologic factor was not related to one of those mentioned etiologies were categorized as "patients with an unknown etiology of liver cirrhosis", and vice versa (Tesfaye *et al*., 2021).

3.9. Ethical considerations

In accordance with the Helsinki Declaration, this study was carried out. The Institutional Review Board of Addis Ababa University's College of Health Sciences (Protocol No. 064/19/SOP and Reference No. CHS/RTTD/229/2020) and the National Ethical Review Committee of the former Ministry of Science and Higher Education (Reference No. 04/246/680/21) both granted ethical approval. Written consent was obtained after participants were informed about the goals, advantages, and risks of the study. Confidentiality and anonymity

were ensured by limiting data access and removing the identifiers of study participants (Appendix A).

4. RESULTS

4.1. Study participants recuritment process

Initially, 163 and 287 patients with probable and no signs of liver disease were identified as potential cases and controls, respectively. After excluding 32 (from the case group) and 28 (from the control group) patients who did not fulfill the inclusion criteria, 131 cases and 258 controls were enrolled in the study. From the case group, 4 patients were further excluded, as 3 of them refused to provide blood and it was impossible to draw blood from one patient. Whereas, from the control group, 5 patients were further excluded as they refused to provide blood. Hence, data for 127 cases and 253 controls were used for analysis (Figure 2).

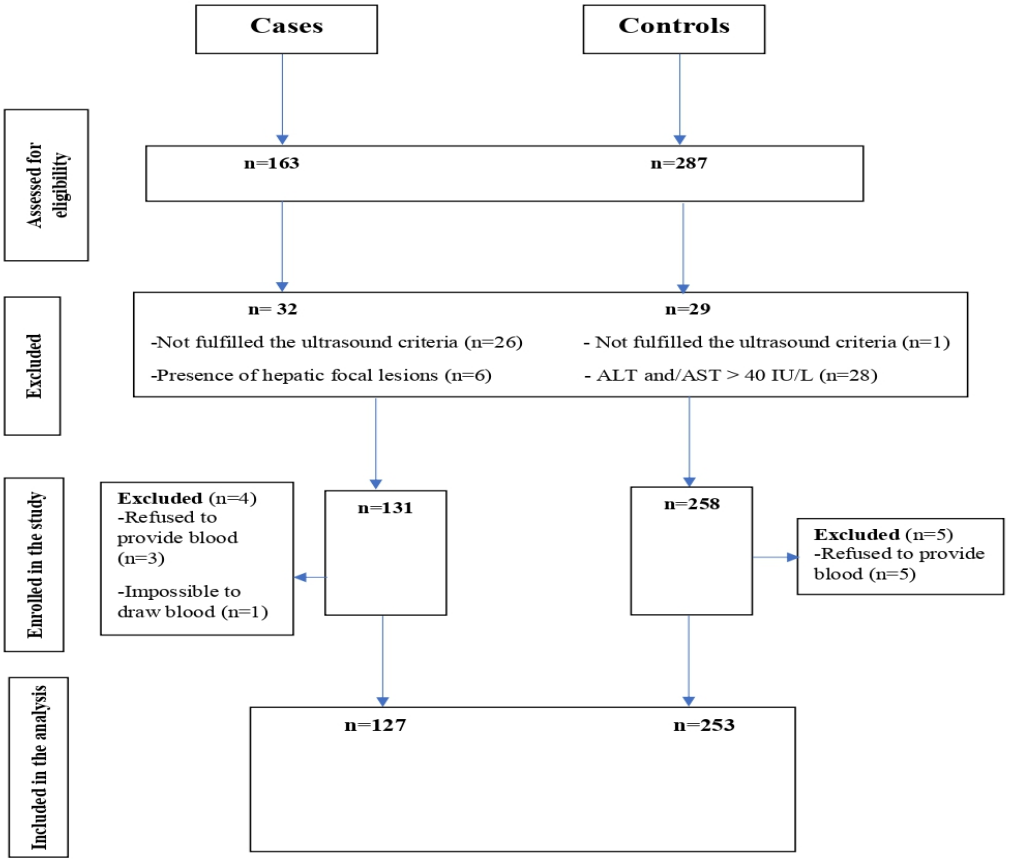

Figure 2: Recruitment process of liver cirrhosis cases and controls, Eastern Ethiopia, 2020/21

4.2. Characteristics of the study participants

The cases and controls included in the study had a median age of 32 years (28–45) and 35 years (25–50), respectively. Certain characteristics of the participants included in this study are shown in Table 1 below.

Table 1: Selected characterstics of study participants, Eastern Ethiopia, 2020/21

Variable	Category	Paper I			Paper II			Paper III		
		Unknown	Known	*p*-value*	Cases	Controls	*p*-value*	Cases	Controls	*p*-value*
Gender	Male	63 (64)	21 (72)	>0.05	84 (66)	144 (57)	>0.05	71 (67)	54 (52)	<0.05
	Female	35 (36)	8 (28)		43 (34)	109 (43)		35 (33)	50 (48)	
Age	<35	51 (52)	14 (48)	>0.05	65 (51)	125 (49)	>0.05	54 (51)	52 (49)	>0.05
	35-44	19 (20)	4 (14)		23 (18)	48 (19)		17 (16)	22 (21)	
	45-54	13 (13)	5 (17)		18 (14)	32 (13)		16 (15)	8 (8)	
	55 and above	15 (15)	6 (21)		21 (17)	48 (19)		19 (18)	22 (21)	
Residence	Urban	14 (14)	6 (21)	>0.05	20 (16)	78 (31)	<0.001	17 (16)	32 (31)	<0.05
	Rural	84 (86)	23 (79)		107 (84)	175 (69)		89 (84)	72 (69)	
Education	No formal education	78 (80)	24 (83)	>0.05	102 (80)	140 (55)	<0.001	86 (81)	55 (53)	<0.001
	Formal education	20 (20)	5 (17)		25 (20)	113 (45)		20 (19)	49 (47)	
Occupation	Not farmer	17 (17)	6 (21)	>0.05	23 (18)	120 (47)	<0.001	20 (19)	49 (47)	<0.001
	Farmer	81 (83)	23 (79)		104 (82)	133 (53)		86 (81)	55 (53)	
Family history of liver disease	No	90 (92)	21 (72)	<0.001	111 (87)	245 (97)	<0.001	95 (90)	100 (96)	> 0.05
	Yes	8 (8)	8 (28)		16 (13)	8 (3)		11 (10)	4 (4)	
HBV positive	No	98 (100)	2 (7)	NA	100 (79)	239 (94.5)	<0.001	84 (79)	96 (92)	<0.05
	Yes	0 (0)	27 (93)		27 (21)	14 (5.5)		22 (21)	8 (8)	
Staple Food	Rice/Teff/pasta/corn	14 (14)	11 (38)	<0.001	25 (20)	106 (42)	<0.001	19 (18)	43 (41)	<0.001
	Sorghum	84 (86)	18 (62)		102 (80)	147 (58)		87 (82)	61 (59)	

* *p*-values from chi-square or Fisher exact test. HBV, Hepatities B virus; NA, not applicable.

4.3. Predictors of unknown etiology of liver cirrhosis

Cases with a family history of liver disease had 76% (AOR = 0.24; 95% CI: 0.06, 0.91) lower odds of developing liver cirrhosis with an unknown cause than those who did not. Patients who relied on sorghum as a staple food were 3.8 times (AOR = 3.8; 95% CI: 1.2, 12.5) more likely to be diagnosed with liver cirrhosis of unknown etiology. The odds of having liver cirrhosis with an unknown cause were also 4.0 times (AOR = 4.0; 95% CI: 1.1, 14.4) higher in splenomegaly patients than in people without the condition (Table 4, paper I).

4.4. Aflatoxin B_1 exposure and liver cirrhosis

Detectable AF-alb adducts were found in 257 (68%) of the 380 total samples, with a median (IQR) level of 8.6 (4.7–21.9) pg/mg of albumin. As shown in Figure 1(Paper II), the median AF-alb adducts of cases were significantly higher than controls [11 pg/mg (5.5–25) vs. 7.0 pg/mg (4.3–20.5)]. The AF-alb adducts detectable rate in case is higher than control group (75% vs 64%, $p<0.05$) with levels ranged from 3.04 to 307.33 pg/mg in cases and from 3.02 pg/mg to 326.87 pg/mg in controls (Table 2).

Table 2: Geometric mean (95% confidence interval) and range of aflatoxin B_1-albumin adduct level (pg/mg) among the study participants, Eastern Ethiopia, 2020/21

	N	Geometric mean (CI)	Range (min-max)
Cases	95	13.22 (10.57, 16.53)	3.04-307.33
Controls	162	10.34 (8.75, 12.21)	3.02-326.87
Total	257	11.32 (9.90, 12.94)	3.02-326.87

CI, confidence interval

4.4.1. Aflatoxin B_1 level and sociodemographic factors

The link between the levels of AFB_1 and sociodemographic factors, considering the total study population as well as based on their status, i.e., cases and controls, was investigated. Accordingly, the median adduct concentration in farmers was substantially higher ($p<0.001$) than in non-farmers considering the total study population [10 pg/mg (5.3–29.4) *vs.* 6.7 pg/mg (4.3–14.7)] (Table S1, Paper II). Moreover, the median adduct concentration was markedly higher ($p<0.001$) in participants who did not attend a formal education compared with those who did [9.9 pg/mg (5.3–28.9) *vs.* 6.2 pg/mg (4.2–14.0)]. When stratified by case and control group,

a significantly higher (p<0.05) median AF-alb adduct was observed in control subjects without formal education than in controls with formal education [9.2 pg/mg (4.5–31.8) *vs.* 5.9 pg/mg (4.2–12.7)], but no significant difference was found in the case group (Table S1, Paper II). Furthermore, the median AF-alb adduct level was significantly lower (p<0.05) in participants who were from urban than rural areas [6.2 pg/mg (3.9–13.1) *vs.* 9.5 pg/mg (5.1–27.1)]. Similarly, when stratified by case and control group, a significantly lower (p<0.05*)* median AF-alb adduct was detected in control subjects who resided in urban areas than in controls from rural areas [5.6 pg/mg (3.9–12.7) *vs.* 7.9 pg/mg (4.7–27.1)], but no significant difference was found in the case group (Table S1, Paper II).

4.4.2. Contribution of AFB_1 exposure to liver cirrhosis

According to multivariable analysis, participants who were exposed to a high (≥8.6 pg/mg) level of AF-alb adduct had a 2-fold increased risk of developing liver cirrhosis in comparison to those whose levels were undetectable (AOR=2.0; 95% CI: 1.1, 3.7). Patients with HBV seropositivity had four-time greater odds of developing hepatic cirrhosis than those without HBV (AOR = 4.0; 95% CI: 1.9, 8.8). Patients aged 55 years and older had a 60% lower (AOR = 0.4; 95% CI: 0.2, 0.8) risk of developing hepatic cirrhosis than those under the age of 35. The odds of developing hepatic cirrhosis were three times higher (AOR = 3.0; 95% CI: 1.5, 6.0) in individuals who were farmers by occupation in contrast to those who were not farmers. Those who had a family history of hepatic disease had 2.9 times greater (AOR = 2.9; 95% CI: 1.1, 7.9) odds of developing hepatic cirrhosis than those without a family history (Table 4, Paper II).

4.5. Copy number variation in *GSTM1* and *GSTT1* genes

Both *GSTM1* and *GSTT1* null alleles were more frequent in cases (0.45; 95% CI: 0.36, 0.55 and 0.40; 95% CI: 0.30, 0.50, respectively) than controls (0.33; 95% CI: 0.24, 0.43 and 0.25; 95% CI: 0.17, 0.34, respectively). The overall frequency of *GSTM1* and *GSTT1* null alleles were 0.39 (95% CI: 0.32, 0.46) and 0.32 (95% CI: 0.26, 0.39) in the study population, respectively. In comparison to controls (11%; 95% CI: 0.05, 0.18), cases (16%; 95% CI: 0.10, 0.24) had a comparatively higher percentage of dual null *GSTM1* and *GSTT1* alleles (13%; 95% CI: 0.09, 0.19). Additionally, more than two copies of the *GSTM1* and *GSTT1* genes were found in 1.9% (95% CI: 0.01, 0.05) and 1.4% (95% CI: 0.01, 0.04) of the study population, respectively (Figure 1, Paper III).

4.5.1. *GSTM1* and *GSTT1* genes copy numbers and risk of liver cirhosis

Our study demonstrated that *GSTT1* carriers were far less likely (p<0.05) to develop hepatic cirrhosis than people with null alleles (AOR = 0.47; 95% CI: 0.25, 0.86). Additional investigation showed that individuals with one copy of the *GSTT1* gene had a considerably lower (p<0.05) chance of developing hepatic cirrhosis than individuals with null alleles. Furthermore, compared to patients with a dual null genotype, the probability of developing hepatic cirrhosis was significantly lower (p<0.05) in those patients who had 2 or more copies of combined *GSTM1* and *GSTT1* (AOR = 0.38; 95% CI: 0.16, 0.91). There was also a significant gene-dose relationships were also found (p-trend < 0.001), Table 2 (Paper III).

4.5.2. Risk of liver cirrhosis in relation to AFB1 exposure and *GSTM1* and *GSTT1* copy numbers

To prove the hypothesis that the effect of AFB_1 exposure on liver cirrhosis risk might vary according to *GSTM1* and *GSTT1* copy numbers, we further did a stratified analysis (Table 3-5). Accordingly, as the copy number of *GSTM1* increased (from 0 to 2 or more), AFB_1 exposure resulted in a tendency toward a decrease in the risk of liver cirrhosis from 14.85- to 3.19-fold (Table 3). However, the lowest AOR obtained in those having 2 or more copy numbers of *GSTM1* was not statistically significant (AOR=3.19; 95% CI: 0.65, 15.75). However, the interaction between AFB_1 exposure and *GSTM1* genotype was statistically significant (p=0.04). Furthermore, as shown in Table 3, the risk of developing liver cirrhosis in AFB_1-exposed patients with *GSTM1* null genotype (AOR=14.85; 95% CI: 3.99, 55.14) was significantly higher than patients who were carriers of *GSTM1* genotypes (AOR=4.41; 95% CI: 1.95, 9.97).

Table 3. Risk of liver cirrhosis in relation to AFB_1 exposure, stratified by *GSTM1* gene copy number and genotype, Eastern Ethiopia, 2020/21.

Copy numbers		AFB_1 unexposed	AFB_1 exposed
0	Cases/controls	5/21	43/13
	OR (95%CI) [a]	1.00	14.85 (3.99, 55.14) *
1	Cases/controls	16/32	25/16
	OR (95%CI) [a]	1.00	5.55 (1.97, 15.67) *
≥2	Cases/controls	7/17	10/5
	OR (95%CI) [a]		3.19 (0.65, 15.75)
Null	Cases/controls	5/21	43/13
	OR (95%CI) [a]	1.00	14.85 (3.99, 55.14) *
Carrier	Cases/controls	23/49	35/21
	OR (95%CI) [a]	1.00	4.41 (1.95, 9.97) *
P interaction between genotype and exposure to AFB_1 = 0.04			

*p value < 0.05. [a] adjusted for gender, age, and Hepatitis B virus exposure. AFB1, aflatoxin B1; OR, Odds ratio

Likewise, as shown in Table 4 the risk of developing liver cirrhosis in AFB_1-exposed *GSTT1* carrier patients (AOR=5.13; 95% CI: 2.35, 11.21) was significantly lower than in patients with null genotype (AOR=12.46; 95% CI: 3.30, 47.05). The interaction between AFB_1 exposure and *GSTT1* genotype was statistically significant (p=0.01). On the other hand, as the copy number of *GSTT1* increases from 0 to 2 or more, AFB_1 exposure results in a decreasing tendency of causing liver cirrhosis. However, the lowest OR obtained in those having 2 or more copy numbers of *GSTT1* was not statistically significant (AOR=2.84; 95% CI: 0.52, 15.48).

Table 4. Risk of liver cirrhosis in relation to AFB_1 exposure, stratified by *GSTT1* gene copy number and genotype, Eastern Ethiopia, 2020/21.

Copy number		**AFB_1 unexposed**	**AFB_1 exposed**
0	Cases/controls	7/17	35/9
	OR (95%CI) [a]	1.00	12.46 (3.30, 47.05) *
1	Cases/controls	13/40	36/20
	OR (95%CI) [a]	1.00	7.32 (2.83, 18.90) *
≥2	Cases/controls	8/13	7/5
	OR (95%CI) [a]	1.00	2.84 (0.52, 15.48)
Null	Cases/controls	7/17	35/9
	OR (95%CI) [a]	1.00	12.46 (3.30, 47.05) *
Carrier	Cases/controls	21/53	43/25
	OR (95%CI) [a]	1.00	5.13 (2.35, 11.21) *
P interaction between genotype and exposure to AFB1 = 0.01			

*p value < 0.05. [a] adjusted for gender, age, and Hepatitis B virus exposure. AFB_1, aflatoxin B_1; OR, Odds ratio

Furthermore, as shown in Table 5 AFB_1 exposure associated risk of liver cirrhosis was reduced in those who were carriers of either *GSTM1* or *GSTT1* gene (AOR=5.53; 95% CI: 2.79, 10.94) compared with those who had double null genotype of both *GSTM1* and *GSTT1* gene (AOR=15.87: 95% CI: 1.16, 218.06). Besides, having two or more copies of the combined *GSTM1* and *GSTT1* gene (AOR=3.67; 95% CI: 1.54, 8.77) was associated with a significant reduction in risk of liver cirrhosis compared with having one (AOR= 13.04; 95% CI: 2.96, 60.65) or zero-copy (AOR=15.87; 95% CI: 1.16, 218.06) of the genes. Moreover, the interaction between AFB_1 exposure and combined *GSTM1* and *GSTT1* genotypes was statistically significant (p=0.00).

Table 5. Risk of liver cirrhosis in relation to AFB_1 exposure, stratified by the sum of *GSTM1* and *GSTT1* genotype, Eastern Ethiopia, 2020/21.

Genotype		AFB_1 unexposed	AFB_1 exposed
Sum of *GSTM1/GSTT1*			
0	Cases/controls	2/7	15/4
	OR (95%CI) [a]	1.00	15.87 (1.16, 218.06) *
1	Cases/controls	5/16	37/9
	OR (95%CI) [a]	1.00	13.04 (2.96, 60.65) *
≥2	Cases/controls	21/47	26/21
	OR (95%CI) [a]	1.00	3.67 (1.54, 8.77) *
Null/Null	Cases/controls	2/7	15/4
	OR (95%CI) [a]	1.00	15.87 (1.16, 218.06) *
Carrier/Carrier	Cases/controls	26/63	63/30
	OR (95%CI) [a]	1.00	5.53 (2.79, 10.94) *
P interaction between genotype and exposure to AFB1 = 0.00			

*p value < 0.05. [a] adjusted for gender, age, and Hepatitis B virus exposure. AFB1, aflatoxin B1; OR, Odds ratio

4.5.3. Level of hepatic enzymes and *GSTM1* and *GSTT1* genes copy numbers

Compared to null alleles, liver cirrhosis patients with 2 or more copies of *GSTM1* had a 31% (β = 0.69; 95% CI: 0.48, 0.99; $p<0.05$) lower serum level of ALT (Table 3, Paper III). Furthermore, serum levels of AST were decreased by 39% (β = 0.61; 95% CI: 0.41, 0.90; $p<0.05$) in those with two or more copies of *GSTT1* genes than individuals with the null allele (Table 3, Paper III). Likewise, serum levels of AST displayed a substantial drop ($p<0.05$) in liver cirrhosis cases who carried the *GSTT1* gene in contrast to those wit null alleles (β = 0.76; 95% CI: 0.58, 0.98) (Table 3, Paper III).

5. DISCUSSION

Liver cirrhosis is a major health issue in Eastern Ethiopia, despite insufficient information on its etiology (Orlien *et al*., 2018a; Orlien *et al*., 2018b). The high morbidity and mortality rates associated with this condition in the area emphasize the pressing need for further investigation and improved interventions. In an area with a high incidence of unexplained instances of cirrhosis, this study investigated the role of AFB_1 exposure in the etiology of liver cirrhosis to provide a drive for further investigation and action. Our study key findings include: i) The majority (77%) of liver cirrhosis patients had an unknown etiology, which was strongly predicted by splenomegaly and consumption of sorghum as a staple food; ii) We have found detectable AF-alb adduct levels in a significantly higher percentage of liver cirrhosis cases (75%) than controls (64%); iii) the study further revealed being a farmer by occupation, living in rural areas, and having no formal education as strong predictors of AFB_1 exposure; iv) Exposure to a high level ($\geq$ 8.6 pg/mg) of AFB_1 was strongly associated with liver cirrhosis; v) Single copy number of *GSTT1* and two or more copies of the combined *GSTM1* and *GSTT1* genes were shown to decrease the risk of hepatic cirrhosis; and vi) Those who had two or more copies of the *GSTM1* or *GSTT1* genes showed a significant drop in their serum levels of ALT and AST, respectively.

Our present study revealed a strong and significant association between sorghum consumption and liver cirrhosis of unknown etiology. In support of this, similar studies done in Gambia, Guatemala, and Kenya have demonstrated AFB_1 exposure and the risk of liver disease through consumption of maize-based products and groundnut as a staple diet (Kuniholm *et al*., 2008; Kroker-Lobos *et al*., 2019; Alvarez *et al*., 2020a; Omara *et al*., 2021). Moreover, our current study further found splenomegaly as a predictive factor of hepatic cirrhosis of unknown etiology. In this context, a study that enrolled Kenyan schoolchildren reported a 43% increase in the prevalence of hepatomegaly or hepatosplenomegaly for every natural log-unit rise in AF-alb adduct level after adjustment for *S. mansoni* and *Plasmodium* infections (Gong *et al*., 2012). The high prevalence of a diet high in AFB_1-contaminated staple foods, the lack of dietary variety, inadequate nutrition, and the linkage between AFB_1 and splenomegaly suggested a possible

association between AFB_1 and liver cirrhosis in Eastern Ethiopia (Mekonnen *et al*., 2020; Aweke *et al*., 2022; Gebremichael *et al*., 2022).

Biomarker-based studies are indispensable to prove the exposure burden of AFB_1 and its etiologic significance in this part of Ethiopia, which has a high burden of cryptogenic hepatic cirrhosis. Accordingly, we found significantly higher median AF-alb adducts in cases than controls. Furthermore, multivariable analysis revealed that exposure to high levels of AFB_1 was strongly associated with liver cirrhosis. The present finding was consistent with other studies reported from different regions of the world known to have a high burden of AFB_1-contaminated staple diets. For example, a case-control study done in Guatemala reported a positive association between AFB_1 exposure and cirrhosis, with a significantly higher median AF-alb adduct level among cases (11.4 pg/mg) than controls (5.1 pg/ mg). The study further revealed the association of increased levels of AF-alb adduct and liver cirrhosis (Alvarez *et al*., 2020a). Moreover, other studies reported from Gambia (Kuniholm *et al*., 2008), Egypt (Hosny *et al*., 2008), Turkey (Aydın *et al*., 2015), and Taiwan (Chen *et al*., 2007; Chu *et al*., 2017) reported a markedly elevated proportion of AFB_1-related mutation in TP53, an increased mean concentration of AF-alb adduct, and a positive relation in the adduct concentration and ultrasonographic changes in cases with chronic liver disease or hepatic cirrhosis.

Moreover, studies reported from Gambia and Taiwan found a synergistic interaction between AFB_1 exposure and infection with HBV in the development of hepatic cirrhosis (Kuniholm *et al*., 2008; Chu *et al*., 2017). In this regard, the present study revealed a strong relationship between HBV infection and hepatic cirrhosis in Eastern Ethiopia. Hence, co-exposure to AFB_1 and HBV infections could further fuel the burden of hepatic cirrhosis in the area. Moreover, our study revealed a strong association between young adults (<35 years) and the risk of developing liver cirrhosis. In sub-Saharan Africa, it is regarded that HCC (typically developed in patients with liver cirrhosis) is an illness of young adults, in contrast to North Africa and America, Western Europe, and Japan, where it is predominant in the elderly (Kedar *et al*., 2021). This discrepancy could be partly explained by the fact that sub-Saharan Africa has a relatively high prevalence of HBV infections, with vertical or horizontal transmission of the virus during childhood as a major means of exposure to the virus (Sonderup and Spearman, 2022; Ansari *et al*., 2023). Indeed, investigations also reported the impact of age at infection on the onset of HBV infection and its progression to hepatic cirrhosis, or HCC (Edmunds *et al*., 1993; Shimakawa *et al*., 2013). Exposure to AFB_1 is also known to have begun in utero (Ismail *et al*.,

2021). This coexposure to HBV and AFB_1 at an early age could hasten the disease occurrence at a younger age in sub-Saharan Africa, contrary to the rest of the globe.

The possible mechanisms of synergy between AFB_1 and HBV include the induction of CYP3A4 that metabolizes AFB_1 to AFBO either by chronic hepatitis attributable to HBV or by the presence of the virus itself, based on experimental evidence using HBV transgenic mice (Gemechu-Hatewu *et al*., 1997; Kew, 2003). Furthermore, chronic HBV infection has also been shown to generate ROS formation that further escalates AFB_1-related ROS buildup and the associated sequelae (Popa and Popa, 2022). Besides, low-level AFB_1 exposure could increase susceptibility to HBV infection or its replication (Wild and Gong, 2010). AFB_1 treatment has shown a significant increase in the expression of the HBV gene in ducklings and the HBsAg level in HepG2 cells transfected with recircularized HBV DNA (Barraud *et al*., 1999; Banerjee *et al*., 2000). According to studies, AFBO-induced DNA damage might pave the way for viral DNA integration into the host genome, in addition to being a possible mechanism (Dandri *et al*., 2002; Jiang *et al*., 2005). For instance, a study that explored the immune modulation effects of dietary exposure to AFB_1 in Kenyan children who received HBV vaccination reported a drop in anti-HBV antibodies by 0.91 mIU/ml for every unit rise in serum level of AF-alb adduct (Githang'a *et al*., 2019). Chronic exposure to AFB_1 has also been shown to increase susceptibility to other infectious diseases due to its immunosuppressive effects by interfering in the innate cell functions, activation and proliferation of lymphocytes, and the expression of cytokines and chemokines (Jolly *et al*., 2013; Bbosa *et al*., 2013; Ya *et al*., 2021).

The current study further scrutinized sociodemographic factors associated with AFB_1 exposure and accordingly, being a farmer by occupation, having no formal education, and living in rural areas as strong predictors of AFB_1 exposure in our study population. Similarly, studies done in Tanzania reported the positive effect of education on aflatoxin*s* awareness and higher rates of AFB_1 exposure in rural populations (Ndwata *et al*., 2022; Gichohi-Wainaina *et al*., 2023). The later study suggested poor dietary diversity and a staple-based diet consisting of maize, sorghum, groundnuts, and other foods prone to AFB_1 contamination in rural populations as possible reasons for the high rates of AFB_1 exposure. Moreover, in our study, multivariable analysis revealed farmer occupation as a risk factor for liver cirrhosis. In this context, farmers face a significant risk of AFB_1 airborne exposure in addition to dietary exposure while managing infected grains as well as when processing and handling animal feed (Malik *et al*., 2014; Wangia *et al*., 2019). This is more fueled by lack of awareness about mycotoxins, especially in resource-

limited settings. Moreover, our study also revealed being a farmer by occupation as a predictive factor of liver cirrhosis in Eastern Ethiopia. In this regard, apart from the observed high level of AF-alb adduct, potential co-exposure to pesticides like DDT might put farmers at increased risk of liver cirrhosis, as discussed in Paper II. Taken together, AFB_1 exposure coupled with occupational exposure to pesticides (VoPham *et al*., 2017; Derso and Dagnew, 2019; Alebachew *et al*., 2023) might be possible factors that put farmers at higher risk of developing liver cirrhosis than non-farmers.

In an effort to explore the significance of gene-environment interactions in the multifactorial pathogenesis of hepatic cirrhosis, we also investigated the link between AFB_1 metabolizing enzymes (*GSTM1* and *GSTT1*) polymorphisms and hepatic cirrhosis. The current study found that *GSTM1* and *GSTT1* null alleles occur more commonly in hepatic cirrhosis cases than controls. In the study population, the overall frequencies of *GSTM1* and *GSTT1* null alleles were 0.39 and 0.32, respectively. In addition, double *GSTM1* and *GSTT1* null alleles were found in 13% of the study population. Besides, 3.3% (1.9% for *GSTM1* and 1.4% for *GSTT1*) of the study population had duplicate copies of the genes.

The genes that encode the key AFB_1 detoxifying enzymes, *GSTM1* and *GSTT1,* vary among populations. Studies have shown that the frequency of the *GSTM1* null genotype is higher in Asians, Caucasians, and Arabs than in Africans (0.23 to 0.66, 0.45 to 0.57, 0.50 to 0.63, and 0.11 to 0.44, respectively) (Alshagga *et al*., 2011; Piacentini *et al*., 2011; Salem *et al*., 2011; Mansour *et al*., 2015; Nakanishi *et al*., 2022). The *GSTT1* null allele was reported to be highly prevalent in Asians, Africans, and Arabs than in Caucasians, with frequencies of 0.35 to 0.52, 0.20 to 0.47, 0.20 to 0.37, and 0.14 to 0.28, respectively (Alshagga *et al*., 2011; Piacentini *et al*., 2011; Salem *et al*., 2011; Mansour *et al*., 2015; Nakanishi *et al*., 2022). On the other hand, dual *GSTM1* and *GSTT1* null alleles were found in Asians, Arabs, and Africans at frequencies of 0.27, 0.21, and 0.15, respectively (Guo et al., 2008; Salem et al., 2011; Kiendrebeogo et al., 2019). Furthermore, *GSTM1* or *GSTT1* gene duplications have been found at a greater percentage among Caribbeans originated from Africa (3.4%) than in Caucasians (0.2%) (Nørskov *et al*., 2011; Emeville *et al*., 2014). In fact, our study results on the frequency of *GSTM1* or *GSTT1* and dual null genotypes, as well as the proportion of *GSTM1* or *GSTT1* gene duplication, were in line with prior studies that investigated Africans and populations descended from the continent.

It has been suggested that null alleles encoding phase II metabolizing enzymes, including *GSTM1* and *GSTT1* has been related with greater risk of various disease that involved oxidative

stress as a pathogenesis (Nørskov *et al*., 2011; Emeville *et al*., 2014). Similarly, our study demonstrated a substantially reduced risk of hepatic cirrhosis in *GSTT1* carriers than in those with null alleles, and specifically, having a single copy of the *GSTT1* gene was protective against hepatic cirrhosis compared with null alleles. Besides, two or more copies of combined *GSTM1* and *GSTT1* genes were shown to reduce the risk of hepatic cirrhosis in comparison with double null allele. In this context, no observational studies examining the relationship between *GSTT1* and *GSTM1* copy numbers and hepatic cirrhosis are available for comparison. Meanwhile, previous studies which merely compared carriers (irrespective of the exact copy number of the genes) and null alleles of *GSTM1* and *GSTT1* have reported lower risk of hepatic disease, including drug-induced hepatic injury (Li *et al*., 2013; Chbili *et al*., 2018), alcoholic cirrhosis (Ladero *et al*., 2005), viral and non-viral HCC (Li et al., 2019), non-alcoholic fatty liver disease (Damavandi and Zeinali, 2021), and HCV related hepatic cirrhosis (Araujo *et al*., 2021). Our finding also revealed a substantial gene dose association between the combined *GSTM1* and *GSTT1* copies and the development of hepatic cirrhosis. The current finding may support the idea that the activity of the enzymes encoded by the *GSTM1* and *GSTT1* genes is directly correlated with their copy numbers (Sprenger *et al*., 2000; Girault *et al*., 2005). Consequently, having more copy numbers could be considered protective against the development of oxidative stress and related inflammation due to viral and non-viral etiologies of liver cirrhosis.

Likewise, several epidemiological investigations have suggested that the potential toxicity of AFB_1 varies with the ability of the exposed individual to detoxify its reactive metabolite, AFB1, exo-8,9-epoxide (Chen *et al*., 1996; Wild *et al*., 2000; Sun *et al*., 2001; Li *et al*., 2014). Accordingly, we did a stratified analysis based on *GSTM1* and *GSTT1* copy numbers, and the effect of AFB_1-exposure on liver cirrhosis risk showed a decreasing tendency as the copy numbers increased from zero to 2 or more copies. Though, the lowest risk observed in those having 2 or more copy numbers was not statistically significant. This could be because of the smaller size of the sample. However, significant risk reduction was observed in those having 2 or more combined copies of *GSTM1* and *GSTT1* genes. Besides, the interaction between AFB_1 exposure and *GSTM1* or *GSTT1 or* combined *GSTM1 and GSTT1* genotypes was statistically significant. In this regard, no observational studies exploring the relation between *GSTT1* and *GSTM1* copy numbers and the effect of AFB_1 exposure on the risk of liver cirrhosis are available for comparison. Thus, having more copy numbers could be protective against AFB_1-exo-8, 9-epoxide-induced oxidative stress and accompanied risk of liver cirrhosis in exposed individuals.

A disturbance in the level of hepatic enzymes might be indirect evidence of the extent of hepatic insult following exposure to toxins like AFB_1 (Saad-Hussein *et al*., 2016). In the current study, we also explored whether the genotypes of *GSTM1* and *GSTT1* indirectly affect the levels of hepatic enzymes. In this regard, our study finding showed a marked drop in ALT and AST levels in individuals with two or more copies of *GSTM1* and *GSTT1* genes, respectively. Currently, no studies have investigated the influence of *GSTM1* and *GSTT1* CNVs on the level of hepatic enzymes in hepatic cirrhosis patients. Meanwhile, few studies have attempted to examine the impact of the *GSTM1* and *GSTT1* carrier and null genotypes on the level of hepatic enzymes.

For instance, a study that involved HCV-infected HCC and liver cirrhosis patients reported no significant difference in the level of hepatic enzymes between carriers and null alleles of *GSTT1* and/or *GSTM1* (Araujo *et al*., 2021). However, our study revealed a significant decrease in AST levels among *GSTT1* carriers of hepatic cirrhosis patients compared with null alleles, in addition to the finding associated with two or more copies of *GSTM1* and *GSTT1*. In addition to disparities in race and the etiologic factors of cirrhosis, one explanation for this divergence could be that our investigation sought to determine the true impact of the exact copy numbers of the genes. HCV infection and excess alcohol intake are the leading causes of HCC and hepatic cirrhosis in America, Oceania, and Europe, while exposure to HBV and AFB_1 is the predominant cause in Africa and Asia (Fassio *et al*., 2010; El-Kassas and Elbadry, 2022; Díaz *et al*., 2022). In fact, our study demonstrated HBV infection and AFB_1 exposure as strong predictors of hepatic cirrhosis in Eastern Ethiopia.

In this regard, few investigations have reported the relevance of *GSTM1* and *GSTT1* null and carrier genotypes to the blood concentrations of ALT and AST in individuals exposed to hepatotoxic drugs known to produce reactive free radicals as transient metabolites (Roy *et al*., 2001; Watanabe *et al*., 2003; Chbili *et al*., 2018). For example, a study that involved Tunisian epileptic patients taking carbamazepine, known to have 10, 11-epoxy carbamazepine as a reactive metabolite, reported a marked elevation in both ALT and AST concentrations in association with the *GSTM1* null genotype. Besides, the study revealed a significant increase in AST levels in those with *GSTT1* null genotypes (Chbili *et al*., 2018). Hence, carrying normal

GSTM1 and *GSTT1* genes could influence the concentration of AST and ALT, at least by reducing oxidative stress and related insult in the liver due to ROS formed by drugs and environmental toxins, including AFB_1. To support these findings, however, more research with a larger sample size is needed.

6. LIMITATIONS AND STRENGTH OF THE STUDY

To the best of our knowledge, this study is the first to provide an impetus for further investigation and action on the association between AFB_1 and the risk of cirrhosis of unknown etiology in Ethiopia, where people feed on a diet contaminated with a high burden of AFB_1. Cases and controls were recruited using ultrasound examination that has up to 80% and 97% sensitivity and specificity, respectively, versus liver biopsy to diagnose liver cirrhosis (Kelly *et al*., 2018; Yen *et al*., 2019). Moreover, the ultrasound examination reduced the chance of including study participants with undiagnosed liver disease in the control group. Nevertheless, a number of factors may have influenced the results obtained in this study.

Selection bias cannot be completely avoided due to the inherent nature of the study design. For various socioeconomic, cultural, and practical reasons, it's possible that some liver cirrhosis patients might be overlooked during the recruitment of cases. Besides, using hospital controls might introduce Berkson's bias due to the varying rates of hospital admissions (Nahler, 2009). However, this potential bias might not be very problematic, since both incident liver cirrhosis cases and controls were recruited from the same unit (internal medicine) that served most patients who visited the hospital with a great variety of diseases caused by numerous exposures. Moreover, the statistical power of our study also improved due to the inclusion of more than one control per case in the study.

Another limitation of the current study was that we were not able to conduct the genetic analysis study using all the samples. As it is difficult to obtain enough blood from all the study participants to be used for preliminary tests conducted during recruitment and for further tests. Moreover, unavailability of enough kits and reagents further limited us to analyze all available samples. However, the number of samples analyzed in the genetic association study could not as such affect the statistical power of the study.

Additional potential limitation of this study was its inability to evaluate the association between liver cirrhosis and polymorphisim in *mEH*, and *AFAR* enzyme genes. However, both enzymes are known to involve in AFB_1 detoxification though their contribution was minor as reported by various studies (Deng *et al*., 2018; Wang *et al*., 2022; Lootens *et al*., 2023).

7. CONCLUSIONS

The study found a high burden of hepatic cirrhosis of unidentified etiology in an area where sorghum is used as a staple food. The finding that the marker for the fungal toxin AFB_1 was higher in cases than controls and the association between sorghum and unexplained liver cirrhosis, as well as the higher chance of sorghum being contaminated by the toxin, strongly implicate the toxin as an etiologic factor. Moreover, our findings indicate the link between CNVs in AFB_1 metabolizing enzymes *GSTM1* and *GSTT1* and the risk of hepatic cirrhosis. In this regard, a copy of *GSTT1*, and two or more copies of combined *GSTM1* and *GSTT1* were linked with lower risk of hepatic cirrhosis. In addition, a marked reduction in ALT and AST level were observed in those patients with two or more copies of *GSTM1* and *GSTT1* genes, respectively. These results underscore the significance of gene-environment interactions in the multifactorial pathogenesis of the disease.

8. RECOMMENDATIONS

Hepatic cirrhosis is a major public health problem worldwide and the main reason of morbidity and mortality in young adults in Eastern Ethiopia. Hence, there is a pressing need for more studies to pave the way for public health efforts to combat the burden of liver cirrhosis in sub-Saharan Africa in general and in this region of Ethiopia in particular.

- Our study has reported AFB_1 and HBV exposure as major culprits of liver cirrhosis, both of which are preventable. However, in addition to the evidence generated from our hospital-based study design with certain limitations, further community-based studies are needed to explore the extent and further potential sources of AFB_1 exposure in this region for effective aflatoxin control policy design and implementation.

- Our study also revealed a significant association between liver cirrhosis and rural subsistence farming communities. In this regard, apart from the already observed high level of AFB_1, there could be a potential co-exposure with insecticides like DDT, as studies have reported improper use of it in rural farming communities. Thus, biomarker studies are warranted in Eastern Ethiopia to further explore the missing etiologies of hepatic cirrhosis in an area with a great burden of cryptogenic cases.

- In our study, young adults were at significantly higher risk of hepatic cirrhosis potentially due to early life exposure to HBV, AFB_1 and other possible hepatotoxins. Hence, prevention strategies should give emphasis effective measures to safeguard this segment of the population in Eastern Ethiopia as well as in sub-Saharan Africa.

- The relation between chronic exposures to AFB_1 and the development of HCC has been well established, and increasing reports implicate it in the etiology of liver cirrhosis. Hence, our study findings have sought to strengthen the existing level of evidence linking liver cirrhosis and AFB_1 exposure. Moreover, the study contributed by adding new findings regarding *GSTM1* and *GSTT1* genes to the aspects of the gene-environment interactions in the multifactorial pathogenesis hepatic cirrhosis. In this regard, further studies with larger sample size are required for substantiating these genetic evidences.

- Finally, AFB_1 exposure in Ethiopia should be viewed as a public health issue as a result, and various intervention techniques should be used, beginning on the farm. The

maximum permissible level of AFB_1 contamination in food and feed should be strictly governed by laws and regulations. It is crucial to launch coordinated initiatives to educate and change how farmers, traders, and consumers view the pre- and post-harvest procedures that lead to AFB_1 contamination.

REFERENCES

Abrehame S, Manoj VR, Hailu M, et al. (2023) Aflatoxins: Source, Detection, Clinical Features and Prevention. *Processes* 11(1): 204.

Alameri MM, Kong AS-Y, Aljaafari MN, et al. (2023) Aflatoxin Contamination: An Overview on Health Issues, Detection and Management Strategies. *Toxins* 15(4): 246.

Alebachew F, Azage M, Kassie GG, et al. (2023) Pesticide use safety practices and associated factors among farmers in Fogera district wetland areas, south Gondar zone, Northwest Ethiopia. *PLoS One* 18(1): e0280185.

Alshagga MA, Mohamed N, Nazrun Suhid A, et al. (2011) Frequencies of glutathione s-transferase (GSTM1, GSTM3 AND GSTT1) polymorphisms in a Malaysian population. *Arch Med Sci* 7(4): 572-578.

Alvarez CS, Hernández E, Escobar K, et al. (2020a) Aflatoxin B(1) exposure and liver cirrhosis in Guatemala: a case-control study. *BMJ Open Gastroenterol* 7(1).

Alvarez CS, Ortiz J, Bendfeldt-Avila G, et al. (2020b) Analysis of TP53 aflatoxin signature mutation in hepatocellular carcinomas from Guatemala: A cross-sectional study (2016-2017). *Health Sci Rep* 3(2): e155.

Ansari A, Vincent JP, Moorhouse L, et al. (2023) Risk of early horizontal transmission of hepatitis B virus in children of uninfected mothers in sub-Saharan Africa: a systematic review and meta-analysis. *The Lancet Global Health* 11(5): e715-e728.

Araujo OC, de Paula VS, do Ó KM, et al. (2021) Association of Polymorphisms in the Glutathione S-Transferase Theta-1 Gene with Cirrhosis and Hepatocellular Carcinoma in Brazilian Patients with Chronic Hepatitis C. *Vaccines (Basel)* 9(8).

Ashenafi W, Eshetu F, Assefa N, et al. (2017) Trend and causes of adult mortality in Kersa health and demographic surveillance system (Kersa HDSS), eastern Ethiopia: verbal autopsy method. *Population Health Metrics* 15(1): 22.

Assefa D (2018) Natural Occurrence of Toxigenic Fungi Species and Aflatoxin in Freshly Harvested Groundnut Kernels in Tigray, Northern Ethiopia. 5: 377-384.

Aweke CS, Sassi M, Lahiff E, et al. (2022) Seasonality and food security among smallholder rural households in eastern Ethiopia: Evidence from panel data analysis. *Cogent Economics & Finance* 10(1): 2035492.

Ayalew A (2010) Mycotoxins and surface and internal fungi of maize from Ethiopia. *African Journal of Food, Agriculture, Nutrition and Development* 10: 4109-4123.

Ayalew A, Fehrmann H, Lepschy J, et al. (2006) Natural occurrence of mycotoxins in staple cereals from Ethiopia. *Mycopathologia* 162(1): 57-63.

Baclig MO, Alvarez MR, Lozada XM, et al. (2012) Association of glutathione S-transferase T1 and M1 genotypes with chronic liver diseases among Filipinos. *Int J Mol Epidemiol Genet* 3(2): 153-159.

Banerjee R, Caruccio L, Zhang YJ, et al. (2000) Effects of carcinogen-induced transcription factors on the activation of hepatitis B virus expression in human hepatoblastoma HepG2 cells and its implication on hepatocellular carcinomas. *Hepatology* 32(2): 367-374.

Barraud L, Guerret S, Chevallier M, et al. (1999) Enhanced duck hepatitis B virus gene expression following aflatoxin B1 exposure. *Hepatology* 29(4): 1317-1323.

Bbosa GS, Kitya D, Lubega A, et al. (2013) Review of the Biological and Health Effects of Aflatoxins on Body Organs and Body Systems.

Benkerroum N (2020) Aflatoxins: Producing-Molds, Structure, Health Issues and Incidence in Southeast Asian and Sub-Saharan African Countries. *Int J Environ Res Public Health* 17(4).

Besaratinia A, Kim SI, Hainaut P, et al. (2009) In vitro recapitulating of TP53 mutagenesis in hepatocellular carcinoma associated with dietary aflatoxin B1 exposure. *Gastroenterology* 137(3): 1127-1137, 1137.e1121-1125.

Boni S, Beed F, Kimanya M, et al. (2021) Aflatoxin contamination in Tanzania: quantifying the problem in maize and groundnuts from rural households. *World Mycotoxin Journal* 14: 1-12.

Bräse S, Encinas A, Keck J, et al. (2009) Chemistry and biology of mycotoxins and related fungal metabolites. *Chem Rev* 109(9): 3903-3990.

Carvajal-Campos A, Manizan AL, Tadrist S, et al. (2017) Aspergillus korhogoensis, a Novel Aflatoxin Producing Species from the Côte d'Ivoire. *Toxins (Basel)* 9(11).

Chala A, Mohammed A, Ayalew A, et al. (2013) Natural occurrence of aflatoxins in groundnut (Arachis hypogaea L.) from eastern Ethiopia. *Food Control* 30(2): 602-605.

Chala A, Taye W, Ayalew A, et al. (2014) Multimycotoxin analysis of sorghum (Sorghum bicolor L. Moench) and finger millet (Eleusine coracana L. Garten) from Ethiopia. *Food Control* 45: 29-35.

Chapot B and Wild CP (1991) ELISA for quantification of aflatoxin-albumin adducts and their application to human exposure assessment. *Techniques in Diagnostic Pathology* 2: 135-155.

Chauhan NM, Washe AP and Minota T (2016) Fungal infection and aflatoxin contamination in maize collected from Gedeo zone, Ethiopia. *Springerplus* 5(1): 753.

Chbili C, Hassine A, Fathallah N, et al. (2018) Glutathione S-transferase M1 and T1 polymorphisms and the risk of mild hepatotoxicity induced by carbamazepine in a tunisian population study. *BMC Neurology* 18(1): 24.

Chen CJ, Yu MW, Liaw YF, et al. (1996) Chronic hepatitis B carriers with null genotypes of glutathione S-transferase M1 and T1 polymorphisms who are exposed to aflatoxin are at increased risk of hepatocellular carcinoma. *Am J Hum Genet* 59(1): 128-134.

Cheng K, Niu J, Zheng X, et al. (2023) Aflatoxin-B1-Exposure-Induced Hepatic Injury Could Be Alleviated by Polydatin through Reducing Oxidative Stress, Inhibiting Inflammation and Improving Mitophagy. *Toxics* 11(4).

Chittmittrapap S, Chieochansin T, Chaiteerakij R, et al. (2013) Prevalence of aflatoxin induced p53 mutation at codon 249 (R249s) in hepatocellular carcinoma patients with and without hepatitis B surface antigen (HBsAg). *Asian Pac J Cancer Prev* 14(12): 7675-7679.

Chu YJ, Yang HI, Wu HC, et al. (2017) Aflatoxin B(1) exposure increases the risk of cirrhosis and hepatocellular carcinoma in chronic hepatitis B virus carriers. *Int J Cancer* 141(4): 711-720.

Dai C, Tian E, Hao Z, et al. (2022) Aflatoxin B1 Toxicity and Protective Effects of Curcumin: Molecular Mechanisms and Clinical Implications. *Antioxidants (Basel)* 11(10).

Damavandi N and Zeinali S (2021) Association of xenobiotic-metabolizing enzymes (GSTM1 and GSTT 1), and pro-inflammatory cytokines (TNF-α and IL-6) genetic polymorphisms with non-alcoholic fatty liver disease. *Mol Biol Rep* 48(2): 1225-1231.

Dandri M, Burda MR, Bürkle A, et al. (2002) Increase in de novo HBV DNA integrations in response to oxidative DNA damage or inhibition of poly(ADP-ribosyl)ation. *Hepatology* 35(1): 217-223.

Deng J, Zhao L, Zhang N-Y, et al. (2018) Aflatoxin B1 metabolism: Regulation by phase I and II metabolizing enzymes and chemoprotective agents. *Mutation Research/Reviews in Mutation Research* 778: 79-89.

Derso AG and Dagnew GG (2019) Exposure and Health Risk Assessment of Farmers to DDT during Khat Production in Chiro Woreda, West Hararghe Zone: Ethiopia. *World Journal of Agricultural Research* 7: 29-35.

Díaz LA, Ayares G, Arnold J, et al. (2022) Liver Diseases in Latin America: Current Status, Unmet Needs, and Opportunities for Improvement. *Curr Treat Options Gastroenterol* 20(3): 261-278.

Eaton DL, Bammler TK and Kelly EJ (2001) Interindividual differences in response to chemoprotection against aflatoxin-induced hepatocarcinogenesis: implications for human biotransformation enzyme polymorphisms. *Adv Exp Med Biol* 500: 559-576.

Edmunds WJ, Medley GF, Nokes DJ, et al. (1993) The influence of age on the development of the hepatitis B carrier state. *Proc Biol Sci* 253(1337): 197-201.

El-Kassas M and Elbadry M (2022) Hepatocellular Carcinoma in Africa: Challenges and Opportunities. *Front Med (Lausanne)* 9: 899420.

Emeville E, Broquère C, Brureau L, et al. (2014) Copy Number Variation of GSTT1 and GSTM1 and the Risk of Prostate Cancer in a Caribbean Population of African Descent. *PLOS ONE* 9(9): e107275.

Eshete M, Gebremedhin S, Alemayehu FR, et al. (2021) Aflatoxin contamination of human breast milk and complementary foods in southern Ethiopia. *Maternal & Child Nutrition* 17(1): e13081.

Fassio E, Díaz S, Santa C, et al. (2010) Etiology of hepatocellular carcinoma in Latin America: a prospective, multicenter, international study. *Annals of Hepatology* 9(1): 63-69.

Gebremichael B, Misgana T, Tamiru D, et al. (2022) Undernutrition and associated factors among rural pregnant women in Eastern Ethiopia. *SAGE Open Med* 10: 20503121221104430.

Gemechu-Hatewu M, Platt KL, Oesch F, et al. (1997) Metabolic activation of aflatoxin B1 to aflatoxin B1-8,9-epoxide in woodchucks undergoing chronic active hepatitis. *Int J Cancer* 73(4): 587-591.

Gichohi-Wainaina WN, Kimanya M, Muzanila YC, et al. (2023) Aflatoxin Contamination, Exposure among Rural Smallholder Farming Tanzanian Mothers and Associations with Growth among Their Children. *Toxins (Basel)* 15(4).

Girault I, Lidereau R and Bièche I (2005) Trimodal GSTT1 and GSTM1 genotyping assay by real-time PCR. *Int J Biol Markers* 20(2): 81-86.

Githang'a D, Wangia RN, Mureithi MW, et al. (2019) The effects of aflatoxin exposure on Hepatitis B-vaccine induced immunity in Kenyan children. *Current Problems in Pediatric and Adolescent Health Care* 49(5): 117-130.

Gong YY, Watson S and Routledge MN (2016) Aflatoxin Exposure and Associated Human Health Effects, a Review of Epidemiological Studies. *Food Saf (Tokyo)* 4(1): 14-27.

Gong YY, Wilson S, Mwatha JK, et al. (2012) Aflatoxin exposure may contribute to chronic hepatomegaly in Kenyan school children. *Environ Health Perspect* 120(6): 893-896.

Gu X and Manautou JE (2012) Molecular mechanisms underlying chemical liver injury. *Expert Rev Mol Med* 14: e4.

Guo J, Yan W-R, Tang J-K, et al. (2022) Dietary phillygenin supplementation ameliorates aflatoxin B1-induced oxidative stress, inflammation, and apoptosis in chicken liver. *Ecotoxicology and Environmental Safety* 236: 113481.

Guo X, O'Brien SJ, Zeng Y, et al. (2008) GSTM1 and GSTT1 gene deletions and the risk for nasopharyngeal carcinoma in Han Chinese. *Cancer Epidemiol Biomarkers Prev* 17(7): 1760-1763.

Hagström H (2017) Alcohol Consumption in Concomitant Liver Disease: How Much is Too Much? *Current Hepatology Reports* 16(2): 152-157.

Heikkinen H, Jallinoja P, Saarni SI, et al. (2008) The impact of smoking on health-related and overall quality of life: a general population survey in Finland. *Nicotine Tob Res* 10(7): 1199-1207.

Hidese S, Ota M, Matsuo J, et al. (2022) Association of body mass index and its classifications with gray matter volume in individuals with a wide range of body mass index group: A whole-brain magnetic resonance imaging study. *Frontiers in Human Neuroscience* 16.

Hua Z, Liu R, Chen Y, et al. (2021) Contamination of Aflatoxins Induces Severe Hepatotoxicity Through Multiple Mechanisms. *Frontiers in Pharmacology* 11.

Huang DQ, Terrault NA, Tacke F, et al. (2023) Global epidemiology of cirrhosis — aetiology, trends and predictions. *Nature Reviews Gastroenterology & Hepatology* 20(6): 388-398.

IARC IAfRoC (2012) Chemical agents and related occupations. A review of Human Carcinogens. *IARC Monogr Eval Carcinog Risks Hum* 100(Pt F): 9-562.

Ibrahim AM, Ahmed HS, Alazizi NM, et al. (2016) Glutathione S-Transferase M1 and T1 Gene Polymorphisms and the Outcome of Chronic Hepatitis C Virus Infection in Egyptian Patients. *Ann Hum Genet* 80(1): 32-37.

Ismail A, Naeem I, Gong YY, et al. (2021) Early life exposure to dietary aflatoxins, health impact and control perspectives: A review. *Trends in Food Science & Technology* 112: 212-224.

Jiang Y, Jolly PE, Ellis WO, et al. (2005) Aflatoxin B1 albumin adduct levels and cellular immune status in Ghanaians. *Int Immunol* 17(6): 807-814.

Jolly PE, Inusah S, Lu B, et al. (2013) Association between high aflatoxin B(1) levels and high viral load in HIV-positive people. *World Mycotoxin J* 6(3): 255-261.

Jung M, Kim J and Ahn SM (2020) Factors Associated with Frequency of Peanut Consumption in Korea: A National Population-Based Study. *Nutrients* 12(5).

Kapahtia S, Hazam RK, Asim M, et al. (2018) Role of Glutathione S Transferase M1 and T1 Gene Polymorphism in Hepatitis B Related Liver Diseases and Cryptogenic Cirrhosis. *J Clin Exp Hepatol* 8(2): 169-172.

Karita E, Ketter N, Price MA, et al. (2009) CLSI-Derived Hematology and Biochemistry Reference Intervals for Healthy Adults in Eastern and Southern Africa. *PLOS ONE* 4(2): e4401.

Kedar M VVP, Sewram V, Ndlovu N, et al. (2021) Hepatocellular Carcinoma in Sub-Saharan Africa. *JCO Glob Oncol* 7: 756-766.

Kelly EMM, Feldstein VA, Parks M, et al. (2019) An assessment of the clinical accuracy of ultrasound in diagnosing cirrhosis in the absence of portal hypertension. *Gastroenterol Hepatol (N Y)*14(6):367-373.

Kew MC (2003) Synergistic interaction between aflatoxin B1 and hepatitis B virus in hepatocarcinogenesis. *Liver International* 23(6): 405-409.

Kiendrebeogo IT, Zoure AA, Sorgho PA, et al. (2019) Glutathione S-transferase M1 (GSTM1) and T1 (GSTT1) variants and breast cancer risk in Burkina Faso. *Biomol Concepts* 10(1): 175-183.

Kirk GD, Turner PC, Gong Y, et al. (2005) Hepatocellular carcinoma and polymorphisms in carcinogen-metabolizing and DNA repair enzymes in a population with aflatoxin exposure and hepatitis B virus endemicity. *Cancer Epidemiol Biomarkers Prev* 14(2): 373-379.

Kroker-Lobos MF, Alvarez CS, Rivera-Andrade A, et al. (2019) Association between aflatoxin-albumin adduct levels and tortilla consumption in Guatemalan adults. *Toxicol Rep* 6: 465-471.

Kuniholm MH, Lesi OA, Mendy M, et al. (2008) Aflatoxin exposure and viral hepatitis in the etiology of liver cirrhosis in the Gambia, West Africa. *Environ Health Perspect* 116(11): 1553-1557.

Ladero JM, Martínez C, García-Martin E, et al. (2005) Polymorphisms of the glutathione S-transferases mu-1 (GSTM1) and theta-1 (GSTT1) and the risk of advanced alcoholic liver disease. *Scand J Gastroenterol* 40(3): 348-353.

Leggieri MC, Toscano P and Battilani P (2021) Predicted Aflatoxin B1 Increase in Europe Due to Climate Change: Actions and Reactions at Global Level. *Toxins* 13(4): 292.

Li C, Long J, Hu X, et al. (2013) GSTM1 and GSTT1 genetic polymorphisms and risk of anti-tuberculosis drug-induced hepatotoxicity: an updated meta-analysis. *Eur J Clin Microbiol Infect Dis* 32(7): 859-868.

Li S, Xue F, Zheng Y, et al. (2019) GSTM1 and GSTT1 null genotype increase the risk of hepatocellular carcinoma: evidence based on 46 studies. *Cancer Cell Int* 19: 76.

Lin DY, Sheen IS, Chiu CT, et al. (1993) Ultrasonographic changes of early liver cirrhosis in chronic hepatitis B: a longitudinal study. *J Clin Ultrasound* 21(5): 303-308.

Lingappan K (2018) NF-κB in Oxidative Stress. *Curr Opin Toxicol* 7: 81-86.

Liu Y, Chang CC, Marsh GM, et al. (2012) Population attributable risk of aflatoxin-related liver cancer: systematic review and meta-analysis. *Eur J Cancer* 48(14): 2125-2136.

Liu Y and Wu F (2010) Global burden of aflatoxin-induced hepatocellular carcinoma: a risk assessment. *Environ Health Perspect* 118(6): 818-824.

Liu YB and Chen MK (2022) Epidemiology of liver cirrhosis and associated complications: Current knowledge and future directions. *World J Gastroenterol* 28(41): 5910-5930.

Long XD, Huang HD, Huang XY, et al. (2015) XPC codon 939 polymorphism is associated with susceptibility to DNA damage induced by aflatoxin B1 exposure. *Int J Clin Exp Med* 8(1): 1197-1204.

Long XD, Ma Y, Wei YP, et al. (2005) [Study on the detoxication gene gstM1-gstT1-null and susceptibility to aflatoxin B1 related hepatocellular carcinoma in Guangxi]. *Zhonghua Liu Xing Bing Xue Za Zhi* 26(10): 777-781.

Long XD, Ma Y, Wei YP, et al. (2006) The polymorphisms of GSTM1, GSTT1, HYL1*2, and XRCC1, and aflatoxin B1-related hepatocellular carcinoma in Guangxi population, China. *Hepatol Res* 36(1): 48-55.

Lootens O, De Boevre M, Ning J, et al. (2023) Building a Human Physiologically Based Pharmacokinetic Model for Aflatoxin B1 to Simulate Interactions with Drugs. *Pharmaceutics* 15(3): 894.

Ma J, Liu Y, Guo Y, et al. (2021) Transcriptional Profiling of Aflatoxin B1-Induced Oxidative Stress and Inflammatory Response in Macrophages. *Toxins (Basel)* 13(6).

Mahdiani S, Omidkhoda N, Heidari S, et al. (2022) Protective effect of luteolin against chemical and natural toxicants by targeting NF-κB pathway. *BioFactors* 48(4): 744-762.

Malik A, Ali S, Shahid M, et al. (2014) Occupational exposure to Aspergillus and aflatoxins among food-grain workers in India. *Int J Occup Environ Health* 20(3): 189-193.

Mamo FT, Abate BA, Tesfaye K, et al. (2020) Mycotoxins in Ethiopia: A Review on Prevalence, Economic and Health Impacts. *Toxins (Basel)* 12(10).

Mannervik B, Board P, Hayes J, et al. (2005) Nomenclature for Mammalian Soluble Glutathione Transferases. *Methods in enzymology* 401: 1-8.

Mansour AA, Saleh OM, Askar T, et al. (2015) Frequency of glutathione-S-transferase null-M1 and null-T1 genotypes among the Turabah population in Saudi Arabia. *Genet Mol Res* 14(4): 16863-16871.

Marie S (2017) *Level of Aflatoxin in Sorghum Injera from Eastern Ethiopia*. Addis Ababa University, Addis Ababa, Ethiopia.

Mazari AMA, Zhang L, Ye Z-W, et al. (2023) The Multifaceted Role of Glutathione S-Transferases in Health and Disease. *Biomolecules* 13(4): 688.

McGlynn KA, Rosvold EA, Lustbader ED, et al. (1995) Susceptibility to hepatocellular carcinoma is associated with genetic variation in the enzymatic detoxification of aflatoxin B1. *Proc Natl Acad Sci U S A* 92(6): 2384-2387.

McIlwain CC, Townsend DM and Tew KD (2006) Glutathione S-transferase polymorphisms: cancer incidence and therapy. *Oncogene* 25(11): 1639-1648.

Meijer N, Kleter G, de Nijs M, et al. (2021) The aflatoxin situation in Africa: Systematic literature review. *Comprehensive Reviews in Food Science and Food Safety* 20(3): 2286-2304.

Mekonnen DA, Talsma EF, Trijsburg L, et al. (2020) Can household dietary diversity inform about nutrient adequacy? Lessons from a food systems analysis in Ethiopia. *Food Security* 12(6): 1367-1383.

Mekuria AN, Routledge MN, Gong YY, et al. (2020) Aflatoxins as a risk factor for liver cirrhosis: a systematic review and meta-analysis. *BMC Pharmacology and Toxicology* 21(1): 39.

Meneely JP, Kolawole O, Haughey SA, et al. (2023) The Challenge of Global Aflatoxins Legislation with a Focus on Peanuts and Peanut Products: A Systematic Review. *Exposure and Health* 15(2): 467-487.

Mohammed A, Bekeko Z, Yusufe M, et al. (2022a) Fungal Species and Multi-Mycotoxin Associated with Post-Harvest Sorghum (Sorghum bicolor (L.) Moench) Grain in Eastern Ethiopia. *Toxins* 14(7): 473.

Mohammed A, Faustinelli PC, Chala A, et al. (2021) Genetic fingerprinting and aflatoxin production of Aspergillus section Flavi associated with groundnut in eastern Ethiopia. *BMC Microbiol* 21(1): 239.

Mohammed F, Geda B, Assebe Yadeta T, et al. (2022b) Exploring the trend of Schizophrenia at Hiwot Fana specialized university referral hospital, Eastern, Ethiopia (2016-2020): A 5-year retrospective analysis. *SAGE Open Med* 10: 20503121221132160.

Mokdad AA, Lopez AD, Shahraz S, et al. (2014) Liver cirrhosis mortality in 187 countries between 1980 and 2010: a systematic analysis. *BMC Medicine* 12(1): 145.

Murcia HW and Diaz GJ (2021) Protective effect of glutathione S-transferase enzyme activity against aflatoxin B(1) in poultry species: relationship between glutathione S-transferase enzyme kinetic parameters, and resistance to aflatoxin B(1). *Poult Sci* 100(8): 101235.

Nahler G (2009) Berkson's bias. In: Nahler G (ed) *Dictionary of Pharmaceutical Medicine*. Vienna: Springer Vienna, pp.14-14.

Nakanishi G, Pita-Oliveira M, Bertagnolli L, et al. (2022) Worldwide Systematic Review of GSTM1 and GSTT1 Null Genotypes by Continent, Ethnicity, and Therapeutic Area. *OMICS: A Journal of Integrative Biology* 26.

Nasir U, Naeem I, Asif M, et al. (2021) Assessment of aflatoxins exposure through urinary biomarker approach and the evaluation of the impacts of aflatoxins exposure on the selected health parameters of the children of Multan city of Pakistan. *Food Control* 123: 107863.

Ndwata A, Suleiman R and Chaula D (2022) Aflatoxins B1 contamination levels in maize and awareness of aflatoxins among main maize stakeholders in Chemba and Kondoa Districts, Tanzania. *African Journal of Microbiology Research* 16: 223-237.

Negash A and Demissie M (2021) Alcohol Use Disorders among Adults at Primary Care, South-West Ethiopia. *J Psychiatry* 24: 480.

Nleya N, Ngoma L, Adetunji MC, et al. (2020) Biodiversity of Aflatoxigenic Aspergillus Species in Dairy Feeds in Bulawayo, Zimbabwe. *Front Microbiol* 11: 599605.

Nørskov MS, Frikke-Schmidt R, Bojesen SE, et al. (2011) Copy number variation in glutathione-S-transferase T1 and M1 predicts incidence and 5-year survival from prostate and bladder cancer, and incidence of corpus uteri cancer in the general population. *Pharmacogenomics J* 11(4): 292-299.

Nørskov MS, Frikke-Schmidt R, Loft S, et al. (2009) High-throughput genotyping of copy number variation in Glutathione S-Transferases M1 and T1 using real-time PCR in 20,687 individuals. *Clinical Biochemistry* 42(3): 201-209.

Omara T, Kiprop AK, Wangila P, et al. (2021) The Scourge of Aflatoxins in Kenya: A 60-Year Review (1960 to 2020). *Journal of Food Quality* 2021: 8899839.

Orlien SMS, Ismael NY, Ahmed TA, et al. (2018a) Unexplained chronic liver disease in Ethiopia: a cross-sectional study. *BMC Gastroenterol* 18(1): 27.

Orlien SMS, Sandven I, Berhe NB, et al. (2018b) Khat chewing increases the risk for developing chronic liver disease: A hospital-based case-control study. *Hepatology* 68(1): 248-257.

Pellicelli A (2023) Underlying Liver Disease. In: Ettorre GM (ed) *Hepatocellular Carcinoma*. Cham: Springer International Publishing, pp.27-34.

Piacentini S, Polimanti R, Porreca F, et al. (2011) GSTT1 and GSTM1 gene polymorphisms in European and African populations. *Mol Biol Rep* 38(2): 1225-1230.

Pickova D, Ostry V, Toman J, et al. (2021) Aflatoxins: History, Significant Milestones, Recent Data on Their Toxicity and Ways to Mitigation. *Toxins* 13(6): 399.

Polimanti R, Piacentini S and Fuciarelli M (2011) HapMap-based study of human soluble glutathione S-transferase enzymes: the role of natural selection in shaping the single nucleotide polymorphism diversity of xenobiotic-metabolizing genes. *Pharmacogenet Genomics* 21(10): 665-672.

Popa GL and Popa MI (2022) Oxidative Stress in Chronic Hepatitis B—An Update. *Microorganisms* 10(7): 1265.

Qin-Qin L, Xiao-Qin W and Jin-Guang Y (2019) The Blood AFB1-DNA Adduct Acting as a Biomarker for Predicting the Risk and Prognosis of Primary Hepatocellular Carcinoma. In: Xi-Dai L (ed) *Aflatoxin B1 Occurrence, Detection and Toxicological Effects*. Rijeka: IntechOpen, pp.Ch. 6.

Rose-Zerilli MJ, Barton SJ, Henderson AJ, et al. (2009) Copy-Number Variation Genotyping of GSTT1 and GSTM1 Gene Deletions by Real-Time PCR. *Clinical Chemistry* 55(9): 1680-1685.

Routledge MN, Kimanya ME, Shirima CP, et al. (2014) Quantitative correlation of aflatoxin biomarker with dietary intake of aflatoxin in Tanzanian children. *Biomarkers* 19(5): 430-435.

Roy B, Chowdhury A, Kundu S, et al. (2001) Increased risk of antituberculosis drug-induced hepatotoxicity in individuals with glutathione S-transferase M1 'null' mutation. *J Gastroenterol Hepatol* 16(9): 1033-1037.

Saad-Hussein A, Shahy EM, Shaheen W, et al. (2016) Comparative Hepatotoxicity of Aflatoxin B1 among Workers Exposed to Different Organic Dust with Emphasis on Polymorphism Role of Glutathione S-Transferase Gene. *Open Access Maced J Med Sci* 4(2): 312-318.

Salem AH, Yaqoob A, Ali M, et al. (2011) Genetic polymorphism of the glutathione S-transferase M1 and T1 genes in three distinct Arab populations. *Dis Markers* 31(5): 311-316.

Shen L, Li JQ, Zeng MD, et al. (2006) Correlation between ultrasonographic and pathologic diagnosis of liver fibrosis due to chronic virus hepatitis. *World J Gastroenterol* 12(8): 1292-1295.

Shimakawa Y, Yan HJ, Tsuchiya N, et al. (2013) Association of early age at establishment of chronic hepatitis B infection with persistent viral replication, liver cirrhosis and hepatocellular carcinoma: a systematic review. *PLoS One* 8(7): e69430.

Smith JW, Matchado AJ, Wu LS, et al. (2022) Longitudinal Assessment of Prenatal, Perinatal, and Early-Life Aflatoxin B(1) Exposure in 828 Mother-Child Dyads from Bangladesh and Malawi. *Curr Dev Nutr* 6(2): nzab153.

Sonderup MW and Spearman CW (2022) Global Disparities in Hepatitis B Elimination-A Focus on Africa. *Viruses* 14(1).

Song K, Yi J, Shen X, et al. (2012) Genetic Polymorphisms of Glutathione S-Transferase Genes GSTM1, GSTT1 and Risk of Hepatocellular Carcinoma. *PLOS ONE* 7(11): e48924.

Sprenger R, Schlagenhaufer R, Kerb R, et al. (2000) Characterization of the glutathione S-transferase GSTT1 deletion: discrimination of all genotypes by polymerase chain reaction indicates a trimodular genotype-phenotype correlation. *Pharmacogenetics* 10(6): 557-565.

Sui Y, Lu Y, Zuo S, et al. (2022) Aflatoxin B1 Exposure in Sheep: Insights into Hepatotoxicity Based on Oxidative Stress, Inflammatory Injury, Apoptosis, and Gut Microbiota Analysis. *Toxins* 14(12): 840.

Sumon AH, Islam F, Mohanto NC, et al. (2021) The Presence of Aflatoxin M(1) in Milk and Milk Products in Bangladesh. *Toxins (Basel)* 13(7).

Sun CA, Wang LY, Chen CJ, et al. (2001) Genetic polymorphisms of glutathione S-transferases M1 and T1 associated with susceptibility to aflatoxin-related hepatocarcinogenesis among chronic hepatitis B carriers: a nested case-control study in Taiwan. *Carcinogenesis* 22(8): 1289-1294.

Taye W, Ayalew A, Chala A, et al. (2016) Aflatoxin B(1) and total fumonisin contamination and their producing fungi in fresh and stored sorghum grain in East Hararghe, Ethiopia. *Food Addit Contam Part B Surveill* 9(4): 237-245.

Tesfaye BT, Feyissa TM, Workneh AB, et al. (2021) Chronic Liver Disease in Ethiopia with a Particular Focus on the Etiological Spectrums: A Systematic Review and Meta-Analysis of Observational Studies. *Can J Gastroenterol Hepatol* 2021: 8740157.

Vivian H, Kelly J and Jef LL (2018) The impact of reducing dietary aflatoxin exposure on child linear growth: a cluster randomised controlled trial in Kenya. *BMJ Global Health* 3(6): e000983.

VoPham T, Bertrand KA, Hart JE, et al. (2017) Pesticide exposure and liver cancer: a review. *Cancer Causes Control* 28(3): 177-190.

Wang L, Huang Q, Wu J, et al. (2022) The metabolism and biotransformation of AFB1: Key enzymes and pathways. *Biochemical Pharmacology* 199: 115005.

Wangia RN, Tang L and Wang J-S (2019) Occupational exposure to aflatoxins and health outcomes: a review. *Journal of Environmental Science and Health, Part C* 37(4): 215-234.

Watanabe I, Tomita A, Shimizu M, et al. (2003) A study to survey susceptible genetic factors responsible for troglitazone-associated hepatotoxicity in Japanese patients with type 2 diabetes mellitus. *Clin Pharmacol Ther* 73(5): 435-455.

Wild CP and Gong YY (2010) Mycotoxins and human disease: a largely ignored global health issue. *Carcinogenesis* 31(1): 71-82.

Wild CP, Jiang YZ, Sabbioni G, et al. (1990) Evaluation of methods for quantitation of aflatoxin-albumin adducts and their application to human exposure assessment. *Cancer Res* 50(2): 245-251.

Xu Y, Gong YY and Routledge MN (2018) Aflatoxin exposure assessed by aflatoxin albumin adduct biomarker in populations from six African countries: REVIEW ARTICLE. *World Mycotoxin J* 11(3): 411-419.

Ya X, Sophie M, Gaoyun C, et al. (2021) Impact of dietary aflatoxin on immune development in Gambian infants: a cohort study. *BMJ Open* 11(7): e048688.

Yen YH, Kuo FY, Chen CH, et al. (2019) Ultrasound is highly specific in diagnosing compensated cirrhosis in chronic hepatitis C patients in real world clinical practice. *Med* 98(27):e16270.

Yenasew A (2019) Aflatoxin Contamination Level of Different Crops in Ethiopia. *International Journal of Bioorganic Chemistry* 4: 42.

Zanotti S, Boot GF, Coto-Llerena M, et al. (2022) The Role of Chronic Liver Diseases in the Emergence and Recurrence of Hepatocellular Carcinoma: An Omics Perspective. *Front Med (Lausanne)* 9: 888850.

Zentai A, Jóźwiak Á, Süth M, et al. (2023) Carry-Over of Aflatoxin B1 from Feed to Cow Milk-A Review. *Toxins (Basel)* 15(3).

Zhang Y, Meng X, Ma Z, et al. (2023) Association of Androgen-Receptor Gene Mutations with the Copy Number of Androgen-Receptor Silk Protein A Complex and Glutathione-S-Transferases T1 and M1 in Prostate Cancer Patients. *Genet Res (Camb)* 2023: 5956951.

Zuñiga-Aguilar E and Ramírez-Fernández O (2022) Fibrosis and hepatic regeneration mechanism. *Transl Gastroenterol Hepatol* 7: 9.

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Paper I: Liver cirrhosis of unknown etiology and its predictors in Eastern Ethiopia

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ORIGINAL RESEARCH

Liver Cirrhosis of Unknown Etiology and Its Predictors in Eastern Ethiopia

Abraham Nigussie Mekuria[1], Teshome Nedi[1], Yun Yun Gong[2], Teferra Abula[1], Ephrem Engidawork[1]

[1]Department of Pharmacology and Clinical Pharmacy, School of Pharmacy, College of Health Sciences, Addis Ababa University, Addis Ababa, Ethiopia; [2]School of Food Science and Nutrition, University of Leeds, Leeds, UK

Correspondence: Ephrem Engidawork; Teferra Abula, Email ephrem.engidawork@aau.edu.et; abula_teferra@yahoo.com

Background: The global burden of liver cirrhosis is increasing, with 2.1 million incident cases and nearly 1.5 million deaths in 2019. Despite the enormous progress in our understanding of the etiology of liver cirrhosis, significant cases of the disease have been reported in Eastern Ethiopia due to unidentified causes. Hence, this study aimed to identify predictors of liver cirrhosis of unknown etiology in Eastern Ethiopia.

Methods: A score of 7 out of 11 possible points on the ultrasound-based cirrhosis scale was used as a diagnostic criterion to include 127 liver cirrhosis patients. The study participants' demographic, dietary, lifestyle, and clinical data were gathered using a structured questionnaire and standardized reporting forms. The associations between the outcome (known and unknown etiology) and independent variables were modeled using binary logistic regression analysis.

Results: The etiology of liver cirrhosis was known in only 23% of patients and attributed to hepatitis B virus (21%), hepatitis C virus (0.8%), and alcohol abuse (0.8%). Sorghum consumption as a staple food (adjusted odds ratio (AOR) =3.8; 95% CI: 1.2, 12.5), splenomegaly (AOR = 4.0; 95% CI: 1.1, 14.4), and a family history of liver disease (AOR = 0.24; 95% CI: 0.06, 0.91) were significantly associated with liver cirrhosis of unknown etiology.

Conclusion: Sorghum consumption was found to be the determinant factor of liver cirrhosis of unknown etiology, suggesting it as a possible source of exposure to aflatoxin B_1.

Keywords: liver cirrhosis, unknown etiology, sorghum, splenomegaly, family history of liver disease, Ethiopia

Introduction

Liver cirrhosis, known to be a well-established risk factor for hepatocellular carcinoma (HCC), represents a late stage of progressive hepatic fibrosis characterized by distortion of the hepatic architecture and formation of regenerative nodules.[1] Globally, the number of incident cases of liver cirrhosis reached 2.1 million and contributed to nearly 1.5 million deaths in 2019.[2] Numerous etiological factors, the prevalence of which varies geographically, have been linked to liver cirrhosis.[3] Among the major causes, alcoholic liver disease, hepatitis C virus (HCV) infection, and non-alcoholic fatty liver disease are the most common causes in Western and industrialized countries, whereas chronic hepatitis B virus

(HBV) infectionis the main etiologic factor in Asia and Africa.[4,5] In sub-Saharan Africa, 69% of liver cirrhosis cases were attributed toHBV, HCV, and alcohol consumption. However, the etiology was unknown in the remaining percentage of cases.[4]Similarly, a systematic review and meta-analysis study revealed that HBV and HCV infections, as well as alcoholconsumption, are the three most important causes of liver cirrhosis in Ethiopia. Nevertheless, the cause is yet to be identified in 45% of the cases.[6]

In Eastern Ethiopia, despite the high burden of liver cirrhosis of unknown etiology, fewer efforts have been made to explore its potential determinants. In this regard, our recent case–control study found aflatoxin B_1 (AFB_1) exposure asa potential missing etiologic factor of liver cirrhosis in Eastern Ethiopia, where significant cases of the disease were cryptogenic.[7] Thus, it is imperative to explore the potential predictive factors for the unknown etiology of liver cirrhosis to obtain clues for further missing etiologies and for potential sources of exposure to AFB_1 and its predictors. Hence, this study aimed to identify the possible sociodemographic, dietary, lifestyle, and clinical factors associated with the unknown etiology of liver cirrhosis in Eastern Ethiopia.

Materials and Methods

Study Setting and Subjects

This is part of a study conducted in a tertiary hospital in Harar, Eastern Ethiopia, to explore risk factors for liver cirrhosis from 1 January 2020 to 31 July 2021.[7] In short, the study included 253 controls who had no history of liver disease and 127 verified instances of hepatic cirrhosis. After explaining the study's objectives to the participants', written consent was obtained, and a professional interviewer completed a thorough questionnaire for each case and control. Only liver cirrhosis patients (127 cases) were included in this study to identify factors associated with the unknown etiology of liver cirrhosis in Eastern Ethiopia with a high burden of unexplained cases.

Operational Definition

Patients were screened for the presence of the most important etiologies of liver cirrhosis in Ethiopia, namely, HBV, HCV, and alcohol abuse.[6] Those patients whose etiologic factor was not related to one of those mentioned etiologies were categorized as "patients with an unknown etiology of liver cirrhosis", and vice versa.

Statistical Analysis

Percentages were used for categorical variables. Continuous variables were thought to have a nonnormal distribution and were expressed as medians (inter-quartile range (IQR)). To compare the features of patients with known and unknown etiologies of liver cirrhosis, the Mann–Whitney *U*-test and $\chi 2$ or Fisher exact tests were used for continuous variables andcategorical variables, respectively. In the univariate analysis, variables with $p<0.25$ were scrutinized and fitted tomultivariable analysis along with biologically relevant variables (age and gender) to identify predictive factors of unknown etiology for liver cirrhosis. Odds ratios (ORs) along with a 95% confidence interval (CI) were calculated to determine the strength of the relationships described above. The level of significance was considered at $p<0.05$ in the final logistic regression model. Statistical analysis was performed using Statistical Package for Social Sciences (SPSS) version 26.0.

Ethical Considerations

Ethical clearance was obtained from the Institutional Review Board of the College of Health Sciences, Addis Ababa University (Protocol No. 064/19/SOP and Reference No. CHS/RTTD/229/2020) and the National Ethical Review Committee of the Ministry of Science and Higher Education (Reference No. 04/246/680/21). This study was conductedin accordance with the Declaration of Helsinki.

Results

The etiology of liver cirrhosis was determined in 29 (23%) of the 127 patients and attributed to HBV (21%), HCV (0.8%), and alcohol abuse (0.8%).

Sociodemographic, Lifestyle and Dietary Characteristics

As shown in Table 1, 66% of the study participants were males, and the median age was 32 years (IQR: 28–45). Majority of the patients were farmers (82%), from a rural area (84%), with no formal education (80%), and had consumed sorghum (80.3%) as a staple food (Table 1). The proportion of patients with no family history of liver disease and consume staple food was significantly higher ($p < 0.05$) in unknown etiology than known etiology group (Table 2).

Table 1 Sociodemographic Characteristics of Liver Cirrhosis Patientsin Eastern Ethiopia, 2020/21

Characteristics	Categories	Etiology	
		Unknown 98 (%)	Known 29 (%)
Gender	Male	63 (64)	21 (72)
	Female	35 (36)	8 (28)
Age	Median (IQR)	32 (27–46)	35 (30–47)
	<35 years	51 (52)	14 (48)
	35–44 years	19 (20)	4 (14)
	45–54 years	13 (13)	5 (17)
	> 55 years	15 (15)	6 (21)
Residence	Urban	14 (14)	6 (21)
	Rural	84 (86)	23 (79)
Formal education	No	78 (80)	24 (83)
	Yes	20 (20)	5 (17)
Occupation	Farmer	81 (82.7)	23 (79.3)
	Housewife	6 (6.1)	1 (3.4)
	Student	5 (5.1)	1 (3.4)
	Public servant	1 (1)	3 (10.3)
	Other[a]	5 (2)	1 (0)

Notes: N= 127; n for unknown =98; n for known =29; [a]Includes unemployed, dailylaborer, and merchant.
Abbreviation: IQR, inter quartile range.

Table 2 Lifestyle, Dietary and Other Characteristics of Liver Cirrhosis Patients in Eastern Ethiopia, 2020/21

Characteristics	Categories	Etiology	
		Unknown 98 (%)	Known 29 (%)
Family history of liver disease*	No	90 (92)	21 (72)
	Yes	8 (8)	8 (28)
Body mass index (BMI)	Normal	59 (60)	14 (48)
	Underweight	34 (35)	11 (38)
	Overweight	5 (5)	4 (14)
Herbal medicine use	No	89 (91)	26 (90)
	Yes	9 (9)	3 (10)
Khat use	No	38 (39)	10 (34)
	Yes	60 (61)	19 (66)
Tobacco use	No	78 (80)	21 (72)
	Yes	20 (20)	8 (28)
History of alcohol use	No	98 (100)	27 (93)
	Yes	0 (0)	2 (7)
Alcohol abuse	No	98 (100)	28 (97)
	Yes	0 (0)	1 (3)
Staple food*	Rice/Teff/Corn	14 (14)	11 (38)
	Sorghum	84 (86)	18 (62)
Source of staple food	Market	41 (42)	18 (62)
	Home	57 (58)	11 (38)
Staple food stored underground[a]	No	28 (49)	7 (64)
	Yes	29 (51)	4 (36)
Peanut intake	No intake	48 (49)	15 (52)
	Intermittent intake[b]	11 (11)	2 (7)
	Frequent intake[c]	39 (40)	12 (41)

Notes: N= 127; n for unknown =98; n for known =29; *p < 0.05 (from chi-square or Fisher exact test); [a] when the source is from home [b] 0.25 to 4 times per week; [c]At least once per day.

Clinical and Ultrasound Findings

Almost all (97%) patients presented with abdominal distention, as shown in Table 3. Patients with unknown etiology of liver cirrhosis were more likely to present with splenomegaly than those in whom the etiology was identified. Otherwise, there were no distinguishing clinical features between the two groups. The major findings on liver ultrasound were heterogeneous liver parenchyma (87%), undulant liver surface (66.0%), spleen size index >20cm^2 (87%), and ascites (96.0%). There was a significant difference (p<0.05) in terms of spleen size index between the two groups.

Laboratory Findings

More or less abnormal lab findings were observed in a somewhat higher percentage of patients with known etiology than patients with unknown etiology of liver cirrhosis. However, there were no significant differences in laboratory findings between the two groups (Table 4).

Table 3 Clinical and Ultrasound Features of Liver Cirrhosis Patients in Eastern Ethiopia, 2020/21

Characteristics	Categories	Etiology	
		Unknown 98 (%)	Known 29 (%)
Clinical features			
Jaundice	No	28 (29)	10 (34.5)
	Yes	70 (71)	19 (65.5)
Weight loss	No	24 (24.5)	10 (34.5)
	Yes	74 (75.5)	19 (65.5)
Epigastric pain	No	31 (42)	9 (31)
	Yes	57 (58)	20 (69)
Abdominal distension	No	3 (3)	1 (3)
	Yes	95 (97)	28 (97)
Fever	No	70 (71)	21 (72)
	Yes	28 (29)	8 (28)
Hematemesis	No	55 (76.5)	21 (72)
	Yes	23 (23.5)	8 (28)
Anorexia	No	18 (18)	3 (10)
	Yes	80 (82)	26 (90)
Splenomegaly*	No	9 (9)	8 (28)
	Yes	89 (91)	21 (72)
Ultrasound features			
Liver surface	Smooth	4 (4)	4 (14)
	Mild uneven surface	28 (29)	7 (24)
	Undulant surface	66 (67)	18 (62)
Liver parenchyma	Homogenous	2 (2)	0 (0)
	Heterogenous	85 (87)	25 (86)
	Coarse	11 (11)	4 (14)
Hepatic vessel	Smooth	62 (63)	17 (59)
	Obscured or blurred	28 (29)	9 (31)
	Irregular & narrowed	8 (8)	3 (10)
Spleen size index*	<20 cm^2	9 (9)	8 (28)
	>20 cm^2	89 (91)	21 (72)
Ascites	No	3 (3)	2 (7)
	Yes	95 (97)	27 (93)

Notes: N= 127; n for unknown =98; n for known =29; *p value < 0.05 from the chi-square or Fisher exact test.

Table 4 Laboratory Findings of Liver Cirrhosis Patients in Eastern Ethiopia, 2020/21

Laboratory Findings	Categories	Etiology	
		Unknown 98 (%)	Known 29 (%)
ALT (U/L)	Median (IQR)	40 (20–52)	48 (20–60)
	Normal	49 (50)	14 (48)
	Abnormal	49 (50)	15 (52)
AST (U/L)	Median (IQR)	48 (31–73)	58 (30–101)
	Normal	38 (39)	9 (31)
	Abnormal	60 (61)	20 (69)

ALP (U/L)	Median (IQR)	142 (89–243)	164 (95–265)
	Normal	56 (57)	15 (52)
	Abnormal	42 (43)	14 (48)
Bilirubin, direct (µmol/L)	Median (IQR)	9 (3–15)	10 (4–18)
	Normal	49 (50)	13 (45)
	Abnormal	49 (50)	16 (55)
Bilirubin, total (µmol/L)	Median (IQR)	18 (7–32)	16 (8–34)
	Normal	80 (82)	23 (79)
	Abnormal	18 (18)	6 (21)
Albumin (g/L)	Median (IQR)	31 (23–39)	27 (24–32)
	Normal	33 (34)	5 (17)
	Abnormal	65 (66)	24 (83)
Creatinine (µmol/L)	Median (IQR)	64 (48–82)	77 (49–96)
	Normal	94 (96)	25 (86)
	Abnormal	4 (4)	4 (14)
Platelet count (10^9/L)	Median (IQR)	185 (123–241)	192 (121–254)
	Normal	68 (69)	21 (72)
	Abnormal	30 (31)	8 (28)
APRI score[a]	< 0.7	43 (44)	10 (34.5)
	> 0.7	55 (56)	19 (65.5)
FIB-4 score[b]	< 3.25	69 (81)	21 (72)
	> 3.25	19 (19)	8 (28)
HBV positive	No	98 (100)	2 (7)
	Yes	0 (0)	27 (93)
HCV positive	No	98 (100)	28 (97)
	Yes	0 (0)	1 (3)

Notes: N= 127; n for unknown= 98; n for known= 29. ALT (8-40 U/L), AST (14-40 U/L), ALP (48-164 U/L), Bilirubin direct (0.4-8.8 µmol/L), Bilirubin total (3-38 µmol/L), Albumin (35-52 g/L), Creatinine (47-109 µmol/L), Platelet count (126-438 × 109/L). [a] APRI= (AST (U/L)/ULN of AST (U/L))/platelet count (109/L) × 100; [b] FIB-4= age (years) x AST (U/L)/ (platelet count (109/L) x √ALT (U/L)).
Abbreviations: ALP, Alkaline Phosphatase; ALT, Alanine Aminotransferase; APRI, Aspartate Aminotransferase to Platelets Ratio Index; AST, Aspartate Aminotransferase; FIB-4, Fibrosis Index Based on 4 Factors; HBV, hepatitis B Virus; HCV, hepatitis C Virus; IQR, inter quartile range.

Predictors for Unknown Etiology of Liver Cirrhosis

Staple foods, body mass index, family history of liver disease, splenomegaly, age, gender, and abnormal serum levels of albumin and creatinine were entered into the final logistic regression model. Family history of liver disease, consumption of sorghum as a staple food, and splenomegaly were found to be significantly associated with unknown etiology of liver cirrhosis (Table 5). Accordingly, patients with a family history of liver disease had a decreased odds (AOR=0.24; 95% CI: 0.06, 0.91) of developing liver cirrhosis of unknown etiology by 76% compared with those without a family history. Patients who consumed sorghum as a staple food were 3.8 times (AOR=3.8; 95% CI: 1.2, 12.5) more likely to be diagnosed with liver cirrhosis of unknown etiology. Likewise, patients with splenomegaly had 4.0 times (AOR=4.0; 95% CI: 1.1, 14.4) higher odds of having liver cirrhosis of unknown etiology than patients without splenomegaly (Table 5).

Table 5 Predictors of Unknown Etiology of Liver Cirrhosis in Eastern Ethiopia, 2020/21

Variables	Categories	Etiology		COR (95% CI)	AOR (95% CI)
		Unknown 98 (%)	Known 29 (%)		
Gender	Male	63 (64)	21 (72)	0.69 (0.28, 1.71)	0.72 (0.23, 2.22)
	Female	35 (36)	8 (28)	1.00	1.00
Age	<35 years	51 (52)	14 (48)	1.00	1.00
	35–44 years	19 (20)	4 (14)	1.30 (0.38, 4.46)	1.75 (0.40, 7.60)
	45–54 years	13 (13)	5 (17)	0.71 (0.22, 2.34)	0.77 (0.19, 3.17)
	> 55 years	15 (15)	6 (21)	0.69 (0.23, 2.10)	0.67 (0.17, 2.72)
Family history of liver disease	No	90 (92)	21 (72)	1.00	1.00
	Yes	8 (8)	8 (28)	0.23 (0.08, 0.69)	0.24 (0.06, 0.91)*
Body mass Index	Normal	59 (60)	14 (48)	1.00	1.00
	Underweight	34 (35)	11 (38)	0.7 (0.3, 1.8)	0.55 (0.19, 1.59)
	Overweight	5 (5)	4 (14)	0.3 (0.07, 1.3)	0.49 (0.09, 2.83)
Staple food	Rice/Teff/ Corn	14 (14)	11 (38)	1.00	1.00
	Sorghum	84 (86)	18 (62)	7.5 (2.2, 25.5)	3.8 (1.2, 12.5)*
Splenomegaly	No	9 (9)	8 (28)	1.00	1.00
	Yes	89 (91)	21 (72)	0.3 (0.09, 0.77)	4.0 (1.1, 14.4)*
Serum albumin	Normal	33 (34)	5 (17)	1.00	1.00
	Abnormal	65 (66)	24 (83)	2.4 (0.85, 6.97)	0.58 (0.18, 1.84)
Serum creatinine	Normal	94 (96)	25 (86)	1.00	1.00
	Abnormal	4 (4)	4 (4)	3.8 (0.88, 16.1)	0.22 (0.04, 1.21)

Notes: N= 127; n for unknown =98; n for known =29; *$p < 0.05$.
Abbreviations: AOR, adjusted odds ratio; CI, confidence interval; COR, crude odds ratio.

Discussion

Despite the assumed heavy burden of liver cirrhosis of unknown etiology, limited data have been available on its predictors in Eastern Ethiopian populations.[8,9] In this study, the magnitude of liver cirrhosis of unknown etiology was 77%, which is higher than that in a previous study performed among chronic liver disease patients in Eastern Ethiopia (55%).[8] This discordance might be due to the focus of our study on the major etiologies of liver cirrhosis in Ethiopia and the difference in the prevalence of HBV infection detected in the studies (21% vs 37%). However, the prevalence of HBV infection in our study was similar to that in a study performed at Addis Ababa (20%).[10] Thus, further investigation is needed to determine other risk factors that contribute to liver cirrhosis in Eastern Ethiopia.

In the current study, majority of the participants consumed sorghum as a staple food (80%), and a strong and significant association was noticed between the consumption of sorghum and liver cirrhosis of unknown etiology. It is known that sorghum flour alone or mixed with other cereals is used for making Injera, a local circular pancake used for serving a common meal known as "La fiso" in Eastern Ethiopia.[11] Meanwhile, majority of the farmers in Eastern Ethiopia store sorghum grain in underground pits. These pits usually elevate grain moisture and storage temperature to levels that favor insect pests and fungi contamination, causing grain spoilage.[12]

In support of this, several studies performed in the area reported a high level of AFB_1 in sorghum grain and sorghum Injera samples,[13–17] when compared against the limits set by the East African Community (EAC) (5 µg/kg for AFB_1 and 10 µg/kg for total aflatoxins).[18] Accordingly, aflatoxin

analysis performed on fresh or stored sorghum samples as well as sorghum injera samples collected from this region revealed AFB_1 concentrations ranging from 10 to 53.3 μg/kg.[15,16]

The present study also revealed that patients with splenomegaly were more likely to have an unknown etiology of livercirrhosis. Although there are no previous observational studies that reported such differences between patients with known and unknown etiology of liver cirrhosis in Ethiopia, splenomegaly has been known to be the most common sign and symptom of liver cirrhosis,[19] and based on the present study, the occurrence seemed to be higher in patients with unknown etiology. Splenomegaly is known to be associated with continued exposure to environmental oxidants[20–23] and parasitic diseases, including *Schistosoma mansoni*.[24] It is generally accepted that *S. mansoni* infection alone does not lead to cirrhosis and is known to be characterized by periportal fibrosis associated with portal hypertension in the presence of preserved liver parenchyma and function.[25] This, however, was not observed in our patients, ruling out the role of *S. mansoni*. A previous study performed in the same area reported toxic liver injury in patients with unknown etiology of the disease and found an association with khat consumption, a commonly used psychostimulant in the Horn of Africa, including Ethiopia.[8,9] However, in our study, there was no detectable difference between patients with known and unknown etiology in terms of khat chewing habits, casting doubt on the role of khat. On the other hand, a preliminary study performed in Kenya involving school children reported a 43% increase in the prevalence of hepatomegaly or hepatosplenomegaly for each natural-log-unit increase in AFB_1-albumin adduct level after adjusting for *S. mansoni* and *Plasmodium* infections.[20]

Patients with a family history of liver disease were less likely to be diagnosed with liver cirrhosis of unknown etiology. According to studies, various established etiologies of liver cirrhosis involve inflammation and oxidative stress as hallmarks of pathogenesis.[26,27] In this regard, variations in genes involved in the antioxidant enzymes,[28] DNA repair pathway,[29] immuno- logically important signaling pathways, and other players in combating oxidative damage were identified as predisposing factors of the disease.[30] Hence, patients with a family history of liver disease would have a lower likelihood of developing cryptogenic hepatic cirrhosis, which is no surprise. To the best of our knowledge, this study is the first to provide an impetus for further investigation and action on the association between dietary staple foods and the risk of cirrhosis of unknown etiology in Ethiopia, where people feed on a diet contaminated with a high burden of AFB_1. However, we acknowledge that due to the inherent limitation of the study design, this study cannot establish an association or possible causality between the exposures and outcome.

Conclusions

In this study, majority of the patients with liver cirrhosis had an unknown etiology. Sorghum consumption, splenomegaly, and family history of liver disease were found to be predictors of unexplained cases of the disease. The strong association of sorghum consumption with liver cirrhosis of unknown etiology and the available evidence of its contamination with AFB_1 suggest sorghum consumption as a possible source of exposure to AFB_1 in Eastern Ethiopia.

Ethics Statement

Ethical clearance was obtained from the Institutional Review Board of the College of Health Sciences, Addis Ababa University (Protocol No. 064/19/SOP and Reference No.

CHS/RTTD/229/2020) and the National Ethical Review Committee of the Ministry of Science and Higher Education (Reference No. 04/246/680/21). This study was conductedin accordance with the Declaration of Helsinki.

Author Contributions

All authors made a significant contribution to the work reported whether that is in the conception, study design, execution, acquisition of data, analysis, and interpretation, or all these areas; took part in drafting, revising, or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

References

1. Zuñiga-Aguilar E, Ramírez-Fernández O. Fibrosis and hepatic regeneration mechanism. *Transl Gastroenterol Hepatol*. 2022;7:9. doi:10.21037/tgh.2020.02.21
2. Lan Y, Wang H, Weng H, et al. The burden of liver cirrhosis and underlying etiologies: results from the Global Burden of Disease Study 2019. *Hepatol Commun*. 2023;7(2):e0026–e0026. doi:10.1097/HC9.0000000000000026
3. Wong MCS, Huang J. The growing burden of liver cirrhosis: implications for preventive measures. *Hepatol Int*. 2018;12(3):201–203. doi:10.1007/s12072-018-9865-y
4. Mokdad AA, Lopez AD, Shahraz S, et al. Liver cirrhosis mortality in 187 countries between 1980 and 2010: a systematic analysis. *BMC Med*. 2014;12(1):145. doi:10.1186/s12916-014-0145-y
5. Zhai M, Long J, Liu S, et al. The burden of liver cirrhosis and underlying etiologies: results from the global burden of disease study 2017. *Aging*. 2021;13(1):279–300. doi:10.18632/aging.104127
6. Tesfaye BT, Feyissa TM, Workneh AB, et al. Chronic Liver Disease in Ethiopia with a Particular Focus on the Etiological Spectrums: a Systematic Review and Meta-Analysis of Observational Studies. *Can J Gastroenterol Hepatol*. 2021;2021:8740157. doi:10.1155/2021/8740157
7. Mekuria A, Xia L, Ahmed TA, et al. Contribution of Aflatoxin B_1 Exposure to Liver Cirrhosis in Eastern Ethiopia: a Case-Control Study. *Int J Gen Med*. 2023;16:3543–3553. doi:10.2147/IJGM.S425992
8. Orlien SMS, Ismael NY, Ahmed TA, et al. Unexplained chronic liver disease in Ethiopia: a cross-sectional study. *BMC Gastroenterol*. 2018;18(1):27.
9. Orlien SMS, Sandven I, Berhe NB, et al. Khat chewing increases the risk for developing chronic liver disease: a hospital-based case-control study. *Hepatol*. 2018;68(1):248–257. doi:10.1002/hep.29809
10. Samson E, Bisrat D, Hailu D, et al. Liver Disease: a Retrospective Hospital Based Study in Addis Ababa-Ethiopia. *J Spleen Liver Res*. 2021;1(4):1–7. doi:10.14302/issn.2578-2371.jslr-21-3912
11. Mekbib F. Folksong based appraisal of bioecocultural heritage of sorghum (Sorghum bicolor (L.) Moench): a new approach in ethnobiology. *J Ethnobiol Ethnomed*. 2009;5:19. doi:10.1186/1746-4269-5-19
12. Dejene M, Yuen J, Sigvald R. Effects of storage methods, storage time and different agro-ecological zones on chemical components of stored sorghum grain in Hararghe, Ethiopia. *J Stored Prod Res*. 2006;42(4):445–456. doi:10.1016/j.jspr.2004.07.006
13. Chala A, Taye W, Ayalew A, et al. Multimycotoxin analysis of sorghum (Sorghum bicolor L. Moench) and finger millet (Eleusine coracana L. Garten) from Ethiopia. *Food Control*. 2014;45:29–35. doi:10.1016/j.foodcont.2014.04.018
14. Taye W, Ayalew A, Chala A, et al. Aflatoxin B(1) and total fumonisin contamination and their producing fungi in fresh and stored sorghum grain in East Hararghe, Ethiopia. *Food Addit Contam Part B Surveill*. 2016;9(4):237–245. doi:10.1080/19393210.2016.1184190
15. Marie S. *Level of Aflatoxin in Sorghum Injera from Eastern Ethiopia. M.Sc. Thesis*. Addis Ababa University; 2017.
16. Mamo FT, Abate BA, Tesfaye K, et al. Mycotoxins in Ethiopia: a Review on Prevalence. *Economic Health Impacts Toxins*. 2020;12(10):65.
17. Mohammed A, Bekeko Z, Yusufe M, et al. Fungal Species and Multi-Mycotoxin Associated with Post-Harvest Sorghum (Sorghum bicolor (L.) Moench) Grain in Eastern Ethiopia. *Toxins*. 2022;14(7):473. doi:10.3390/toxins14070473
18. Boni S, Beed F, Kimanya M, et al. Aflatoxin contamination in Tanzania: quantifying the problem in maize and groundnuts from rural households. *World Mycotoxin J*. 2021;14(4):1–12. doi:10.3920/WMJ2020.2646
19. Li L, Duan M, Chen W, et al. The spleen in liver cirrhosis: revisiting an old enemy with novel targets. *J Transl Med*. 2017;15(1):111. doi:10.1186/s12967-017-1214-8
20. Gong YY, Wilson S, Mwatha JK, et al. Aflatoxin exposure may contribute to chronic hepatomegaly in Kenyan school children. *Environ Health Perspect*. 2012;120(6):893–896. doi:10.1289/ehp.1104357
21. Makhdoumi P, Hossini H, Ashraf GM, et al. Molecular Mechanism of Aniline Induced Spleen Toxicity and Neuron Toxicity in Experimental Rat Exposure: a Review. *Curr Neuropharmacol*. 2019;17(3):201–213. doi:10.2174/1570159X16666180803164238
22. Benkerroum N. Chronic and Acute Toxicities of Aflatoxins: mechanisms of Action. *Int J Environ Res Public Health*. 2020;17(2):423. doi:10.3390/ijerph17020423
23. Sule RO, Condon L, Gomes AV. A Common Feature of Pesticides: oxidative Stress—The Role of Oxidative Stress in Pesticide-Induced Toxicity. *Oxid Med Cell Longev*. 2022;2022:5563759. doi:10.1155/2022/5563759
24. Cosenza-Contreras M, de Oliveira ECRA, Mattei B, et al. The Schistosomiasis SpleenOME: unveiling the Proteomic Landscape of Splenomegaly Using Label-Free Mass Spectrometry. *Front Immunol*. 2018;9:3137. doi:10.3389/fimmu.2018.03137
25. Santos JC, Pereira CLD, Domingues ALC, et al. Noninvasive diagnosis of periportal fibrosis in schistosomiasis mansoni: a comprehensive review. *World J Hepatol*. 2022;14(4):696–707. doi:10.4254/wjh.v14.i4.696

26. Seen S. Chronic liver disease and oxidative stress – a narrative review. *Exp Rev Gastroenterol Hepatol*. 2021;15(9):1021–1035. doi:10.1080/17474124.2021.1949289
27. Conde de la Rosa L, Goicoechea L, Torres S, et al. Role of Oxidative Stress in Liver Disorders. *Livers*. 2022;2(4):283–314.
28. Prysyazhnyuk V, Sydorchuk L, Sydorchuk R, et al. Glutathione-S-transferases genes-promising predictors of hepatic dysfunction. *World J Hepatol*. 2021;13(6):620–633. doi:10.4254/wjh.v13.i6.620
29. Rybicka M, Woziwodzka A, Sznarkowska A, et al. Liver Cirrhosis in Chronic Hepatitis B Patients Is Associated with Genetic Variations in DNA Repair Pathway Genes. *Cancers (Basel)*. 2020;12(11):3295. doi:10.3390/cancers12113295
30. Ashouri S, Khor SS, Hitomi Y, et al. Genome-Wide Association Study for Chronic Hepatitis B Infection in the Thai Population. *Front Genet*. 2022;13:887121. doi:10.3389/fgene.2022.887121

Paper II: Contribution of Aflatoxin B_1 Exposure to Liver Cirrhosis in Eastern Ethiopia: A Case-control Study

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ORIGINAL RESEARCH

Contribution of Aflatoxin B_1 Exposure to Liver Cirrhosis in Eastern Ethiopia: A Case-Control Study

Abraham Mekuria[1], Lei Xia[2], Tekabe Abdosh Ahmed[3], Solomon Bishaw[4], Zelalem Teklemariam[5], Teshome Nedi[1], Tefera Abula[1], Ephrem Engidawork[1], Yun Yun Gong[2]

[1]Department of Pharmacology and Clinical Pharmacy, School of Pharmacy, College of Health Sciences, Addis Ababa University, Addis Ababa, Ethiopia; [2]School of Food Science and Nutrition, University of Leeds, Leeds, UK; [3]Department of Internal Medicine, School of Medicine, College of Health and Medical Sciences, Haramaya University, Harar, Ethiopia; [4]Department of Radiology, School of Medicine, College of Health and Medical Sciences, Haramaya University, Harar, Ethiopia; [5]School of Medical Laboratory Sciences, College of Health and Medical Sciences, Haramaya University, Harar, Ethiopia

Correspondence: Ephrem Engidawork; Yun Yun Gong, Email ephrem.engidawork@aau.edu.et; y.gong@leeds.ac.uk

Background: Liver cirrhosis is a global health problem due to a large number of disability-associated life years and mortality. However, evidence is scarce on its causes in Eastern-Ethiopia, a place where there is a high prevalence of liver cirrhosis of unknown etiology. This study attempted to identify the risk factors related to liver cirrhosis in the area.

Methods: A case-control study was conducted at a tertiary care hospital from January 2020 to July 2021. Following diagnoses using an ultrasound-based cirrhosis scale, a total of 127 cases were identified and compared with 253 control patients. A structured questionnaire and data abstraction form were used to collect demographic, lifestyle, and clinical information. A blood sample was also taken from each participant for clinical chemistry, hepatitis B virus (HBV), and hepatitis C virus tests as well as for an aflatoxin B_1 (AFB_1) albumin adduct (AF-alb) assay. Binary logistic regression analysis was used to determine predictors of liver cirrhosis.

Results: AF-alb levels were detected in 75% of the cases and 64% of the controls, with a median (IQR) level of 11 pg/mg (5.5–25) and 7.0 pg/mg (4.3–20.5), respectively ($p<0.05$). Moreover, the number of subjects with high AF-alb levels (≥8.6 pg/mg) was greater in cases (45%, $p<0.05$)) than controls (28%). Age ≥55 years (adjusted odds ratio (AOR)=0.4; 95% CI: 0.2, 0.8), being a farmer (AOR= 3.0; 95% CI: 1.5, 6.0), family history of liver disease (AOR= 2.9; 95% CI: 1.1, 7.9), HBV seropositivity (AOR=4.0; 95% CI: 1.9, 8.8), and exposure to high levels of AF-alb (AOR=2.0; 95% CI: 1.1, 3.7) were significantly associated with liver cirrhosis.

Conclusion: This study found a strong link between AFB_1 exposure and liver cirrhosis. Mitigation of aflatoxin exposure and a better understanding of additional environmental risk factors like pesticides may be necessary to reduce the disease burden in Ethiopia.

Keywords: liver cirrhosis, aflatoxin, hepatitis B virus, Ethiopia

Introduction

Liver cirrhosis is characterized by distortion of the hepatic architecture and formation of regenerative nodules. It is a leading cause of morbidity and mortality in liver disease patients, accounting for 2.4% of all deaths globally in 2019.[1] In Ethiopia, it is considered the 7th leading cause of mortality, accounting for 24 deaths/100,000 population.[2] The treatment of liver cirrhosis has advanced dramatically in recent years. However, the prognosis remains bleak.[3] Thus, identifying and working against its preventable risk factors should be a priority agenda.

Liver cirrhosis has been known to be associated with obesity, non-alcoholic fatty liver disease, excessive alcohol intake, Hepatitis B or C virus (HBV or HCV) infection, autoimmune disorders,

cholestatic diseases, and iron overload.[1,4] Despite enormous progress in understanding the etiology of the disease, nearly one-third of the cases in sub-Saharan Africa are cryptogenic (cirrhosis of the liver of unknown etiology).[5] Indeed, a study done in the Eastern parts of Ethiopia revealed that up to 55% of liver cirrhosis cases were cryptogenic, and histopathological investigations indicated that these cases were characterized by toxic liver injury.[6]

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In this regard, dietary exposure to aflatoxin B_1 (AFB_1), a fungal toxin produced by *Aspergillus flavus* and *Aspergillus parasiticus*, has been implicated as a potential etiologic factor for liver cirrhosis in numerous epidemiological studies.[7–10] For instance, a recent study done in Guatemala, where maize is the primary staple cereal, known to be contaminated with AFB_1, found a significant association between AFB_1 exposure and liver cirrhosis.[9] In Eastern Ethiopia, several studies have shown severe contamination of principal dietary staple foods and cash crops including, sorghum, maize, and ground nut with AFB_1.[11–15] Despite these studies, there has been little direct evidence linking AFB_1 exposure to the etiology of liver cirrhosis. Human chronic exposure to AFB_1 can be assessed by determining the biomarker, blood aflatoxin albumin adduct (AF-alb), using a competitive enzyme-linked immunosorbent assay (ELISA) method. To this end, this study sought to investigate aflatoxin exposure as a potential risk factor for liver cirrhosis in Eastern Ethiopia.

Materials and Method

Study Setting and Design

A case-control study was conducted in Hiwot Fana Comprehensive Specialized University Hospital (HFCSUH), Harar, Eastern Ethiopia, from 1 January 2020 to 31 July 2021. The teaching hospital is affiliated with Haramaya University and its catchment population is nearly six million, living in Eastern Oromia, Dire Dawa City Administration, Harari, and Somali Regional States.

Study Population and Eligibility

Patients diagnosed with liver cirrhosis through abdominal imaging among those attending the Internal Medicine unit of the hospital during the study period were identified as cases. Patients attending the same unit and without any history or clinical evidence of liver disease were identified as controls. In cases, patients aged ≥18 years old and having a score of

≥7 based on an ultrasound-based cirrhosis scale[16,17] were included, whilst those with evidence of space-occupying hepatic lesions, and unable or unwilling to provide informed consent were excluded. For controls, patients aged ≥18 years old and having ultrasound scores of ≤4 were included, while those with elevated liver function tests (ALT or AST above 40 IU/L) and unable or unwilling to provide informed consent were excluded.

Data Collection and Management

A structured questionnaire was used to gather information on sociodemographic characteristics, alcohol consumption, *Catha edulis* (Vahl) Forssk. Ex Endl. (khat) chewing, and tobacco usage habits. To ensure consistency, the original English version was translated into local languages (Afan Oromo and Amharic) and then back-translated into English by multilingual specialists. Alcohol consumption in the past and present was collected and quantified in grams. Alcohol abuse was defined as daily alcohol use of more than 20 g in women and more than 30 g in men for a minimum of 6 months.[18] The amount of khat, a herb chewed for its amphetamine-like effect, consumed was measured in grams using a visual analog scale. On average, 100–300 g of fresh khat leaves is chewed in a typical session. Thus, khat use refers to chewing at least 200 g of khat daily for one year.[6] Tobacco including cigarette, pipe, and shisha usage[19] in the past and present was recorded.

Body Mass Index (BMI) was calculated to categorize as underweight (<18.5), normal (18.5–24.9), overweight (25.0–29.9), and obese (≥30.0).[20] Food consumption in the previous year was recorded using a food frequency questionnaire (FFQ).

Clinical Examination

A mobile digital color Doppler ultrasound machine (Model: SSI-8000, SonoScape Co. Ltd) was used to perform abdominal ultrasonography following a standard protocol. The diagnosis of liver cirrhosis was made by a physician and a radiologist based on an ultrasonography cirrhosis scale. The intrahepatic vascular type (score 1–3), liver parenchyma (score 1–3), liver surface (score 1–3), and spleen size index (score 1–2, 1 for a spleen size index of 20 cm^2, and 2 for a greater spleen size index) are components of the scoring system. Liver cirrhosis was diagnosed using a score of ≥ 7 out of 11 and normal liver would have a maximum score of 4 in this scale.

Blood Sample Collection and Analysis

A blood sample was drawn via venipuncture using the BD Vacutainer® blood collection system (Becton Dickinson, NJ, USA). The blood was collected in a serum separator tube (3–5 mL) and two EDTA tubes (2 mL and 3 mL). The serum was then used for liver function tests (LFTs), Hepatitis surface antigen B (HBsAg), HCV antibody tests, and serum creatinine tests. The blood collected in the first EDTA tube was used for platelet count. The blood collected in the 2nd EDTA tube was centrifuged at 1100–1300 g for 10 min at 4 °C to separate the plasma and stored at −80°C using a cryotube. The plasma was transported in dry ice to the University of Leeds, United Kingdom for AF-alb analysis.

Clinical Laboratory Tests

Cobas C-311m, a fully automated and closed clinical chemistry analyzer (Roche/Hitachi Cobas C-311, Roche diagnostics GmbH, Mannheim, Germany) was used to perform LFTs including aspartate transaminase (AST), alanine transaminase (ALT), Alkaline phosphatase (ALP), serum bilirubin (total and direct), albumin, and serum creatinine tests. DxH 800 hematology analyzer (Beckman Coulter, Germany) was used to conduct platelet count. Moreover, advanced quality one- step HBsAg test kit (InTec PRODUCTS, INC.) and a one-step HCV test kit (Guangzhou Wondfo Biotech Co. Ltd) were used for HBsAg and anti-HCV screening, respectively.

Aflatoxin B1-Albumin (AF-Alb) Adduct Analysis

The levels of AF-alb in plasma were measured by albumin extraction, enzyme digestion, Sep-Pak C18 column purification, and finally quantification of the adduct using a competitive ELISA method as previously described.[21] Three positive and one negative control samples were analyzed with each batch of plasma samples for quality control. Samples were measured in duplicates on two separate days, and measurements with a coefficient of variations of less than 25% were taken. Moreover, 10% of the total sample was randomly selected and the whole process was repeated to ensure reproducibility of the test. The limit of detection (LOD) was 3 pg AFB_1-lysine equivalents per mg of albumin (pg/ mg). The median detectable adduct levels among the study population was

measured and 8.6 pg/mg of albumin was used as a cutoff for low (<8.6 pg/mg;) and high (≥8.6 pg/mg) levels of AF-alb adduct.

Statistical Analysis

Statistical analysis was performed using Statistical Package for Social Sciences (SPSS) version 26.0. All continuous variables were deemed to have non-normal distribution and expressed as median (IQR). Whereas, percentages were used for categorical variables. To examine differences in the characteristics between cases and controls, the Mann–Whitney *U*-test was used for continuous variables, but $\chi 2$ or Fisher exact tests for categorical variables. Variables with a *p*-value less than 0.25 in the univariate analysis and also biologically relevant variables including gender and age were fitted to the multivariable logistic regression model to determine risk factors associated with liver cirrhosis. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated in the final logistic regression model. The level of significance was set at $p < 0.05$.

Ethical Considerations

This study was conducted following the Declaration of Helsinki. Ethical approval was obtained from both the Institutional Review Board of the College of Health Sciences, Addis Ababa University (Protocol No. 064/19/SOP and Reference No. CHS/RTTD/229/2020) and the National Ethical Review Committee of the Ministry of Science and Higher Education (Reference No. 04/246/680/21). Written informed consent was obtained from participants before the survey.

Results

A total of 163 patients with probable liver disease were initially identified as potential cases. After excluding 32 patients who did not fulfill the inclusion criteria (26 for ultrasound criteria and 6 for having hepatic focal lesions), 131 patients were enrolled in the study. Four patients were further excluded, as three of them refused to provide blood samples and it was impossible to draw blood from one patient. Regarding controls, 287 patients without any signs of liver disease were identified as potential controls. After excluding twenty-nine patients who did not fulfill the inclusion criteria (1 for ultrasound and 28 for LFT), 258 patients were enrolled in the study. Five patients were further excluded as they refused to provide blood samples. As a result, data for 127 cases and 253 controls were used for analysis.

Demographic Characteristics

The demographic characteristics of cases and controls are presented in Table 1. There was a preponderance of males in both cases (66%) and controls (57%), but no significant difference in gender was observed between the two groups (p=0.08). The median age of cases and controls was not statistically different (p=0.73) [32 years (IQR: 28–45) vs 35 years (IQR: 25–50)]. Majority (80%) of the cases and more than half (55%) of the controls had no formal education. A large proportion of cases (82%) were farmers compared to controls (53%). There were significant differences (p<0.05) between cases and controls in terms of residence, education, and occupation (Table 1).

Lifestyle Characteristics

Khat and tobacco use were more commonly observed in cases (72% and 22%) than controls (58% and 13%), with the use being significantly higher (p<0.05) in cases than controls. It is of note that

khat was a substance largely used more than tobacco in both groups (Table 2). Almost all (98%) of the cases and controls did not have a history of alcohol use. About 1/3 of the participants were underweight, and only a small proportion of cases (7%) and controls (5%) were overweight.

Medical and Aflatoxin B1 Exposure Characteristics

As shown in Table 3, a significant proportion of cases were HBV seropositive (23%) and had a family history of liver disease (13%) compared with controls (5.5% and 3%, respectively). HCV infections were found to be less common in

Table 1 Demographic Characteristics of Study Participants, Eastern Ethiopia, 2020/21

Characteristics	Cases 127 (%)	Controls 253 (%)
Gender		
Male	84 (66)	144 (57)
Female	43 (34)	109 (43)
Age, median (IQR)	32 (28–45)	35 (25–50)
<35	65 (51)	125 (49)
35–44	23 (18)	48 (19)
45–54	18 (14)	32 (13)
55 and above	21 (17)	48 (19)
Residence*		
Rural	107 (84)	175 (69)
Urban	20 (16)	78 (31)
Education*		
No formal education	102 (80)	140 (55)
Formal education	25 (18)	113 (45)
Occupation*		
Farmer	104 (82)	133 (53)
Housewife	7 (5)	31 (12)
Student	6 (5)	22 (9)
Public servant	4 (3)	31 (12)
Other[a]	6 (5)	36 (14)
Marital status		
Single	14 (11)	52 (20)
Married	110 (87)	194 (77)
Divorced/widowed	3 (2)	7 (3)

Notes: N= 380; n for cases=127; n for controls =253; IQR, inter quartile range; *p value < 0.05 (from chi-square or Fisher exact test); [a]Includes unemployed, daily laborer, and merchant.

Table 2 Lifestyle Characteristics of Study Participants, Eastern Ethiopia, 2020/21

Characteristics	Cases 127 (%)	Controls 253 (%)
Tobacco use*		
No	99 (78)	221 (87)
Yes	28 (22)	32 (13)
Khat use*		
No	36 (28)	107 (42)
Yes	91 (72)	146 (58)
History of alcohol use		
No	125 (98)	248 (98)
Yes	2 (2)	5 (2)
Alcohol abuse		
No	126 (99.2)	252 (99.6)
Yes	1 (0.8)	1 (0.4)
Body mass index		
Normal	73 (58)	162 (64)
Underweight	45 (35)	82 (32)
Overweight	9 (7)	12 (5)

Notes: N= 380; n for cases=127; n for controls =253; **p* value < 0.05 (from chi-square or Fisher exact test).

Table 3 Medical and Aflatoxin B_1 Exposure Characteristics of Study Participants, Eastern Ethiopia, 2020/21

Characteristics	Cases 127 (%)	Controls 253 (%)
Family history of liver disease*		
No	111 (87)	245 (97)
Yes	16 (13)	8 (3)
Herbal medicine use		
No	115 (91)	233 (92)
Yes	12 (9)	20 (8)
HBV positive*		
No	100 (79)	239 (94.5)
Yes	27 (21)	14 (5.5)
HCV positive		
No	126 (99.2)	252 (99.6)
Yes	1(0.8)	1 (0.4)
Detectable AF-alb adduct*		
No	32 (25)	91 (36)
Yes	95 (75)	162 (64)
AF-alb adduct levels, median (IQR)[a,*]	11 (5.5–25)	7.0 (4.3–20.5)
Low[b]	38 (30)	91 (36)
High[b]	57 (45)	71 (28)*

Notes: N= 380; n for cases=127; n for controls =253; **p* value < 0.05 (from chi-square or Fisher exact test). [a]AF-alb adduct concentration (pg/mg albumin); [b]Low and high were < 8.6 and ≥ 8.6 pg/mg albumin respectively.
Abbreviations: AF-alb adduct, aflatoxin B_1 (AFB_1) albumin adduct; HBV, Hepatitis B Virus; HCV, Hepatitis C Virus; IQR, inter quartile range.

both cases (0.8%) as well as controls (0.4%). AF-alb adducts were detected in 257 (68%) of the total 380 samples with a median level of 8.6 (4.7–21.9) pg/mg of albumin. AF-alb positive rate was significantly higher (p<0.05) in cases (75%) than in controls (64%). As shown in Figure 1 and Table 3, the median AF-alb adduct of cases [11 pg/mg (5.5–25)] was

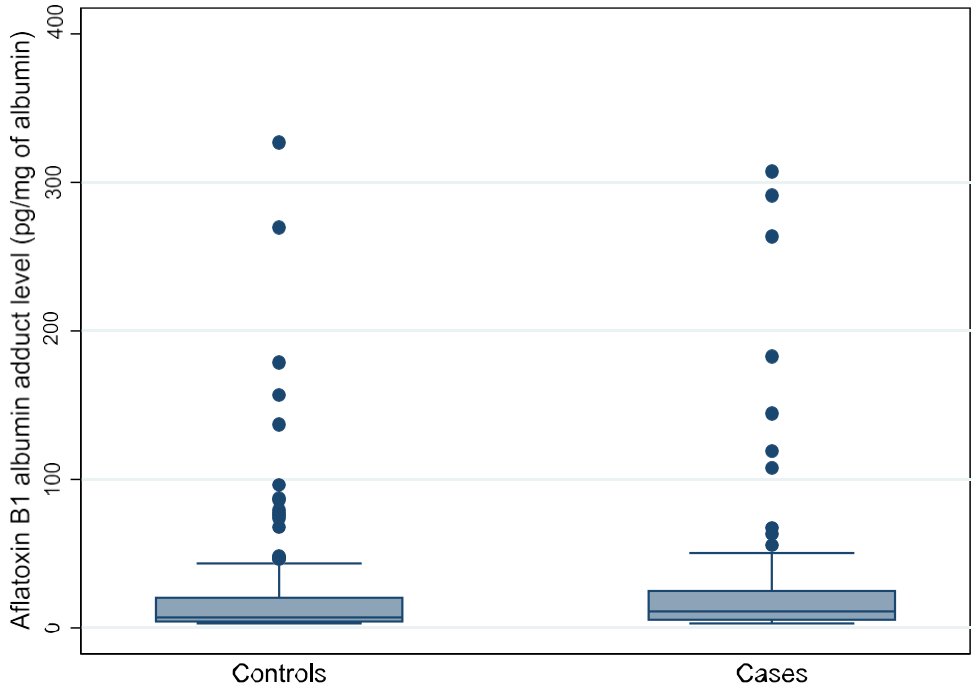

Figure 1 Box and whisker plot showing the distribution of aflatoxin B1-albumin adduct level in cases and controls. Dots represent outlier and extreme values.

significantly higher ($p<0.05$) than controls [7.0 pg/mg (4.3–20.5)]. Moreover, based on the cut-off value used in the present study, a significant proportion of cases (45%) had a higher level ($p<0.05$) of AF-alb compared to controls (28%).

Determinants of Liver Cirrhosis

Age, gender, residence, marital status, formal education, occupation, khat use, tobacco use, BMI, family history of liver disease, HBV status, and AF-alb levels were fitted in the multivariable logistic regression analysis model. Age 55 years and above, being a farmer, family history of liver disease, HBV seropositivity, and high level of AF-alb were found to be significantly associated with liver cirrhosis (Table 4). The odds of developing liver cirrhosis decreased by 60%

Table 4 Multivariable Binary Logistic Regression Analysis of Predictive Factors of Liver Cirrhosis in Eastern Ethiopia, 2020/21

Variables	Category	Cases n (%)	Control n (%)	COR (95% CI)	AOR (95% CI)
Gender	Male	84 (66)	144 (57)	1.5 (0.9, 2.3)	1.0 (0.6, 1.9)
	Female	43 (34)	109 (43)	1.00	1.00
Age category	<35	65 (51)	125 (49)	1.00	1.00
	35–44	23 (18)	48 (19)	0.9 (0.5, 1.6)	0.5 (0.3, 1.1)
	45–54	18 (14)	32 (13)	1.1 (0.6, 2.1)	0.7 (0.3, 1.5)
	55 and above	21 (17)	48 (19)	0.8 (0.5, 1.5)	0.4 (0.2, 0.8)*
Residence	Urban	20 (16)	78 (31)	1.00	1.00
	Rural	107 (84)	175 (69)	2.4 (1.4, 4.1)	1.0 (0.5, 2.0)
Marital status	Never married	14 (11)	52 (20)	0.5 (0.3, 0.9)	0.7 (0.3, 1.6)
	Married	110 (87)	194 (77)	1.00	1.00
	Divorced/Widowed	3 (2)	7 (3)	0.8 (0.2, 3.0)	1.4 (0.3, 7.6)
Formal education	No	102 (80)	140 (55)	1.00	1.00
	Yes	25 (20)	113 (45)	0.3 (0.2, 0.5)	0.6 (0.3, 1.3)

(*Continued*)

Table 4 (Continued).

Variables	Category	Cases n (%)	Control n (%)	COR (95% CI)	AOR (95% CI)
Occupation	Not farmer	23 (18)	120 (47)	1.00	1.00
	Farmer	104 (82)	133 (53)	4.0 (2.4, 6.8)	3.0 (1.5, 6.0)*
Khat use	No	48 (38)	132 (52)	1.00	1.00
	Yes	79 (62)	121 (48)	1.8 (1.2, 2.8)	1.3 (0.7, 2.4)
Tobacco use	No	99 (78)	221 (87)	1.00	1.00
	Yes	28 (22)	32 (13)	2.0 (1.1, 3.4)	1.4 (0.7, 2.8)
Body mass index	Normal	73 (58)	162 (64)	1.00	1.00
	Underweight	45 (35)	82 (32)	1.2 (0.8, 1.9)	1.4 (0.8, 2.3)
	Overweight	9 (7)	9 (4)	2.2 (0.8, 5.8)	1.8 (0.6, 5.7)
Family history of liver disease	No	111 (87)	245 (97)	1.00	1.00
	Yes	16 (13)	8 (3)	3.5 (1.5, 8.0)	2.9 (1.1, 7.9)*
HBV Positive	No	100 (79)	239 (94.5)	1.00	1.00
	Yes	27 (21)	14 (5.5)	4.6 (2.3, 9.2)	4.0 (1.9, 8.8)*
AF-alb adduct level	Nondetectable	32 (25)	91 (36)	1.00	1.00
	Low	38 (30)	91 (36)	1.2 (0.7, 2.1)	1.2 (0.6, 2.2)
	High	57 (45)	71 (28)	2.3 (1.3, 3.9)	2.0 (1.1, 3.7)*

Note: *p value < 0.05.
Abbreviations: AF-alb, aflatoxin B_1 (AFB_1)-albumin adduct; AOR, Adjusted Odds Ratio; COR, Crude Odds Ratio; HBV, Hepatitis B Virus.

(AOR=0.4; 95% CI: 0.2, 0.8) in patients with age 55 years and above compared with patients younger than 35 years. Patients who were farmers by occupation had 3 times higher odds of having liver cirrhosis than those who were not farmers (AOR= 3.0; 95% CI: 1.5, 6.0). The odds of liver cirrhosis were 2.9 times higher among patients with a family history of liver disease compared to patients without a family history (AOR= 2.9; 95% CI: 1.1, 7.9). HBV seropositive patients had 4 times higher odds of developing liver cirrhosis than HBV seronegative patients (AOR=4.0; 95% CI: 1.9, 8.8). Likewise, the odds of liver cirrhosis were 2 times higher among patients who were exposed to a high level of AF- alb adduct compared to patients with undetectable levels (AOR=2.0; 95% CI: 1.1, 3.7).

Discussion

Regardless of the substantial burden of the disease, limited knowledge of the risk factors or etiologic data have been available on liver cirrhosis or chronic liver disease from the Eastern Ethiopian population.[6,22] This study aims to provide a drive for further inquiry and action regarding AFB_1 exposure as an etiologic factor to liver cirrhosis in a region with a high burden of unexplained liver cirrhosis. The current study found that age 55 years and above, being a farmer, family history of liver disease, HBV seropositivity, and exposure to high levels of AF-alb adduct were significantly associated with liver cirrhosis.

In our study, the odds of developing liver cirrhosis were decreased in patients with age 55 years and above compared with patients younger than 35 years. In general, cirrhosis of any etiology is a major risk factor for hepatocellular carcinoma (HCC).[23] Currently, it is believed that HCC in sub-

Saharan Africa is a disease of young adults, unlike in North Africa, Japan, Western Europe, and North America, where it is common in the elderly.[24] In this regard, it is well known that sub-Saharan Africa has a relatively high incidence of HBV, with the majority of cases of exposure occurring during childhood through vertical or horizontal transmission.[25,26] Aflatoxin exposure is also known to have started in the earliest years of life.[27] The combination of these findings may thus partially explain why liver cirrhosis, or HCC, manifests itself at a younger age in sub-Saharan Africa than in the rest of the globe. Indeed, studies also revealed the influence of age at infection for the establishment of HBV infection and development of liver cirrhosis or HCC.[28,29]

On the other hand, patients who were farmers by occupation had higher odds of having liver cirrhosis. In this context, in addition to dietary exposure, farmers face a substantial risk of AFB_1 airborne exposure while managing infected grains as well as processing and handling animal feed.[30,31] This is further fueled by lack of awareness about mycotoxins, especially in resource-limited settings.[32,33] In support of this, our study demonstrated a significant ($p<0.01$) difference in the median levels of AF-alb adduct between patients who were farmers by occupation compared with patients who were not farmers (10.0 pg/mg, 5.3–29.4 vs 6.7 pg/mg, 4.3–14.7) (Supplementary Table 1). These findings together suggest that AFB_1 exposure might be one possible factor that made farmers to be at high risk for developing liver cirrhosis than non- farmers.

Moreover, numerous studies reported the link between hepatic toxicity and occupational exposure to pesticides in farmers residing in Africa and Asia due to poor safety practices.[34–36] In Ethiopia, the use of pesticides in small-scale farms has increased dramatically in the last decades and the situation of inappropriate use of pesticides due to poor knowledge and management of pesticide products is much more serious than commonly visualized.[37–39] In Eastern Ethiopia, the insecticide, Dichlorodiphenyltrichloroethane (DDT), has been extensively used to control insect pests in cash crop production including khat and its inappropriate use and management is of concern.[40,41] To this effect, a study conducted in rural northern Ethiopia to find out the cause of liver disease with unknown etiology detected high levels of DDT in urine and serum samples and inferred its possible association with the disease.[42] Interestingly, DDT exposure has been shown to enhance the toxic effect of environmental toxins, including AFB_1 through induction of CYP3A4.[43,44]

Our study also revealed that patients with a family history of liver disease were more likely to have liver cirrhosis than patients without a family history. Studies indicated that inflammation and oxidative stress play a pathogenic role in liver injury that progresses to liver cirrhosis due to exposure to viral and environmental factors.[45,46] This could be explained by genetic alterations in different cellular signaling pathways linked with DNA repair,[47] and immune response.[48] Furthermore, liver metabolic enzymes, both Phase I and Phase II, have a range of genetic polymorphisms that cause genetic predisposition in activation and/or detoxification functions, influence metabolism, and subsequently increase the toxicity of environmental chemical toxins like AFB_1.[49,50] Thus, it is not surprising that patients with a family history of liver disease would have a higher risk of experiencing liver cirrhosis.

Patients with HBV seropositive had 4 times higher odds of developing liver cirrhosis than HBV seronegative patients. This was consistent with previous findings done in different areas of the world,

including Ethiopia.[51,52] The mechanism by which chronic HBV infection predisposes to liver cirrhosis is still enigmatic. However, in several models of HBV infection, it has been identified that liver injuries in chronic infection are considered to be associated with the activity of HBV-specific T cells, as HBV is not directly cytopathic.[53,54] Moreover, its immuno-pathogenesis depends on an intricate interaction of host factors (such as age, gender, immune status, and genetics),[55] viral,[56] and environmental factors, including AFB_1 exposure.[8]

Our study demonstrated that the median AF-alb level was significantly higher among cases (11.0 pg/mg) than controls (7.0 pg/ mg), and patients exposed to high levels of AF-alb adduct were also at significantly higher risk of developing liver cirrhosis. The results of the present study are consistent with other studies reported from Africa,[7] Asia,[9] and South America,[9] where there is a high burden of aflatoxin exposure. The Gambian study also showed a synergy between AFB_1 exposure and HBV infection on the risk of liver cirrhosis.[7] Moreover, other studies done in Egypt,[57] Turkey,[58] and Taiwan[59] reported a significantly higher proportion of AFB_1 signature mutation in TP53, a higher mean level of AF-alb adduct, and a positive association of AF-alb adduct level and ultrasonographic parenchyma scores among patients with chronic liver disease or liver cirrhosis. As reviewed by Mekuria et al,[10] the toxic effects of AFB_1 against the liver are related to its intermediate metabolic product, AFB_1-exo-8,9-epoxide, formed by CYP3A4 and CYP1A2 enzymes in the hepatocytes and the associated formation of reactive oxygen species, leading to oxidative stress and induction of apoptosis through the mitochondrial signaling pathways.

Cases and controls were recruited using ultrasound examination that has up to 80% and 97% sensitivity and specificity, respectively, versus liver biopsy to diagnose liver cirrhosis.[60,61] Moreover, the ultrasound examination reduced the chance of including study participants with undiagnosed liver disease in the control group. Nevertheless, we acknowledge the following limitations of our study. First, due to the inherent limitation of the study design, this study cannot establish the temporal sequence between exposure and outcome. Second, because participants were asked about their previous experiences, our findings could be influenced by recall bias.

Conclusions

The detection of higher levels of AF-alb adduct in cases and its strong association with liver cirrhosis suggests that this fungal toxin could, at least, in part explain the missing etiologic factor for the high burden of unexplained liver cirrhosis in the region. Interventions to limit aflatoxin exposure, as well as efforts to identify the role of other risk factors for liver cirrhosis, may be essential in reducing the high burden of liver cirrhosis in Eastern Ethiopia.

Ethics Statement

This study was conducted following the Declaration of Helsinki. Ethical approval was obtained from both the Institutional Review Board of the College of Health Sciences, Addis Ababa University (Protocol No. 064/19/SOP and Reference No. CHS/RTTD/229/2020) and the National Ethical Review Committee of the Ministry of Science and Higher Education (Reference No. 04/246/680/21).

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Author Contributions

All authors made a significant contribution to the work reported whether that is in the conception, study design, execution, acquisition of data, analysis, and interpretation, or in all these areas; took part in drafting, revising, or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Disclosure

The authors declare that there are no conflicts of interest in this work.

References

1. Huang DQ, Terrault NA, Tacke F, et al. Global epidemiology of cirrhosis — aetiology, trends and predictions. *Nat Rev Gastroenterol Hepatol*. 2023;20 (6) :388–398.
2. World Health Organization. Global health estimates 2020: deaths by cause, age, sex, by country and by region, 2000–2019. Available from: https://who.int/data/gho/data/themes/mortality-andglobal-health-estimates/ghe-leadingcauses-of-death. Accessed May 28, 2023.
3. Tapper EB, Ufere NN, Huang DQ, et al. Review article: current and emerging therapies for the management of cirrhosis and its complications. *Aliment Pharmacol Ther*. 2022;55(9):1099–1115. doi:10.1111/apt.16831
4. Wang K, Yang F, Zhang P, et al. Genetic effects of iron levels on liver injury and risk of liver diseases: a two-sample Mendelian randomization analysis. *Front Nutr*. 2022;9:964163.
5. Mokdad AA, Lopez AD, Shahraz S, et al. Liver cirrhosis mortality in 187 countries between 1980 and 2010: a systematic analysis. *BMC Med*. 2014;12(1):145. doi:10.1186/s12916-014-0145-y
6. Orlien SMS, Ismael NY, Ahmed TA, et al. Unexplained chronic liver disease in Ethiopia: a cross sectional study. *BMC Gastroenterol*. 2018;18 (1):27. doi:10.1186/s12876-018-0755-5
7. Kuniholm MH, Lesi OA, Mendy M, et al. Aflatoxin exposure and viral hepatitis in the etiology of liver cirrhosis in the Gambia, West Africa. *Environ Health Perspect*. 2008;116(11):1553–1557. doi:10.1289/ehp.11661
8. Chu YJ, Yang HI, Wu HC, et al. Aflatoxin B_1 exposure increases the risk of cirrhosis and hepatocellular carcinoma in chronic hepatitis B virus carriers. *Int J Cancer*. 2017;141(4):711–720. doi:10.1002/ijc.30782
9. Alvarez CS, Hernández E, Escobar K, et al. Aflatoxin B_1exposure and liver cirrhosis in Guatemala: a case–control study. *BMJ Open Gastroenterol*. 2020;7(1):e000380. doi:10.1136/bmjgast-2020-000380
10. Mekuria AN, Routledge MN, Gong YY, et al. Aflatoxins as a risk factor for liver cirrhosis: a systematic review and meta-analysis. *BMC Pharmacol Toxicol*. 2020;21(1):39.

11. Chala A, Mohammed A, Ayalew A, et al. Natural occurrence of aflatoxins in groundnut (Arachis hypogaea L.) from eastern Ethiopia. *Food Control*. 2013;30(2):602–605. doi:10.1016/j.foodcont.2012.08.023
12. Chala A, Taye W, Ayalew A, et al. Multimycotoxin analysis of sorghum (Sorghum bicolor L. Moench) and finger millet (Eleusine coracana L. Garten) from Ethiopia. *Food Control*. 2014;45:29–35. doi:10.1016/j.foodcont.2014.04.018
13. Taye W, Ayalew A, Chala A, et al. Aflatoxin B $_1$ and total fumonisin contamination and their producing fungi in fresh and stored sorghum grain in East Hararghe, Ethiopia. *Food Addit Contam Part B Surveill*. 2016;9(4):237–245. doi:10.1080/19393210.2016.1184190
14. Mamo FT, Abate BA, Tesfaye K, et al. Mycotoxins in Ethiopia: a review on prevalence, economic and health impacts. *Toxins*. 2020;12(10):648. doi:10.3390/toxins12100648
15. Mohammed A, Bekeko Z, Yusufe M, et al. Fungal species and multi-mycotoxin associated with post-harvest Sorghum (Sorghum bicolor (L.) Moench) Grain in Eastern Ethiopia. *Toxins*. 2022;14(7):473. doi:10.3390/toxins14070473
16. Lin D-Y, Sheen I-S, Chiu C-T, et al. Ultrasonographic changes of early liver cirrhosis in chronic hepatitis B: a longitudinal study. *J Clin Ultrasound*. 1993;21(5):303–308. doi:10.1002/jcu.1870210502
17. Shen L, Li JQ, Zeng MD, et al. Correlation between ultrasonographic and pathologic diagnosis of liver fibrosis due to chronic virus hepatitis. *World J Gastroenterol*. 2006;12(8):1292–1295. doi:10.3748/wjg.v12.i8.1292
18. Hagström H. Alcohol consumption in concomitant liver disease: how much is too much? *Curr Hepatol Rep*. 2017;16(2):152–157. doi:10.1007/s11901-017-0343-0
19. Heikkinen H, Jallinoja P, Saarni SI, et al. The impact of smoking on health-related and overall quality of life: a general population survey in Finland. *Nicotine Tob Res*. 2008;10(7):1199–1207. doi:10.1080/14622200802163142
20. Hidese S, Ota M, Matsuo J, et al. Association of body mass index and its classifications with gray matter volume in individuals with a wide range of body mass index group: a whole-brain magnetic resonance imaging study. Original Research. *Front Human Neurosci*. 2022;16. doi:10.3389/fnhum.2022.926804
21. Chapot B, Wild CP. ELISA for quantification of aflatoxin-albumin adducts and their application to human exposure assessment. In: Warhol M, van Velzen D, Bullock GR, editors. *Techniques in Diagnostic Pathology*. San Diego CA: Academic Press; 1991:135–155.
22. Orlien SMS, Sandven I, Berhe NB, et al. Khat chewing increases the risk for developing chronic liver disease: a hospital-based case-control study. *Hepatol*. 2018;68(1):248–257. doi:10.1002/hep.29809
23. Hung TH, Liang CM, Hsu CN, et al. Association between complicated liver cirrhosis and the risk of hepatocellular carcinoma in Taiwan. *PLoS One*. 2017;12(7):e0181858. doi:10.1371/journal.pone.0181858
24. Kedar Mukthinuthalapati VVP, Sewram V, Ndlovu N, et al. Hepatocellular carcinoma in Sub- Saharan Africa. *JCO Glob Oncol*. 2021;7:756–766. doi:10.1200/GO.20.00425
25. Sonderup MW, Spearman CW. Global disparities in hepatitis B elimination-a focus on Africa. *Viruses*. 2022;14(1):82. doi:10.3390/v14010082
26. Ansari A, Vincent JP, Moorhouse L, et al. Risk of early horizontal transmission of hepatitis B virus in children of uninfected mothers in sub-Saharan Africa: a systematic review and meta-analysis. *Lancet Glob Health*. 2023;11(5):e715–e728. doi:10.1016/S2214-109X(23)00131-6
27. Ismail A, Naeem I, Gong YY, et al. Early life exposure to dietary aflatoxins, health impact and control perspectives: a review. *Trends Food Sci Technol*. 2021;112:212–224. doi:10.1016/j.tifs.2021.04.002
28. Edmunds WJ, Medley GF, Nokes DJ, et al. The influence of age on the development of the hepatitis B carrier state. *Proc Biol Sci*. 1993;253:197–201.
29. Shimakawa Y, Yan H-J, Tsuchiya N, et al. Association of early age at establishment of chronic hepatitis B infection with persistent viral replication, liver cirrhosis and hepatocellular carcinoma: a systematic review. *PLoS One*. 2013;8(7):e69430. doi:10.1371/journal.pone.0069430
30. Malik A, Ali S, Shahid M, et al. Occupational exposure to Aspergillus and aflatoxins among food-grain workers in India. *Int J Occup Environ Health*. 2014;20(3):189–193. doi:10.1179/2049396714Y.0000000055
31. Wangia RN, Tang L, Wang J-S. Occupational exposure to aflatoxins and health outcomes: a review. *J Environ Sci Health C*. 2019;37(4):215–234. doi:10.1080/10590501.2019.1664836
32. Udomkun P, Wossen T, Nabahungu NL, et al. Incidence and farmers' knowledge of aflatoxin contamination and control in Eastern Democratic Republic of Congo. *Food Sci Nutr*. 2018;6(6):1607–1620. doi:10.1002/fsn3.735
33. Gichohi-Wainaina WN, Kumwenda N, Zulu R, et al. Aflatoxin contamination: knowledge disparities among agriculture extension officers, frontline health workers and small holder farming households in Malawi. *Food Control*. 2021;121:107672. doi:10.1016/j.foodcont.2020.107672
34. Manfo FPT, Mboe SA, Nantia EA, et al. Evaluation of the effects of agro pesticides use on liver and kidney function in farmers from Buea, Cameroon. *J Toxicol*. 2020;2020:2305764. doi:10.1155/2020/2305764
35. Huang X, Zhang C, Hu R, et al. Association between occupational exposures to pesticides with heterogeneous chemical structures and farmer health in China. *Sci Rep*. 2016;6(1):25190. doi:10.1038/srep25190
36. Prado-Lu JL D. Pesticide exposure, risk factors, and health problems among cut-flower farmers: a cross-sectional study. *J Occup Med Toxicol*. 2007;2:9. doi:10.1186/1745-6673-2-9
37. Nigatu AW, Bråtveit M, Moen BE. Self-reported acute pesticide intoxications in Ethiopia. *BMC Public Health*. 2016;16(1):575. doi:10.1186/s12889-016-3196-5
38. Tessema RA, Nagy K, Ádám B. Pesticide use, perceived health risks and management in Ethiopia and in Hungary: a comparative analysis. *Int J Environ Res Public Health*. 2021;18(19):10431. doi:10.3390/ijerph181910431
39. Alebachew F, Azage M, Kassie GG, et al. Pesticide use safety practices and associated factors among farmers in Fogera district wetland areas, south Gondar zone, Northwest Ethiopia. *PLoS One*. 2023;18(1):e0280185. doi:10.1371/journal.pone.0280185
40. Derso AG, Dagnew GG. Exposure and health risk assessment of farmers to DDT during khat production in Chiro Woreda, West Hararghe Zone: Ethiopia. *World J Agri Res*. 2019;7:29–35.
41. Regassa G, Regassa C. Knowledge and attitude of khat growing farmers on the safe use and handling of pesticides in Haromaya Wereda, Oromia Regional State, Eastern Ethiopia. *Afr J Environ Sci Technol*. 2021;15:16–26. doi:10.5897/AJEST2020.2916
42. Robinson O. *Aetiology of an Unknown Liver Disease in Northern Ethiopia*. Imperial College London; 2012.
43. Coumoul X, Diry M, Barouki R. PXR-dependent induction of human CYP3A4 gene expression by organochlorine pesticides. *Biochem Pharmacol*. 2002;64(10):473–479. doi:10.1016/S0006-2952(02)01298-4

44. Zhao B, Shen H, Liu F, et al. Exposure to organochlorine pesticides is independent risk factor of hepatocellular carcinoma: a case–control study. *J Expo Sci Environ Epidemiol*. 2011;21(6):601–608. doi:10.1038/jes.2011.24
45. Seen S. Chronic liver disease and oxidative stress – a narrative review. *Expert Rev Gastroenterol Hepatol*. 2021;15(9):1021–1035.
46. Conde de la Rosa L, Goicoechea L, Torres S, et al. Role of oxidative stress in liver disorders. *Livers*. 2022;2(4):283–314.
47. Rybicka M, Woziwodzka A, Sznarkowska A, et al. Liver cirrhosis in chronic hepatitis B patients is associated with genetic variations in DNA repair pathway genes. *Cancers*. 2020;12(11):3295. doi:10.3390/cancers12113295
48. Ashouri S, Khor SS, Hitomi Y, et al. Genome-wide association study for chronic hepatitis B infection in the Thai Population. *Front Genet*. 2022;13:887121. doi:10.3389/fgene.2022.887121
49. Deng J, Zhao L, Zhang NY, et al. Aflatoxin B_1 metabolism: regulation by phase I and II metabolizing enzymes and chemoprotective agents. *Mutat Res/Rev Mutat Res*. 2018;778:79–89. doi:10.1016/j.mrrev.2018.10.002
50. Eom SY, Yim DH, Zhang Y, et al. Dietary aflatoxin B1 intake, genetic polymorphisms of *CYP1A2*, *CYP2E1*, *EPHX1*, *GSTM1*, and *GSTT1*, and gastric cancer risk in Korean. *Cancer Causes Control*. 2013;24(11):1963–1972. doi:10.1007/s10552-013-0272-3
51. Alberts CJ, Clifford GM, Georges D, et al. Worldwide prevalence of hepatitis B virus and hepatitis C virus among patients with cirrhosis at country, region, and global levels: a systematic review. *Lancet Gastroenterol Hepatol*. 2022;7(8):724–735. doi:10.1016/S2468-1253(22)00050-4
52. Tesfaye BT, Feyissa TM, Workneh AB, et al. Chronic liver disease in Ethiopia with a particular focus on the etiological spectrums: a systematic review and meta-analysis of observational studies. *Can J Gastroenterol Hepatol*. 2021;2021:8740157. doi:10.1155/2021/8740157
53. Chuang YC, Tsai K-N, Ou JHJ. Pathogenicity and virulence of Hepatitis B virus. *Virulence*. 2022;13(1):258–296. doi:10.1080/21505594.2022.2028483
54. Suhail M, Abdel-Hafiz H, Ali A, et al. Potential mechanisms of hepatitis B virus induced liver injury. *World J Gastroenterol*. 2014;20(35):12462–12472. doi:10.3748/wjg.v20.i35.12462
55. Zhang Z, Wang C, Liu Z, et al. Host genetic determinants of hepatitis B virus infection. *Front Genet*. 2019;10:696. doi:10.3389/fgene.2019.00696
56. Lin J, Wu JF, Zhang Q, et al. Virus-related liver cirrhosis: molecular basis and therapeutic options. *World J Gastroenterol*. 2014;20(21):6457–6469. doi:10.3748/wjg.v20.i21.6457
57. Hosny G, Farahat N, Tayel H, et al. Ser-249 TP53 and CTNNB1 mutations in circulating free DNA of Egyptian patients with hepatocellular carcinoma versus chronic liver diseases. *Cancer Lett*. 2008;264(2):201–208. doi:10.1016/j.canlet.2008.01.031
58. Aydın M, Aydın S, Bacanlı M, et al. Aflatoxin levels in chronic hepatitis B patients with cirrhosis or hepatocellular carcinoma in Balıkesir, Turkey. *J Viral Hepat*. 2015;22(11):926–935. doi:10.1111/jvh.12410
59. Chen CH, Wang MH, Wang JH, et al. Aflatoxin exposure and hepatitis C virus in advanced liver disease in a hepatitis C virus endemic area in Taiwan. *Am J Trop Med Hyg*. 2007;77(4):747–752. doi:10.4269/ajtmh.2007.77.747
60. Yen YH, Kuo FY, Chen CH, et al. Ultrasound is highly specific in diagnosing compensated cirrhosis in chronic hepatitis C patients in real world clinical practice. *Med*. 2019;98(27):e16270. doi:10.1097/MD.0000000000016270
61. Kelly EMM, Feldstein VA, Parks M, et al. An assessment of the clinical accuracy of ultrasound in diagnosing cirrhosis in the absence of portal hypertension. *Gastroenterol Hepatol*. 2018;14(6):367–373.

Table S1: Distribution of Aflatoxin B_1 albumin (AF-alb) adduct level by gender, age, residence, education, and occupation among the study population, Eastern Ethiopia, 2020/21 (n=257)

Variables	Category	AF-alb adduct (pg/mg of albumin)								
		N	Total Median (IQR)	*p* value*	N	Cases Median (IQR)	*p* value*	N	Controls Median (IQR)	*p* value*
Gender	Male	162	8.9 (5.1-22.6)	0.43	62	10.7 (5.9-22.6)	0.89	100	7.7 (4.3-25.6)	0.55
	Female	95	7.9 (4.5-21.9)		33	11.4 (4.8-30.2)		62	6.7 (4.3-19.0)	
Age category	<35	123	6.8 (4.5-18.7)	0.18	48	9.5 (5.3-23.6)	0.77	75	6.1 (4.3-14.8)	0.22
	35-44	57	9.9 (4.9-31.7)		21	10.3 (6.1-52.0)		36	9.9 (4.3-26.0)	
	45-54	32	8.1 (5.3-17.7)		8	11.0 (6.0-35.3)		24	7.5 (4.1-15.7)	
	≥55	45	12.5 (5.2-35.8)		18	12.6 (6.4-21.0)		27	12.5 (4.4-46.8)	
Residence	Urban	57	6.2 (3.9-13.1)	**0.01**	15	8.3 (5.9-15.5)	0.35	42	5.6 (3.9-12.7)	**0.03**
	Rural	200	9.5 (5.1-27.1)		80	11.5 (5.5-28.2)		120	7.9 (4.7-27.1)	
Formal education	No	176	9.9 (5.3-28.9)	**<0.001**	76	11.2 (5.8-24.8)	0.42	100	9.2 (4.5-31.8)	**0.03**
	Yes	81	6.2 (4.2-14.0)		19	8.9 (4.0-24.9)		62	5.9 (4.2-12.7)	
Occupation	Not farmer	87	6.7 (4.3-14.7)	**<0.001**	16	10.1 (4.4-22.4)	0.51	71	6.1 (4.3-12.6)	0.05
	Farmer	170	10.0 (5.3-29.4)		79	11.2 (5.6-25.0)		91	8.5 (4.4-32.0)	

Note: N= 257; n for cases=95; n for controls =162; **p*-value from Mann-Whitney test for variables with two categories and Kruskal-Wallis test for variables with more than two categories. *P*-values less than 0.05 are marked in bold. AF-alb, aflatoxin B_1 albumin adducts; HBV, Hepatitis B virus.

Paper III: Copy number variation in the *GSTM1* and *GSTT1* genes and the risk of liver cirrhosis in Eastern Ethiopia

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ORIGINAL RESEARCH

Copy Number Variation in the *GSTM1* and *GSTT1* Genes and the Risk of Liver Cirrhosis in Eastern Ethiopia

Abraham Nigussie Mekuria[1], Tamrayehu Seyoum[2], Dawit Hailu Alemayehu[2], Markos Abebe[2], Teshome Nedi[1], Tefera Abula[1], Yun Yun Gong[3], Ephrem Engidawork[1]

[1]Department of Pharmacology and Clinical Pharmacy, School of Pharmacy, College of Health Sciences, Addis Ababa University, Addis Ababa, Ethiopia; [2]Department of Biotechnology and Bioinformatics, Armauer Hansen Research Institute, Addis Ababa, Ethiopia; [3]School of Food Science and Nutrition, University of Leeds, Leeds, UK

Correspondence: Ephrem Engidawork, Email ephrem.engidawork@aau.edu.et

Background: Polymorphisms in glutathione S-transferase M1 (*GSTM1*) and T1 (*GSTT1*) can cause an entire gene deletion. The current methodology can accurately identify *GSTM1* and *GSTT1* copy number variants (CNVs), which may shed light on the true contribution of each gene copy to the cellular detoxification process and disease risk. Because liver cirrhosis is becoming a critical worldwide health issue, this study determined the CNVs of *GSTM1* and *GSTT1* and their relationship to the risk of liver cirrhosis.

Methods: In this study, we compared 106 patients with liver cirrhosis to 104 healthy controls. Real-time PCR was used to identify the CNVs of *GSTM1* and *GSTT1*. Logistic and linear regression models were used to estimate the relationship between liver cirrhosis and clinical chemistry variables with the CNVs, respectively.

Results: In 3.3% of the study participants, >2 copies of the *GSTM1* or *GSTT1* genes were detected. *GSTT1* carriers had a significantly lower risk of liver cirrhosis ($p<0.05$) compared with individuals who had homozygous deletion (adjusted odds ratio (AOR) = 0.47; 95% CI: 0.25, 0.86). This risk reduction was significant ($p<0.05$) in patients with a single copy of the *GSTT1* gene (AOR = 0.48; 95% CI: 0.25, 0.91). Those with ≥2 copies of combined *GSTM1* and *GSTT1* also had a significantly ($p<0.05$) lower risk of developing liver cirrhosis compared with double null genotypes (AOR = 0.38; 95% CI: 0.16, 0.91, p trend <0.001). Moreover, ≥2 copies of combined *GSTM1* and *GSTT1* genes were associated with a substantial decrease in alanine amino transferase (ALT) and aspartate aminotransferase (AST) levels, respectively.

Conclusion: A single copy number of *GSTT1*, and ≥2 copies of combined *GSTM1* and *GSTT1* genes were associated with a reduced risk of liver cirrhosis in Ethiopians. These findings underscore the importance of gene–environment interactions in the multifactorial development of liver cirrhosis.

Keywords: liver cirrhosis, copy number variation, glutathione S-transferase genes, Ethiopia

Introduction

Glutathione S-transferase (GST) enzymes are generally known to protect cells from oxidative stress due to metabolic products of endogenous and exogenous chemicals.[1] GST's biochemical defense mechanisms include both the reduction of organic hydroperoxides, which contribute to oxidative stress, and the conjugation of electrophilic chemicals with glutathione, which facilitates their transport out of the cell.[2,3] There are 16 genes coding for the cytosolic form of GST enzymes in humans and are classified into 7 classes: alpha (*GSTA*), mu (*GSTM*), pi (*GSTP*), theta (*GSTT*), omega (*GSTO*), and zeta (*GSTZ*).[1] These genes are known to be highly polymorphic,[4] which can result in

altered detoxification and oxidative stress defense capacity in certain tissues, particularly the liver, which has a central metabolic role.[5,6]

Oxidative stress is thought to be the primary cause of liver injury and progression to liver cirrhosis as well as hepatocellular carcinoma (HCC) associated with aflatoxin B_1 (AFB_1) exposure.[7,8] In our recent study, we found a strong association between AFB_1 exposure and liver cirrhosis.[9] AFB_1 is mainly metabolized by CYP3A4 into the reactive free radical AFB_1-exo-8, 9-epoxide.[10] This intermediate metabolite is typically detoxified by conjugation with glutathione through the action of GST enzymes, mainly GSTM1 and GSTT1 encoded by the *GSTM1* and *GSTT1* gene, respectively.[11] Copy number variation (CNV) has been ascribed to these genes and individuals with zero copy number (null genotype) of *GSTM1* and/or *GSTT1* are more prone to develop HCC associated with AFB_1 exposure.[12,13] However, previous reports failed to distinguish between heterozygous and homozygous bearers of the non-deleted alleles. Hence, the present study aimed at investigating *GSTT1* and *GSTM1* CNVs and the risk for liver cirrhosis in Eastern Ethiopia.

Materials and Methods

Study Subjects

This is a follow-up of a case–control study conducted from January 2020 – July 2021 to assess the risk factors associated with liver cirrhosis in the Internal Medicine Unit of Hiwot Fana Comprehensive Specialized University Hospital (HFCSUH), Harar, Eastern Ethiopia.[9] In brief, the study consisted of 127 confirmed cases of liver cirrhosis and 253 controls without any history or clinical evidence of liver diseases. Written consent from the participants was obtained after explaining aims of the study and a detailed questionnaire was filled out for each case and control by a trained interviewer. A subset of 210 individuals, including all cases (106), for whom blood samples were available, and an equivalent number of controls (104) were randomly selected and included in this genetic association study.

Blood Collection

Two mL of blood was collected in an EDTA tube for genomic DNA extraction. Additional 3–5 mL blood was also taken in a serum separator tube for clinical chemistry tests. Whole blood samples collected for genomic DNA extraction were stored at −80°C until transport and analysis at the Armauer Hansen Research Institute, Addis Ababa, Ethiopia.

DNA Extraction

Genomic DNA was isolated from whole blood using PureLink™ Genomic DNA Mini Kit (Invitrogen) according to the manufacturer's instructions. DNA quality and quantity was assessed using NanoDrop 2000 spectrophotometer (ThermoFisher Scientific, USA). Moreover, 10% of the samples were randomly selected and agarose gel electrophoresis with the aid of a gel imaging system, Gel Doc (Bio-Rad), was performed to ensure quality of the extracted DNA. The genomic DNA samples were stored at −20°C until analysis.

GSTM1 and *GSTT1* Copy Number Analysis

Copy number determination was carried out by a Bio-Rad CFX96 Touch Real-Time System. The reaction mixture (10 uL) was composed of FAM-labeled 20x TaqMan™ Copy Number Assay kit (AB Applied Biosystems) for *GSTM1* (Hs02595872_cn) or *GSTT1* (Hs01731033_cn), VIC-labeled 20X TaqMan™ Reference Assay kit (AB Applied Biosystems: 4403326), TaqPath™ ProAmp™ Master Mix (Applied Biosystems), nuclease-free water and 10 ng genomic DNA template. Thermocycling was initiated at 95°C for 10 min followed by 40 cycles of 15 sec of denaturation at 95°C and 60-sec annealing-extension at 60°C.

To ensure quality, each target assay was done in the same PCR as RNaseP. The amplification of RNaseP and GST genes in the same sample adjusted DNA concentration differences between samples and ensured that no false- negative GST*0/0 genotypes were created due to PCR or pipetting failure or inadequate DNA concentrations in the original sample. The genotyping was done blind to the subject's case or control status. The samples were run twice. In addition, each run contained a no-template control to rule out any contamination. The number of amplification cycles necessary for the fluorescent signal to reach the threshold and surpass the background level is known as the cycle threshold (Ct) value in the qPCR experiment. All samples were quantified in duplicates and average Ct values were normalized to *RNase P* (the reference gene with copy number 2). Copy number estimation was conducted by the ΔΔCt method as follows:

$$\text{Ct target gene} = 2\text{x}2^{-\Delta\Delta\text{Ct}}$$

where ΔΔCt= ΔCtGST sample – ΔCtRNase P and ΔCt was the average Ct value of 2 repeated measurements of the sample. The result obtained from the $2^{-\Delta\Delta Ct}$ calculation was multiplied by 2 because the target genes are known to be diploid.[14,15]

Clinical Chemistry

Liver function test (LFT) including aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), serum bilirubin (total), albumin, and serum creatinine was determined using Cobas C-311 fully automated closed clinical chemistry analyzer (Roche/Hitachi Cobas C-311, Roche diagnostics GmbH, Mannheim, Germany).

Statistical Analysis

Categorical variables were expressed as frequencies and percentages, while continuous variables were expressed as median (IQR). The difference between cases and controls in terms of categorical and continuous variables was tested using χ^2 or Fisher's exact test and the nonparametric Mann–Whitney-*U* test, respectively. Using unconditional logistic regression, odds ratios (ORs) were estimated for *GSTM1* and *GSTT1* genotypes, comparing groups defined by the gene copy number coded as a categorical variable (0, 1, or ≥2 copies), null, and carriers (1, 2 or more copies). Given that gender and age are biological risk factors for liver cirrhosis, we adjusted the OR for these variables as potential confounders. The association between clinical chemistry tests and *GSTM1* and *GSTT1* copy numbers and genotypes was also investigated using linear regression. Since the Shapiro–Wilk test revealed a non-normal distribution of the clinical chemistry data, we used the log-transferred data during regression analysis. The adjustment was made using the potential

confounders (age and gender). $P<0.05$ was considered statistically significant. All statistical analyses were performed using Statistical Package for Social Sciences (SPSS) version 26.0.

Ethical Considerations

This study was conducted following the Declaration of Helsinki. Ethical approval was obtained from both the Institutional Review Board of the College of Health Sciences, Addis Ababa University (Protocol No. 064/19/SOP and Reference No. CHS/RTTD/229/2020) and the National Ethical Review Committee of the Ministry of Science and Higher Education (Reference No. 04/246/680/21). Written consent was obtained following provision of information regarding the study's objectives, benefits, and risks to participants. Confidentiality and anonymity were ensured by restricting data access and removing identifiers, respectively.

Results

Sociodemographic and Clinical Characteristics

Table 1 shows characteristics of the study participants. Males accounted for a higher number of cases (67%) than controls (52%) and we found statistically significant differences ($p<0.05$) regarding gender but not age between the two groups. There was a significant ($p<0.05$) difference between cases and controls in terms of all clinical chemistry parameters (Table 1).

Copy Number Variation

Figure 1 depicts CNV of the study participants. The *GSTM1* null genotype was more common in cases (0.45; 95% CI: 0.36, 0.55) than controls (0.33; 95% CI: 0.24, 0.43), with an overall frequency of 0.39 (95% CI: 0.32, 0.46) in the study population. Similarly, the frequency of *GSTT1* null genotype was significantly higher ($p<0.05$) in cases (0.40; 95% CI: 0.30, 0.50) than controls (0.25; 95% CI: 0.17, 0.34), with an overall frequency of 0.32 (95% CI: 0.26, 0.39). The overall percentage of double null *GSTM1* and *GSTT1* genotypes was 13% (95% CI: 0.09, 0.19) and relatively more common in cases (16%; 95% CI: 0.10, 0.24) than controls (11%; 95% CI: 0.05, 0.18). Moreover, 3.3% (95% CI: 0.01, 0.07) of the study participants had >2 copies of the *GSTM1* or *GSTT1* genes, each accounting for 1.9% (95% CI: 0.01, 0.05) and 1.4% (95% CI: 0.01, 0.04) for *GSTT1*), respectively.

Table 1 Sociodemographic and Clinical Chemistry Characteristics of the Study Participants, Eastern Ethiopia, 2020/21 (N=210)

Characteristics	Cases n (%)	Controls n (%)
Gender*		
Male	71 (67)	54 (52)
Female	35 (33)	50 (48)
Age	32 (28–50)	34 (26–50)
<35	54 (51)	52 (50)
35–44	17 (16)	22 (21)
45–54	16 (15)	8 (8)
>55	19 (18)	22 (21)
ALT (U/L)*	40 (19.8–53.3)	19 (14.7–26)
AST (U/L)*	52.3 (32–76.7)	21 (16.7–28)
ALP (U/L)*	164 (94–261.3)	86 (67–111)
Bilirubin, total (µmol/L)*	18.7 (8.5–32.7)	6.8 (3.8–14.7)
Albumin (g/L)*	29 (22.8–38.3)	41 (33.3–45)
Creatinine (µmol/L)*	63.5 (48.8–81.8)	55 (45.3–65)

Notes: N=210; n for cases=106; n for controls=104; data are presented as number (%) or as median (interquartile range); *p-value <0.05 from the chi-square test or Fisher exact test for categorical and Mann–Whitney *U*-test for continuous variables.
Abbreviations: ALT, alanine aminotransferase; AST, Aspartate aminotrans- ferase; ALP, alkaline phosphatase; g/L, gram per liter; µmol/L, micromole per liter; U/L, units per liter.

Association Studies

As shown in Table 2, *GSTT1* carriers had a significantly (p<0.05) lower risk of liver cirrhosis than individuals with null genotypes (AOR=0.47; 95% CI: 0.25, 0.86). Further analysis also revealed that patients with one copy of the *GSTT1* gene had a significantly (p<0.05) lower risk of liver cirrhosis than patients with null genotypes (AOR=0.48; 95% CI: 0.25, 0.91). Moreover, the risk of liver cirrhosis was markedly (p<0.05) decreased in those patients who had ≥2 combined *GSTM1* and *GSTT1* copies compared with patients who had a dual null genotype (AOR=0.38; 95% CI: 0.16, 0.91). There was also a significant gene dose relationship (p-trend < 0.001).

We further investigated whether *GSTM1* and *GSTT1* copy numbers and genotypes indirectly contribute to the levels of clinical chemistry parameters as shown in Table 3. Consequently, the ALT level was reduced by 31% (β= 0.69; 95% CI: 0.48, 0.99; p<0.05) in cases with ≥2 copies of *GSTM1* in comparison with null genotypes (Table 3). Moreover, AST level was decreased by 39% (β= 0.61; 95% CI: 0.41, 0.90; p<0.05) in cases having ≥2 copies of *GSTT1* than those with

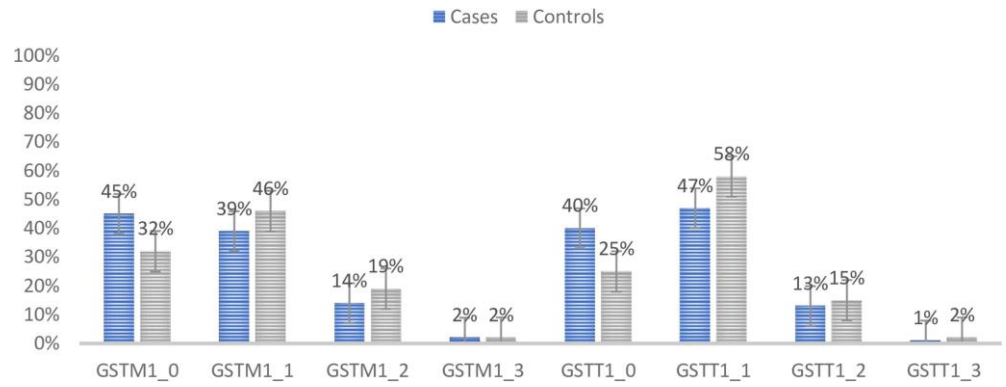

Figure 1 Frequency of *GSTM1* and *GSTT1* copy numbers among the study participants, Eastern Ethiopia, 2020/21 (n=210).

Table 2 Risk of Liver Cirrhosis in Relation to *GSTM1* and *GSTT1* Genes Copy Numbers and Genotypes, Eastern Ethiopia, 2020/21 (N=210)

Copy Numbers and Genotype	Cases (%)	Controls n (%)	Crude OR	Adjusted OR[a]
GSTM1				
0	48 (45)	34 (33)	1.00	1.00
1	41 (39)	48 (46)	0.61 (0.33, 1.11)	0.57 (0.31, 1.07)
≥2	17 (16)	22 (21)	0.55 (0.25, 1.18)	0.56 (0.25, 1.25)
p trend			0.17	0.16
Null	48 (45)	34 (32)	1.00	1.00
Carrier	58 (55)	70 (67)	0.62 (0.34, 1.03)	0.57 (0.32, 1.02)
GSTT1				
0	42 (40)	26 (25)	1.00	1.00
1	49 (46)	60 (58)	0.51 (0.27, 0.94)	0.48 (0.25, 0.91) *
≥2	15 (14)	18 (17)	0.52 (0.22, 1.20)	0.43 (0.18, 1.04)
p trend			0.08	0.05
Null	42 (40)	26 (25)	1.00	1.00
Carrier	64 (60)	78 (75)	0.51 (0.28, 0.92)	0.47 (0.25, 0.86) *
Sum of *GSTM1* and *GSTT1*[b]				
0	17 (16)	11 (11)	1.00	1.00
1	42 (40)	25 (24)	1.09 (0.44, 2.69)	0.92 (0.37, 2.43)
≥2	47 (44)	68 (65)	0.45 (0.19, 1.04)	0.38 (0.16, 0.91) *
p trend			0.01	<0.001
Null/Null	17 (16)	11 (11)	1.00	1.00
Carrier/Carrier	89 (84)	93 (89)	0.62 (0.28, 1.40)	0.54 (0.23, 1.25)

Notes: N=210; n for cases=106; n for controls=104; **p*-value <0.05; [a]Odds ratio adjusted for gender and age; [b]0=$GSTM1_0$ + $GSTT1_0$, 1=$GSTM1_0$ + $GSTT1_1$ or $GSTM1_1$ + $GSTT1_0$, 2= $GSTM1_1$ + $GSTT1_1$ or $GSTM1_0$ + $GSTT1_2$ or $GSTM1_2$ + $GSTT1_0$, ≥3= $GSTM1_0$ + $GSTT1_3$ or $GSTM1_3$ + $GSTT1_0$ or $GSTM1_2$ + $GSTT1_2$ or $GSTM1_1$ + $GSTT1_2$ or $GSTM1_2$ + $GSTT1_1$ or $GSTM1_1$ + $GSTT1_3$ or $GSTM1_3$ + $GSTT1_1$. **Abbreviations**: *GSTM1*, glutathione S-transferase M; *GSTT1*, glutathione S-transferase T1; OR, odds ratio.

Table 3 Linear Regression of the Association Between ALT, AST, *GSTM1*, and *GSTT1* Copy Numbers and Genotypes, Eastern Ethiopia, 2020/21 (N=210)

Copy Numbers and Genotype	ALT (U/L)		AST (U/L)	
	Cases β (95% CI)[a]	Controls β (95% CI)[a]	Cases β (95% CI)[a]	Controls β (95% CI)[a]
GSTM1				
0	1.00	1.00	1.00	1.00
1	1.18 (0.90, 1.55)	1.09 (0.89, 1.34)	1.25 (0.95, 1.63)	0.99 (0.82, 1.20)
≥2	0.69 (0.48, 0.99)*	1.15 (0.90, 1.48)	0.70 (0.49, 1.01)	0.90 (0.70, 1.13)
Null	1.00	1.00	1.00	
Carrier	1.01 (0.79, 1.31)	1.11 (0.91, 1.33)	1.05 (0.81, 1.36)	0.96 (0.80, 1.14)
GSTT1				
0	1.00	1.00	1.00	1.00
1	0.89 (0.64, 1.16)	0.89 (0.72, 1.09)	0.81 (0.62, 1.06)	0.93 (0.76, 1.13)
≥2	1.01 (0.68, 1.51)	0.68 (0.52, 1.12)	0.61 (0.41, 0.90)*	0.79 (0.62, 1.03)
Null	1.00	1.00	1.00	
Carrier	0.91 (0.70, 1.19)	0.84 (0.68, 1.02)	0.76 (0.58, 0.98)*	0.90 (0.74, 1.08)
Sum of *GSTM1* and *GSTT1*[b]				
0	1.00	1.00	1.00	1.00
1	0.83 (0.57, 1.21)	0.79 (0.57, 1.11)	1.19 (0.82, 1.72)	0.86 (0.63, 1.16)
≥2	0.83 (0.57, 1.21)	0.86 (0.64, 1.16)	0.82 (0.57, 1.17)	0.84 (0.64, 1.09)
Null/Null	1.00	1.00	1.00	
Carrier/Carrier	0.83 (0.59, 1.17)	0.84 (0.63, 1.13)	0.98 (0.68, 1.39)	0.84 (0.64, 1.09)

Notes: N= 210; n for cases=106; n for controls=104; **p*-value <0.05; [a]Adjusted for age and gender; [b]0=$GSTM1_0$ + $GSTT1_0$, 1=$GSTM1_0$ + $GSTT1_1$ or $GSTM1_1$ + $GSTT1_0$, 2= $GSTM1_1$ + $GSTT1_1$ or $GSTM1_0$ + $GSTT1_2$ or $GSTM1_2$ + $GSTT1_0$, ≥3= $GSTM1_0$ + $GSTT1_3$ or $GSTM1_3$ + $GSTT1_0$ or $GSTM1_2$ + $GSTT1_2$ or $GSTM1_1$ + $GSTT1_2$ or $GSTM1_2$ + $GSTT1_1$ or $GSTM1_1$ + $GSTT1_3$ or $GSTM1_3$ + $GSTT1_1$.

Abbreviations: ALT, alanine aminotransferase; AST, Aspartate aminotransferase. *GSTM1*, glutathione S-transferase M; *GSTT1*, glutathione S-transferase T1; U/L, units per liter.

a null genotype. Similarly, AST level showed a significant (p<0.05) reduction in cases who were carriers of the *GSTT1* genotype compared to null genotypes (β= 0.76; 95% CI: 0.58, 0.98) (Table 3).

Discussion

Previous studies suggest that polymorphisms in genes encoding Phase II metabolizing enzymes, such as *GSTM1* and *GSTT1* may be linked to an increased vulnerability to various disorders associated with oxidative stress.[16,17] To the best of our knowledge, this is the first study showing the contribution of the exact CNV of *GSTM1* and *GSTT1* to liver cirrhosis. In our study, both *GSTM1* and *GSTT1* null genotypes were more frequently detected in liver cirrhosis patients than controls and the overall frequency was 0.39 and 0.32, respectively. Furthermore, 13% of the study population had double *GSTM1* and *GSTT1* null genotypes, whereas 3.3% (1.9% for

GSTM1 and 1.4% for *GSTT1*) had more than two copies of the *GSTM1* or *GSTT1* genes.

The frequency of *GSTM1* and *GSTT1* null genotypes varies in different populations. Consequently, *GSTM1* null genotype was more frequent in Asians (0.23 to 0.66),[16,18] Caucasians (0.45 to 0.57),[16,19] and Arabs (0.50 to 0.63)[20,21] compared to Africans (0.11 to 0.44).[19] On the other hand, *GSTT1* null genotype was shown to be more frequent among Asians (0.35 to 0.52),[16,18] Africans (0.20 to 0.47)[19] and Arabs (0.20 to 0.37)[20,21] in contrast to Caucasians (0.14 to 0.28).[16,19] The highest frequency of double *GSTM1* and *GSTT1* null genotypes was observed in Asians (0.27),[22] followed by Arabs (0.21),[21] and Africans (0.15).[23] Furthermore, evidence for *GSTM1* or *GSTT1* gene duplication has been identified at a significantly higher frequency in a study involving a Caribbean population descended from Africa (3.4%) than Caucasians (0.2%).[24,25] Indeed, our findings on the frequency of individual and combined (double) null genotypes and the percentage of more than two copies of the genes are consistent with previous studies involving Africans and populations descended from Africa.

In our study, *GSTT1* carriers had a significantly lower risk of liver cirrhosis than individuals with null genotypes, and specifically, patients with one copy of the *GSTT1* gene had a significantly lower risk of liver cirrhosis compared with null genotypes. Furthermore, patients with ≥2 combined *GSTM1* and *GSTT1* copies had a significantly lower risk of liver cirrhosis than those with a dual null genotype. In this regard, no observational studies exploring the relation between *GSTT1* and *GSTM1* copy numbers and the risk of liver cirrhosis are available for comparison. However, several studies have reported the association between *GSTM1* and *GSTT1* polymorphisms and the risk of liver disease, including drug-induced liver injury,[6,26] alcoholic cirrhosis,[27] viral and non-viral HCC,[28] non-alcoholic fatty liver disease,[29] and HCV associated cirrhosis.[30] However, all previous studies reported the association based on comparing carriers (irrespective of copy number of the genes) and null genotypes of *GSTM1* and *GSTT1*. For instance, a recent meta-analysis reported an increased risk of HCC in patients with *GSTM1* or *GSTT1* null genotypes, and double null genotypes of *GSTM1* and *GSTT1*.[28]

Our study also demonstrated a significant gene dose relationship between the sum of *GSTM1* and *GSTT1* copies and the risk of liver cirrhosis. This could support the notion that the activity of enzymes encoded by *GSTM1* and *GSTT1* genes is directly proportional to their copy numbers.[31,32] Thus, having more copy numbers

could be protective against oxidative stress and the associated inflammation due to various insults to the hepatocytes, including viral and non-viral agents.

We further investigated whether *GSTM1* and *GSTT1* genotypes indirectly contribute to the levels of clinical chemistry tests. Accordingly, ≥2 copies of combined *GSTM1* and *GSTT1* genes were associated with a significant reduction in ALT and AST levels, respectively. Moreover, a significant decrease in AST level was observed in *GSTT1* carriers compared with null genotypes. To the best of our knowledge, there are no studies focusing on the impact of *GSTM1* and *GSTT1* CNVs on clinical chemistry parameters of liver cirrhosis patients. There are, however, few studies that attempted to investigate the contribution of *GSTM1* and *GSTT1* to the liver biochemistry tests. For example, a study conducted among HCV-infected Brazilian cirrhosis and HCC patients reported no significant difference between carriers and null genotypes of these genes in terms of the blood level of clinical chemistry parameters.[30] By contrast, our study demonstrated significant difference in AST levels between *GSTT1* carriers and null genotypes of liver cirrhosis patients apart from the observed differences in ≥2 copies of combined *GSTM1* and *GSTT1* mentioned above. This discrepancy might arise from the fact that the mentioned study compared only null vs carriers of the genes but our study further investigated the copy numbers in carriers of the genes. Besides, differences in race and etiology of liver cirrhosis could also explain the discrepancies. Exposure to HBV and AFB_1 are the most important etiologies of HCC and liver cirrhosis in Africa and Asia, whereas HCV infection and alcohol consumption are the main drivers in America, Oceania, and Europe.[33–35] Indeed, in our recent study, HBV infection and AFB_1 exposure were the major culprits of liver cirrhosis in Eastern Ethiopia.[9]

On the other hand, few studies have reported the significance of null genotypes of the *GSTM1* and *GSTT1* genes for changes in the serum levels of ALT and AST due to exposure to hepatotoxic drugs, which have reactive free radicals as intermediate metabolites.[6,36,37] For instance, a study done among Tunisian epileptic patients treated with carbamazepine, known to have 10, 11-epoxy carbamazepine as a reactive metabolite, reported the association between *GSTM1* null genotype and elevation in ALT and AST, whereas AST elevation was associated with *GSTT1* null genotype.[6] Thus, functional *GSTM1* and *GSTT1* genes might indirectly contribute to the level of AST and ALT, at least by minimizing oxidative stress and associated damage due to reactive oxygen species produced as a result of drugs and environmental toxins, including AFB_1 in the hepatocytes. However, additional

studies with larger sample size and inclusion of controls with a risk factor would be required for substantiating these observations.

Conclusions

Our findings suggest that a single copy number of *GSTT1*, and ≥2 copies of combined *GSTM1* and *GSTT1* are associated with reduced risk of liver cirrhosis in Ethiopians. Besides, ≥2 copies of *GSTM1* and *GSTT1* genes were associated with a substantial decrease in ALT and AST levels, respectively. These findings highlight the significance of gene–environ- ment interactions in the multifactorial development of liver cirrhosis.

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Author Contributions

All authors made a significant contribution to the work reported whether that is in the conception, study design, execution, acquisition of data, analysis, and interpretation, or in all these areas; took part in drafting, revising, or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Disclosure

The authors declare that there are no conflicts of interest in this work.

References

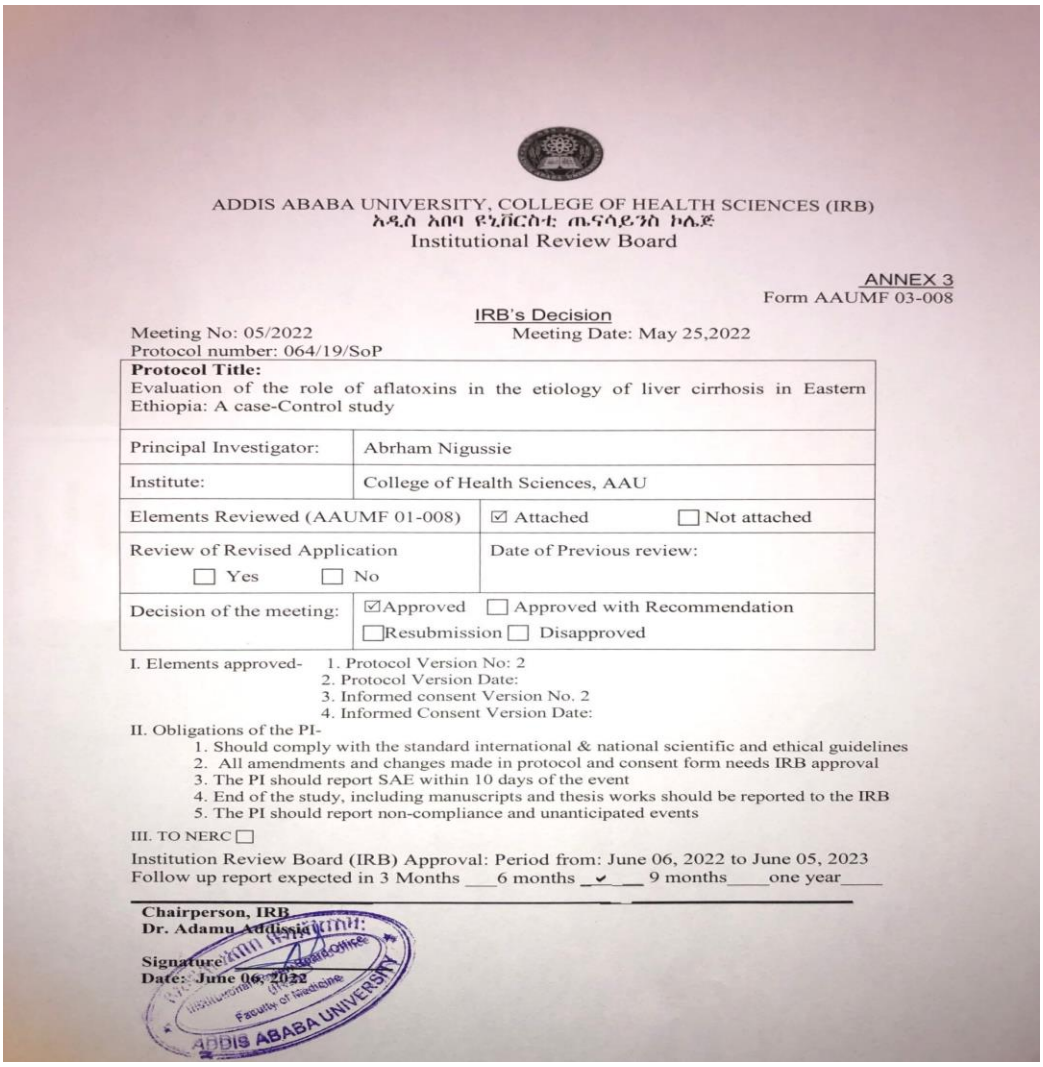
1. Mazari AMA, Zhang L, Ye Z-W, et al. The multifaceted role of glutathione s-transferases in health and disease. *Biomolecules*. 2023;13(4):688. doi:10.3390/biom13040688
2. Ge B, Song Y, Zhang Y, et al. Glutathione S-transferase M1 (GSTM1) and T1 (GSTT1) null polymorphisms and the risk of hypertension: a meta-analysis. *PLoS One*. 2015;10(3):e0118897. doi:10.1371/journal.pone.0118897
3. Qu K, Liu SS, Wang ZX, et al. Polymorphisms of glutathione S-transferase genes and survival of resected hepatocellular carcinoma patients. *World J Gastroenterol*. 2015;21(14):4310–4322. doi:10.3748/wjg.v21.i14.4310
4. Ginsberg G, Smolenski S, Hattis D, et al. Genetic Polymorphism in Glutathione Transferases (GST): population distribution of GSTM1, T1, and P1 conjugating activity. *J Toxicol Environ Health B Crit Rev*. 2009;12(5–6):389–439. doi:10.1080/10937400903158375
5. Chaudhary P, Janmeda P, Docea AO, et al. Oxidative stress, free radicals and antioxidants: potential crosstalk in the pathophysiology of human diseases. Review. *Front Chem*. 2023;11:1158198.
6. Chbili C, Hassine A, Fathallah N, et al. Glutathione S-transferase M1 and T1 polymorphisms and the risk of mild hepatotoxicity induced by carbamazepine in a Tunisian population study. *BMC Neurol*. 2018;18(1):24.

doi:10.1186/s12883-018-1013-8
7. Cichoż-Lach H, Michalak A. Oxidative stress as a crucial factor in liver diseases. *World J Gastroenterol*. 2014;20(25):8082–8091. doi:10.3748/wjg. v20.i25.8082
8. Kapahtia S, Hazam RK, Asim M, et al. Role of glutathione S transferase M1 and T1 gene polymorphism in hepatitis B related liver diseases and cryptogenic cirrhosis. *J Clin Exp Hepatol*. 2018;8(2):169–172. doi:10.1016/j.jceh.2017.05.208
9. Mekuria A, Xia L, Ahmed TA, et al. Contribution of aflatoxin B1 exposure to liver cirrhosis in Eastern Ethiopia: a case-control study. *Int J Gen Med*. 2023;16:3543–3553. doi:10.2147/IJGM.S425992
10. Cao W, Yu P, Yang K, et al. Aflatoxin B1: metabolism, toxicology, and its involvement in oxidative stress and cancer development. *Toxicol Mech Methods*. 2022;32(6):395–419. doi:10.1080/15376516.2021.2021339
11. Dohnal V, Wu Q, Kuča K. Metabolism of aflatoxins: key enzymes and interindividual as well as interspecies differences. *Arch Toxicol*. 2014;88 (9):1635–1644. doi:10.1007/s00204-014-1312-9
12. Sun CA, Wang LY, Chen CJ, et al. Genetic polymorphisms of glutathione S-transferases M1 and T1 associated with susceptibility to aflatoxin-related hepatocarcinogenesis among chronic hepatitis B carriers: a nested case-control study in Taiwan. *Carcinogenesis*. 2001;22 (8):1289–1294. doi:10.1093/carcin/22.8.1289
13. Long XD, Ma Y, Wei YP, et al. Study on the detoxication gene GSTM1-GSTT1-null and susceptibility to aflatoxin B1 related hepatocellular carcinoma in Guangxi. *Zhonghua Liu Xing Bing Xue Za Zhi*. 2005;26(10):777–781.
14. Zhang Y, Meng X, Ma Z, et al. Association of androgen-receptor gene mutations with the copy number of androgen-receptor silk protein A complex and Glutathione-S-Transferases T1 and M1 in prostate cancer patients. *Genet Res*. 2023;2023:5956951. doi:10.1155/2023/5956951
15. Mawick A, Pfeiffer H, Vennemann M. Sudden infant death syndrome: deletions of glutathione-S-transferase genes M1 and T1 and tobacco smoke exposure. *Int J Legal Med*. 2021;135(4):1375–1383. doi:10.1007/s00414-021-02556-5
16. Nakanishi G, Pita-Oliveira M, Bertagnolli LS, et al. Worldwide systematic review of GSTM1 and GSTT1 null genotypes by continent, ethnicity, and therapeutic area. *Omics*. 2022;26(10):528–541. doi:10.1089/omi.2022.0090
17. Nugrahaningsih DAA, Wihadmadyatami H, Widyarini S, et al. A review of the *GSTM1* null genotype modifies the association between air pollutant exposure and health problems. *Int J Genomics*. 2023;2023:4961487. doi:10.1155/2023/4961487
18. Alshagga MA, Mohamed N, Nazrun SA, et al. Frequencies of glutathione s-transferase (GSTM1, GSTM3 and GSTT1) polymorphisms in a Malaysian population. *Arch Med Sci*. 2011;7(4):572–578. doi:10.5114/aoms.2011.24123
19. Piacentini S, Polimanti R, Porreca F, et al. GSTT1 and GSTM1 gene polymorphisms in European and African populations. *Mol Biol Rep*. 2011;38 (2):1225–1230. doi:10.1007/s11033-010-0221-0
20. Mansour AA, Saleh OM, Askar T, et al. Frequency of glutathione-S-transferase null-M1 and null-T1 genotypes among the Turabah population in Saudi Arabia. *Genet Mol Res*. 2015;14(4):16863–16871. doi:10.4238/2015.December.14.13
21. Salem AH, Yaqoob A, Ali M, et al. Genetic polymorphism of the glutathione S-transferase M1 and T1 genes in three distinct Arab populations. *Dis Markers*. 2011;31(5):311–316. doi:10.1155/2011/796520
22. Guo X, O'Brien SJ, Zeng Y, et al. GSTM1 and GSTT1 gene deletions and the risk for nasopharyngeal carcinoma in Han Chinese. *Cancer Epidemiol Biomarkers Prev*. 2008;17(7):1760–1763. doi:10.1158/1055-9965.EPI-08-0149
23. Kiendrebeogo IT, Zoure AA, Sorgho PA, et al. Glutathione S-transferase M1 (GSTM1) and T1 (GSTT1) variants and breast cancer risk in Burkina Faso. *Biomol Concepts*. 2019;10(1):175–183. doi:10.1515/bmc-2019-0020
24. Nørskov M, Frikke-Schmidt R, Bojesen S, et al. Copy number variation in glutathione-S-transferase T1 and M1 predicts incidence and 5-year survival from prostate and bladder cancer, and incidence of corpus uteri cancer in the general population. *Pharmacogenomics J*. 2011;11 (4):292–299. doi:10.1038/tpj.2010.38
25. Emeville E, Broquère C, Brureau L, et al. Copy number variation of GSTT1 and GSTM1 and the risk of prostate cancer in a Caribbean population of African descent. *PLoS One*. 2014;9(9):e107275. doi:10.1371/journal.pone.0107275
26. Li C, Long J, Hu X, et al. GSTM1 and GSTT1 genetic polymorphisms and risk of anti-tuberculosis drug-induced hepatotoxicity: an updated meta-analysis. *Eur J Clin Microbiol Infect Dis*. 2013;32(7):859–868. doi:10.1007/s10096-013-1831-y
27. Ladero JM, Martínez C, García-Martin E, et al. Polymorphisms of the glutathione S-transferases mu-1 (GSTM1) and theta-1 (GSTT1) and the risk of advanced alcoholic liver disease. *Scand J Gastroenterol*. 2005;40(3):348–353. doi:10.1080/00365520510012109
28. Li S, Xue F, Zheng Y, et al. GSTM1 and GSTT1 null genotype increase the risk of hepatocellular carcinoma: evidence based on 46 studies. *Cancer Cell Int*. 2019;19(1):76. doi:10.1186/s12935-019-0792-3
29. Damavandi N, Zeinali S. Association of xenobiotic-metabolizing enzymes (GSTM1 and GSTT 1), and pro-inflammatory cytokines (TNF-α and IL- 6) genetic polymorphisms with non-alcoholic fatty liver disease. *Mol Biol Rep*. 2021;48(2):1225–1231. doi:10.1007/s11033-021-06142-1
30. Araujo OC, de Paula VS, Do Ó KM, et al. Association of Polymorphisms in the Glutathione S-Transferase Theta-1 gene with cirrhosis and hepatocellular carcinoma in Brazilian patients with chronic hepatitis C. *Vaccines*. 2021;9(8):831. doi:10.3390/vaccines9080831
31. Sprenger R, Schlagenhaufer R, Kerb R, et al. Characterization of the glutathione S-transferase GSTT1 deletion: discrimination of all genotypes by polymerase chain reaction indicates a trimodular genotype-phenotype correlation. *Pharmacogenetics*. 2000;10(6):557–565. doi:10.1097/00008571- 200008000-00009
32. Girault I, Lidereau R, Bièche I. Trimodal GSTT1 and GSTM1 genotyping assay by real-time PCR. *Int J Biol Markers*. 2005;20(2):81–86. doi:10.1177/172460080502000201
33. Fassio E, Díaz S, Santa C, et al. Etiology of hepatocellular carcinoma in Latin America: a prospective, multicenter, international study. *Ann Hepatol*. 2010;9(1):63–69. doi:10.1016/S1665-2681(19)31681-3
34. El-Kassas M, Elbadry M. Hepatocellular carcinoma in Africa: challenges and opportunities. *Front Med*. 2022;9:899420.

doi:10.3389/ fmed.2022.899420
35. Díaz LA, Ayares G, Arnold J, et al. Liver Diseases in Latin America: current status, unmet Needs, and opportunities for improvement. *Curr Treat Options Gastroenterol*. 2022;20(3):261–278. doi:10.1007/s11938-022-00382-1
36. Roy B, Chowdhury A, Kundu S, et al. Increased risk of antituberculosis drug-induced hepatotoxicity in individuals with glutathione S-transferase M1 'null' mutation. *J Gastroenterol Hepatol*. 2001;16(9):1033–1037.
37. Watanabe I, Tomita A, Shimizu M, et al. A study to survey susceptible genetic factors responsible for troglitazone-associated hepatotoxicity in Japanese patients with type 2 diabetes mellitus. *Clin Pharmacol Ther*. 2003;73(5):435–455.

APPENDICES

Appendix A: Ethical clearance letters



ADDIS ABABA UNIVERSITY, COLLEGE OF HEALTH SCIENCES (IRB)
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Institutional Review Board

ANNEX 3
Form AAUMF 03-008

IRB's Decision

Meeting No: 05/2022 Meeting Date: May 25,2022
Protocol number: 064/19/SoP

Protocol Title: Evaluation of the role of aflatoxins in the etiology of liver cirrhosis in Eastern Ethiopia: A case-Control study		
Principal Investigator:	Abrham Nigussie	
Institute:	College of Health Sciences, AAU	
Elements Reviewed (AAUMF 01-008)	☑ Attached	☐ Not attached
Review of Revised Application ☐ Yes ☐ No	Date of Previous review:	
Decision of the meeting:	☑Approved ☐ Approved with Recommendation ☐Resubmission ☐ Disapproved	

I. Elements approved- 1. Protocol Version No: 2
2. Protocol Version Date:
3. Informed consent Version No. 2
4. Informed Consent Version Date:

II. Obligations of the PI-
1. Should comply with the standard international & national scientific and ethical guidelines
2. All amendments and changes made in protocol and consent form needs IRB approval
3. The PI should report SAE within 10 days of the event
4. End of the study, including manuscripts and thesis works should be reported to the IRB
5. The PI should report non-compliance and unanticipated events

III. TO NERC ☐

Institution Review Board (IRB) Approval: Period from: June 06, 2022 to June 05, 2023
Follow up report expected in 3 Months ___6 months _✔___ 9 months____one year____

Chairperson, IRB
Dr. Adamu Addissie

Signature
Date: June 06, 2022

Faculty of Medicine
ADDIS ABABA UNIVERSITY

የኢትዮጵያ ፌደራላዊ ዲሞክራሲያዊ ሪፐብሊክ
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FEDERAL DEMOCRATIC REPUBLIC OF ETHIOPIA
MINISTRY OF EDUCATION

ስ: +251 11 155 3133
ፓ.ሳ.ቁ 1367፣ አራዳ ክፍለ-ከተ
አዲስ አበባ፣ ኢትዮጵያ
T: +251 11 155 3133
P.O. BOX 1367, ARADA SUB-CITY
ADDIS ABABA, ETHIOPIA
www.moe.gov.et
www.facebook.com/fdremoe
@fdremoe
info@moe.gov.et

ቀን/DATE: 21 JUN 2022
ቁጥር/REF NO: 02/846/927/22

Addis Ababa University College of Health Sciences
Addis Ababa

Subject: Letter of renewal

We are writing this letter in reference to your renewal request letter with Ref.No. CHS/RTTD/42/2022 dated June 19, 2022. After having in depth review of your request on **"Evaluation of the role of aflatoxins in the etiology of liver cirrhosis in Eastern Ethiopia: a case-control study design"** the Ministry of Education (MoE) via its National Research Ethics Review Committee has reviewed and approved your renewal request for one year (**June 20, 2022 - June 19, 2023**).

This is therefore, to notify that the ethical approval is renewed and your group can proceed in accordance to the latest approved document. Please make sure that you submit a biannual report and an annual renewal application 30 days prior to expiry date. We are confident that your esteemed organization and the PI (copied) of the project should monitor the ethical implication of the project as stipulated in the latest approved document.

Sincerely

Samuel Kifle Kidane (PhD)
State Minister

Cc

- State Minister (Sector for Higher Education Development)
- CEO, Research & Community Engagement Affairs
- Research Ethics Desk
  Ministry of Education

- Mr.Abraham Nigussie (PI)
  AAUCHS

Appendix B: Material transfer agreement

MATERIAL TRANSFER AGREEMENT

Addis Ababa University, College of Health Sciences, School of Pharmacy, Department of Pharmacology

Material Transfer Agreement

This Material Transfer Agreement (MTA) has been prepared for use by **Department of Pharmacology and Clinical Pharmacy** in all transfer of research material (samples, derivatives, and specimens) related to the protocol: **"Evaluation of the role of aflatoxins in the etiology of liver cirrhosis in Eastern Ethiopia: A case control study."**

Provider: Department of Pharmacology & Clinical Pharmacy, School of Pharmacy, College of Health Sciences, Addis Ababa University, Addis Ababa, Ethiopia

Recipient: University of Leeds, School of Food Science and Nutrition, Leeds, United Kingdom

1. Provider agrees to transfer to recipient's designated (human biological sample) the following research materials (specimen) **blood sample.**

The research material will only be used for research purposes as described in the protocol by recipient's investigator in designated laboratory for the research project described below, under suitable containment conditions. This research material will not be used for commercial purposes such as screening, production or sale for which a commercialization license may be required. Recipient agrees to comply with all National and International guidelines rules and regulations applicable to the Research Project and the handling of the Research Material.

a) Are the Research materials of human origin?

Yes [X] No [ ]

b) If yes, will they be collected according to the details in the protocol and in adherence to National Health Research Ethics Review Committee and Addis Ababa University, College of Health Sciences Ethics Review Committee recommendations and their approval?

Yes [X] No [ ]

2. This research material and its derivatives will be used by recipient's investigator solely in connection with the following research project described with specificity as follows

"Evaluation of the role of aflatoxins in the etiology of liver cirrhosis in Eastern Ethiopia: A case control study."

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3. In all presentations or written publications concerning the research projects, recipient will seek agreement of provider and acknowledge provider's contribution of this research material unless requested otherwise.

4. This research material represents a significant contribution on the part of provider and is considered proprietary to provider. Recipient therefore agrees to retain control over this research material and further agrees not to transfer the research material to other people not under her/his direct supervision without advance written approval of provider. The research material will be disposed of as agreed upon per protocol at the end of completion of the project.

5. The provider does not take any responsibility for loss, damage, wastage or spoilage of the research material during or after shipment to the address provided by the recipient under conditions agreed to in the protocol on shipment of the samples. This research material is provided as a service to research community. IT IS BEING SUPPLIED TO RECIPIENT WITH NO WARRANTIES, EXPRESS OR IMPLIED, INCLUDING ANY WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE. Provider makes no representations that the use of the research material will not infringe any patent or proprietary right of third parties.

6. The recipient shall notify the provider in writing of any intention, improvement, modification discovery or development to the material or the information made by recipient or parities, collaborating with recipient, herein after referred to an "invention". Nothing in this agreement shall, however, be construed as conveying to the provider any rights under any patents or other intellectual property to such invention, other than as explicitly provided herein. At its option the provider shall be entitled to receive sample of any materials derived from the Materials for its own research and evaluation purposes only.

7. The under-signed provider and Recipient expressly certify any affirm that the contents of any statements made herein are truthful and accurate.

8. Any additional terms (use an attached page if necessary):

9. The provider maintains, ownership right of the research material and its derivatives unless stated otherwise. The provider will retain a copy (aliquot) of every sample sent abroad as much as possible for local research needs.

Material Transfer Agreement signature page

For Recipient:

Recipient's Investigator

Professor Yun Yun Gong

Signature:

Da 21/01/2020

Mailing Address for Material

School of Food Science and Nutrition
University of Leeds
Leeds, LS2 9JT

Tel: +44(0)113 3431415

Fax:

Duly Authorized

School of Food Science and Nutrition
University of Leeds, United Kingdom

Frances Cameron
SENIOR CONTRACTS MANAGER
THE UNIVERSITY OF LEEDS

Signature/Stamp

Date 21/01/2020

Mailing Address for Notices:

Director of Commercialisation, RIS,
University of Leeds, Leeds, LS29JT

Tel:

Fax: Commercialisation@leeds.ac.uk

For Provider:

Provider's Investigator

Abraham Nigussie Mekuria

Date 11/11/2019

Mailing Address

Department of Pharmacology & Clinical Pharmacy, School of Pharmacy, Addis Ababa University, Addis Ababa, Ethiopia

P.O. Box 1176

Tel: 251911194519

Email: abrishn@yahoo.com

Duly Authorized

Department of Pharmacology & Clinical Pharmacy, School of Pharmacy, Addis Ababa University

Signature/Stamp

Date 12/11/2019

Mailing Address for Notices

Department of Pharmacology & Clinical Pharmacy, School of Pharmacy, Addis Ababa University, Addis Ababa, Ethiopia

P.O. Box 9086

Tel: 2510118265147

Fax: 251111558566

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Appendix C: Participant information sheet and informed voluntary consent forms

Participant information sheet and informed voluntary consent form (English version)

My name is _____. I am working as a data collector for the study being conducted in this hospital by Abraham Nigussie who is studying for his Doctorial degree at Addis Ababa University, College of Health Sciences, School of Pharmacy. I kindly request you to lend me your attention to explain you about the study and being selected as the study participant.

1. The study/project title:

Evaluation of the role of aflatoxins in the etiology of liver cirrhosis in Eastern Ethiopia: A case-control study.

2. Purpose/aims of the study:

The findings of this study can be of paramount importance for health bureaus of Eastern Ethiopia to plan interventions program along with other sector bureaus including Agriculture, food quality control and food processing firms to plan intervention programs to prevent liver disease that has been associated with consumption of aflatoxins contaminated foods in your community; there by improve health and survival, in general. Moreover, the aim of this study is to write a thesis as a requirement for partial fulfillment of Doctorial program in Pharmacology for the principal investigator.

3. Procedures and duration

I will be interviewing you using a questionnaire to obtain pertinent data that is helpful for the study. The interview will take about 45 minutes. Then, you will be examined by a well-experienced physician and radiologist (using ultrasound) and the examination may take 30 minutes and then, if you are willing, a well-trained medical laboratory technologist will insert a needle into your vein in one of your arms and draw two teaspoonful of blood, this may take 5-10 minutes. Your blood sample will be tested for hepatitis B virus, hepatitis C virus, liver function markers, alpha-fetoprotein level, aflatoxin-albumin adduct, and also a screening of 249ser TP53 mutation (change that occurs in your DNA sequence) and genotyping for *glutathione –S- transferase (GST) and human microsomal epoxide hydrolase (EPHX)* genes.

4. Risks and benefits:

The risk of being participating in this study is beyond minimal risk. You may feel a slight discomfort while the blood sample is taken and you may have some bruising at the place where the blood sample is taken which would disappear in short duration. To prevent any risk of infection and minor discomfort, blood sample will be withdrawal with universal precautions. It also takes few minutes from your time.

You will not have to pay any fee for any test done for this study. You will also not be given any fee for participating in this study. However, the findings from this research may reveal important information to plan appropriate interventions that will help to reduce the risk of liver cirrhosis associated with aflatoxin.

5. Confidentiality:

The information that will be collected from this study will be fully confidential**.** There will be no information that will identify you. The findings of the study will be general for the study community and will not reflect anything particular of individual persons or housing. The data that we will be gathered from this study will exclude showing names. No reference will be made in oral or written reports that could link participants to the research.

6. Rights:

Participation for this study is fully voluntary. You have the right to declare to be involved in this study or not. You may choose to stop participating in this research at any time that you wish to, without having to explain yourself. You may also choose not to answer any question or not to undergo any procedure you find uncomfortable or private. There will be no consequence, loss of benefit or care to you if you choose to withdraw from the study.

7. Person to contact:

If you have any problems or questions about this study you should feel free to ask now or anytime throughout the study by contacting: Abraham Nigussie, mobile number; 0911194519, email address; abrishn@yahoo.com; as well as contact address of the responsible Institutional Review Board (IRB), Addis Ababa University, using the details below for further advice and information: Telephone 011 896 1396; email: chs.irb@aau.edu.et; P.o.Box 9086, Addis Ababa, Ethiopia.

8. Declaration of informed voluntary consent:

I have read/ was read to me the participant information sheet. I have clearly understood the purpose of the research, the procedures, the risks and benefits, issues of confidentiality, the rights of participating and the contact address for any queries. I have been given the opportunity to ask questions for things that may have been unclear. I was informed that I have the right to withdraw from the study at any time or not to answer any question or not cooperate to undergo any procedure that I do not want. Therefore, I declare my voluntary consent to participate in this study with my initials (signature).

Name and signature of the study participant: _____ Date _____

Name and signature of data collector: _____ Date_____

N.B

- This is signed face to face in the presence of the data collector.
- Please provide a copy of this signed consent to the participant.
- If the participant is a lay person and cannot sign initials, can put his/her thumb print in front of a competent witness; and the witness has to sign alongside (with his/her name and address).

Participant information sheet and informed voluntary consent form (Afann Oromo version)

Maqaa kiyyaa_____ kan tahe Universitii Finfinnee Kolleejii Fayyaa mana barumsa Farmaasitii Doktoraal Digriifiin barachuti kan jiru Abrahaam Niguseetiif akka sassabdu raga hojiachutin jira. Yeroo waa'ee qorannoo filatame ibsu xiyyeeffa nnoo akka naf kanifanifi akka nahordoftan kabajaedhan gaaffadha.

1. Mataa Duree Qorannoo

Baha Itophiyaa keesati jiran keesa, gamaagama shora, Aflatoxin madda Dhukkuba tiruu (Liver cirrhosis) qabu qorachudha, Qorannoo fakkisa (case-control study).

2. Kaayyoo Qorannoo

Argannoo qorannoo kana irra anqamu Biiroolee fayya, Bahaa Itophiyaa keessati argaman, qorannoo argannoo isan gageesanifi Biiroolee qonnar, qulqulina nyaata warsnaalee nyaata qoppeesan, nyaata Aflatoxininitiin kan faalame wa'iqabate rekkoolee dhukkuba tiruu ummata keessati jiran ittisuf hojii isan gageesan gargaaruf shora gudda qaba. Karaa kanaan ifjireenya fi fayyaa ummata akka egamu nigargaara. Kana irrati dabalate abbicha qorannoo kana gageesu Barumsaa faarmaasii akke argatu ulaaga akka gutu gargaara.

3. **Adeemsa fi yeroo**

Ragaalee Qorannoo kana wajjiin waldhufeenya qaban fi Qorannoo kana gargaaruf gaaffannoo itti fayyadamudhan gaafilee isin gaafana. Gaafileen daqiiqaa 45 fudhachu dandaan. Kana booda Haakimaa muxxannoo qabanifi Radioyologistii (altaasaawundi itti fayyadamudhan) Niqoratamtan. Qorannoon dhaqiqaa 30 fudhata. Kanati ansee fedhii yoo qabaatan ogeesa Laaboraatorii tecknologistiin Harkaa tokkaa lilman dirudhan dedebistu dhiga irra dhiga fele'anaa sahyi lemaa fudhata, kuniis dekikan 5-10 ni fudhataa. dhignii dhukkuba tirruu vaayrasii hepatitis B, vaayrasii Hepatitis C, liver function markarsii, alpha feto protein level, Aflatoxin-albumin adduct, fi screening of 249serTP53 mutation (Jijiremaa sequencii DNA) fi genotyping for *glutathione –S- transferase (GST)* fi *human microsomal epoxide hydrolase (EPHX)* genes torechuf tejaajilaa.

4. Milkaa'uu fi Danta bu'aalee

Milkaa'uu hirmaannaa qorannoo kana keessati jiraachu dandaa'n xiiqaa miiti. Hoguu Dhigni fudhatamu hirkaa dhigaa fudhatamu irrati dhibii sidhaga'ama. Bakka dhigan fudhatamutis, dirmaamuu xiiqaa jiraachu danda'aa. Garuu dirmamnii yeroo tikaa bodaa ni bedaa. Balaa dhukkuba qabsistu fi jija ankachu ahabu ittisuf yeroo fakkisaa dhiga fudhatamu ofeggannoo barbaachisa hunda nigodhama. Yeroo keessani irratis daqiqaa xiiqaa fudhata. Hirmaannaa kanaaf kafalti kafalamu hinjiru ken isinif kefelemus hinjiru.Haatauu malee argannoo qorannoo kana balaa dhukkuba tiru (liver cirrhosis) fi dhukkuboota aflatoxin wajjiin walqunamti qaban ittisuf giddu seenaa godhamuf odeeffnnoo barbaachisa tahe nikenaa.

5. Iccitumaa

Ragaalee qorrannoo irra sassaabaman iccitumaa isaani gutuma guttutii kan egame taha. Raga eenyuma keessan kan ibsu kan ifaa godhamu hinjiru.Argannoo qorannoo kana qaamulee itti fayyadamanif demsaa snaati kenama malee maqaa hirmaatoota fi haarka isani hinibsamu.Raga kan hirmaatoota wajjiin walqabata Raga baru tahe kan afaani kan ibsamu hinjiru.

6. Mirga

Hirmaatootan hirmaannaa isan godhan gutumaa gututi fedhi isaan irrati kan hundaa'e dha. Qorannoo kana hirmaachuti hirnaachu dhabun filanoon kan isaaniti qorannoo kana irrati hirmaachuti hirmachu dhabun filanoon kan isaaniti Qorannoo kana irrati sadarka kamiyuu irrati osoo enyumaa keessan hinibsin dhaabu nidendeesan. Gaafii isinif hintolee yokaan adeemsa isin hinnijoyne hindeebisu yokaan inhirmaadhu jechuf mirga qabdan. Hirmachu dhabu keesanif yokaan debisu dhabu keessanif tarkaanfi fudhatamu hinjiru. Yokaan wan dhabtan hinjiru.

7. Teesso-waliti dhufeenya

Qorannoo kana ilaalchise gaafii kammiyu kan isin barbaadan kana Abrhaam Nigusee lekofsa mobaayila 0911194519. Emaili abrishn@yahoo.com fi teesoo institushinii iffi gaafatamtuma qabu Institutional Review Board (IRB), Addis Ababa Universitylekofsaa Bilbilaa 0118961396; email chs.irb@aau.edu.et or SCB 9086, Addis Ababa walqunamu dandeesan.

8. Ibsa Namoota qindeesoota fedhi qabeensa

Waraqaa odeeffanno hirmaatoota dubbisetin/ naf dubbifame jira. Kaayyoo Qorannoo, adeemsa Qorannoo, hikaa'uufi danta Bujaalee, dhimmi Iccituma, mirga Hirmaatootafi gaafi kamiyutiif tessoo walqunamti karaa sirritiin hubadhefiin jira. Dhimmi naf ifaa hintaanef gaafii akaa dhikeeso carraa naf kaname jira. Yerro barbaaduti qorannoo kana irra hirmaana kiyya akka dhaabu danda'uu, garfiilee naf dhihaa tanif deebisa kennu dhabu akka danda'uu naf ibsame jira. Kanaafu fedhi kiyyaatiin yaada hirmaatoota walifi fiidu irrati hirmaachu kiyya mallattoo kiyyatiin mirkaneesa.

Maqaafi mallatto Hirmaata qorannoo _____ Guyya_____

Maqaafi mallatto sassaabdu qorannoo _____ Guyya____

Yaddannoo

- Mallattoo kana Bakka sessaabdu Qorannoo jiruti ifaati mallataa'ee
- Koopii yaada Qindeesate hirmaatootaf akka kanamu. Yaadechisna
- Hirmaataan nama hinbarranne yoo tahe fi mallatusu kan hindandenye yoo tahe bakka, nama amanamu jiruti qubaan mallatteesu nidanda'a Isa cinaaf Ragaan. Isa cinaafi mallatteesu qebe. (maqaa Isa/Ishe wajjiiti)

Participant information sheet and informed voluntary consent form (Amharic version)

ስሜ _____ይባላል። በ አዲስ አበባ ዩኒቨርስቲ ጤና ሳይንስ ኮሌጅ፡ ፋርማሲ ት/ቤት፡ የ ፋርማኮሎጂ እና ክሊኒካል ፋርማሲ ት/ክፍል፡ የድህረ ምረቃ ተማሪ የሆኑት አቶ አብርሃም ንጉሴ በአሁኑ ሰዓት የመመረቂያ ጥናታቸዉን በ————————ሆስፒታል እየሰሩ ሲሆን: እኔም የጥናቱ አጋር በመሆን ለጥናቱ ከተመረጡ ግለሰቦች ላይ መረጃዎች እሰበስባለሁ፡ እናም ለጥናቱ ከተመረጡ ግለሰቦች መሀል አንዱ አርሶ ስለሆኑ ስለጥናቱ ምንነትና አላማ ገለፃ እንዳደርግሎት እንዲፈቅዱልኝ በትህትና እጠይቃለሁ።

1. የጥናቱ / የፕሮጀክቱ ርዕስ:

በምስራቅ ኢትዮጲያ ስላለው የ ጉበት በሽታ መንስኤ የ አፍላቶክሲን ሚናን ማጥናት::

2. የጥናቱ አስፈላጊነት/ አላማዎች:

የዚህ ጥናት ግኝት/ቶች ለምስራቅ ኢትዮጵያ የጤና ቢሮዎች ከሌሎች ቢሮዎች ጋር ማለትም የግብርና፣ የምግብ ጥራት ቁጥጥር ፣ እንዲሁም የምግብ ማቀነባበሪያ ተቋማት ጋር በመሆን በአፍላቶክሲን በተበከሉ ምግቦች ተያይዞ የሚመጣን የጉበት በሽታን ለመከላከል የሚያስችሉ ጣልቃ-ገብ መርሃ-ግብሮችን ለማቀድ ከፍተኛ ጠቀሜታ አለው:: በአጠቃላይ በአካባቢዎ ያሉትን ማህበረሰቦች ጤንነትን እና ህይወት የሚያሻሽል ይሆናል:: በተጨማሪም የዚህ ጥናት ዓላማ ለዋናው ተመርማሪ የዶክትሬት ድግሪያቸዉን በፋርማኮሎጂ ትምህርት ዘርፍ ለማጠናቀቅ የሚረዳቸዉን ጥናታዊ ጹሁፍ ለመስራት ይጠቅማቸዋል::

3. ሂደቶች እና ቆይታ:

ለጥናቱ ጠቃሚ የሆኑ መረጃዎችን ለመሰብሰብ መጠይቅ በመጠቀም ቃለ መጠይቅ አደርግልሀለሁ/ሻለሁ፡ ቃለ-መጠይቁም አንድ 45 ደቂቃ ያህል ይወስዳል። ከዚያም በደንብ ልምድ ባላቸው የዉስጥ-ደዌ ሐኪም እና ራዲዮሎጂስት (አልትራሳውንድ በመጠቀም) ምርመራ ይደረግልዎታል። ምርመራውም 30 ደቂቃ ሊፈጅ ይችላል። ከዚያም በ በደንብ በሰለጠነ የህክምና ላቦራቶሪ ቴክኒሻያን መርፌ በመጠቀም ከአንደኛው እጅዎ ደም ስር ላይ ሁለት የሻይ ማንኪያ ደም ይወሰዳል፡ ይህም ከ 5-10 ደቂቃ ሊወስድ ይችላል። የተወሰደውም የደም ናሙናን በመጠቀም ሄፐታይተስ ቢ ቫይረስ፣ ሄፓታይተስ ሲ ቫይረስ፣ የጉበት ተግባር ጠቋሚ ኢንዛይሞች መጠን ፣ የአልፋ-ፌቶ ፕሮቲን መጠን ፣ አፍላቶክሲን መጠን ፣ እና ዘረ መልዎ 249^{ser}TP53 ሽግግር (በዘረ መልዎ ቅደም ተከተልዎ ውስጥ የሚከሰት ለውጥ) እና የ ግሉታታቴን ትራንስፈሬዝ እና ኢፖክሳይድ ሀይድሮሌዝ ኢንዛይሞች ጂኖች አይነት ጥናት ይውላል።

4. አደጋዎች እና ጥቅሞች:

በዚህ ጥናት ውስጥ መሳተፍ አደጋው ከዝቅተኛ በላይ ነው። የደም ናሙና በሚወሰድበት ጊዜ ትንሽ ህመም ሊሰማዎት ይችላል፡ ልላው የደም ናሙናው በተወሰደበት ቦታ ላይ የተወሰነ እብጠት ለአጭር ግዜ ሊኖርዎት ይችላል። በደም የሚተላለፉ በሽታዎች የመያዝ አደጋን ለማስቀረት ና የ ህመም ስሜትን ለመቀነስ ደም በሚወስዱበት ጊዜ ሁለንተናዊ ጥንቃቄዎች ይወሰዳል። ሌላው ከእርስዎ ሰዓት ላይ ትንሽ ደቂቃዎች ይወስዳል።

በዚህ ጥናት ውስጥ በሚሳተፍበት ግዜ ለሚደረጉ ምርመራዎች ምንም አይነት የገንዘብ ክፍያ አይከፍሉም። በዚህ ጥናት ውስጥ ለመሳተፍ ምንም የገንዘብ ክፍያ አይሰጥዎትም። ይሁን እንጂ ከጥናቱ የሚገኙ ግኝቶች ከአፍላቶክሲን ጋር ተያይዞ የሚመጡ በሽታዎችን ለመቀነስ የሚረዱ ተገቢ ጣልቃ-ገብ መፍትሄዎችን ለመፈለግ ጠንካራ መሰረት ይጥላል።

5. ሚስጥራዊነት:

ከዚህ ጥናት ጋር ተያይዞ የምንሰበስበው መረጃ ሙሉ በሙሉ ሚስጥራዊ ነው፡ እርስዎን የሚለይ መረጃ አይኖርም። የጥናቱ ግኝቶች ለጥናቱ ማህበረሰብ አጠቃላይ ይሆናሉ። እናም የግለሰብን ወይም የቤቱን መለያ የሚያንጸባርቅ አይደለም። ከዚህ ጥናት የምንሰበስበው መረጃ መለያ ስሞችን በጭራሽ አይዝም። በጥናቱ ውስጥ ተሳታፊዎችን ሊያሳዩ የሚችሉ የቃልም ሆነ የጽሑፍ ሪፖርቶች አይኖሩም።

6. መብቶች:

የዚህ ጥናት ተሳትፎ ሙሉ በሙሉ በፈቃደኝነት ነው። በዚህ ጥናት ውስጥ ለመሳተፍ ወይም ላለመሳተፍ የመወሰን መብት ሙሉ በሙሉ የእርስዎ ነው። በዚህ ምርምር ለይ በማንኛውም ጊዜ ላለመቀጠል ከመረጡ ምንም አይነት

ምክንያት ሳይሰጡ ማቋረጥ ይችላሉ። ለእርስዎ የማይመቹ ወይም የግል ለሆኑ ጥያቄዎች መልስ አለመስጠት ይችላሉ። እንዲሁም ያልተመቸዎት የጥናቱ ሂደት አለማከናዎን ይችላሉ። ከጥናቱ ለመውጣት ከመረጡ ምንም አይነት የሚያጡት የህከምና ጥቅም ወይም እንክብካቤ አይኖርም።

7. ጥያቄ ካሎዎት:

ይህን ጥናት በተመለከተ ወይም ከዚህ ጋራ በተዛመደ መልኩ ስለሚያጋጥሙ ችግሮች ወይም ጥያቄ ካሎት በሚከተለዉ አድራሻ ይጠቀሙ። አብርሃም ንጉሴ: ሞባይል ስልክ ቁጥር: 0911194519፣ ኢሜል abrishn@yahoo.com ወይም Institutional Review Board (IRB), Addis Ababa University, በቢሮ ስልክ ቁጥር 011 896 1396, ኢሜል: chs.irb@aau.edu.et ወይም ፖሳቁሩ 9086, አዲስ አበባ በሚል አድራሻዎችን ይፃፉልን።

8. በበጎ ፈቃደኝነት ላይ የተመሠረተ ስምምነት-

የተሳታፊዎችን የመረጃ ዝርዝር አንብቤአለሁ / ተነቦልኛል። የጥናቱንም አላማ፣ ስነ-ሂደቶቶች፣ አደጋዎች እና ጥቅሞች፣ ሚስጢራዊነት ፣ የመሳተፍ መብቶች እና ማናቸውም ጥያቄዎች ካሉኝ የማሳወቂያ አድራሻውን መጠቀም እንደምችል በግልጽ ተረድቼያለሁ። ምናልባት ግልጽ ባልሆኑልኝ ነገሮች ላይ ጥያቄዎችን ካሉኝ ለመጠየቅ እድል ተሰጥቶኝ ነበር። በዚህ ምርምር ለይ በማንኛውም ጊዜ ላለመቀጠል ከወሰንኩ የማቋረጥ መብት እንዳለኝ ተረድቻለሁ። ይችላሉ። እንዲሁም ለእኔ የማይመቹ ወይም የግል ለሆኑ ጥያቄዎች መልስ አለመስጠት፡ ያልተመቹኝን/የማልፈልጋቸውን የጥናቱ ሂደቶችን ያለማከናዎን እንደምችል ተረድቻለሁ። ስለሆነም፡ በዚህ ጥናት ለመሳተፍ በጎ ፈቃደኝነቴን በፊርማዬ አረጋግጣለሁ።

የተሳተፊው ስም እና ፊርማ: _____ ቀን _____

የመረጃ ስብሳቢው ስም እና ፊርማ: _____ ቀን _____

- ይህ ፊርማ መረጃ ሰብሳቢው ባለበት ፊት ለፊት ተፈርሟል::
- ለተሳታፊው የተፈረመውን ስምምነት ቅጂ እባክዎን ያስጡ::
- ተሳታፊው ሰው የመጀመሪያ ፊርማቸውን መፈረም ካልቻሉ የእራሱን / የእራሳን ህትመት በሚታወቅ ምስክር ፊት ያቅርቡ እና ምስክሩም ፊርማቸውን ያስቀምጡ ከእሳቸው / ከሷ ስም እና አድራሻ ጋር::

Appendix D: Questionnaires

Questionnaire (English version)

Addis Ababa University, Collage of Health Sciences, School of Pharmacy, Department of Pharmacology and Clinical Pharmacy, questionnaire to be used for collection of data regarding socio-demographic, tobacco use, alcohol drinking, Khat chewing, and dietary history from each participant (cases and controls) enrolled in the study that aims at evaluating the role of aflatoxins in the etiology of liver cirrhosis in Eastern Ethiopia.

Introduction

Thank you for signing the informed consent to participate in this study entitled by "evaluation of the role of aflatoxins in the etiology of liver cirrhosis in Eastern Ethiopia". We are conducting this research in HFCSUH and we will interview many patients attending this hospital to achieve the objectives of the study. As we have discussed, the first thing that we will do is to fill in the questionnaire concerning this study which comprises, basic information, socio-demographic details, tobacco use, khat chewing, alcohol drinking, and last year's dietary habits. I will also measure your height and take your weight. I would like to reassure you that the interview will be strictly confidential and that the information disclosed will only be used without any personal name or identifiers. Any potential benefits of the study for the well-being of the population rely on the accuracy of your answers. Therefore, if you do not understand the meaning of any of the questions, please don't hesitate to ask. At any time you may refuse to continue or to answer specific questions.

Do you have any question/concern? |__| Yes |__| No

[If the study participant has any concern, the interviewer will give an explanation to clarify or reassure] If you don't have any question, can we start now?

Part 1: Basic information

Interviewer: please fill out this part before the interview starts, after consent has been obtained.

1.	Study Number	|__|__|__|
2.	Status of study participant	|__| (1) Case (2) Control
3.	Date of interview	|__|__|/|__|__|/|__||__||__||__| (dd/mm/yy)
4.	Gender	|__| (1) Male (2) Female

Part 2: Socio-demographic details

5.	What is your age? (completed years)	|__|__|__| years Encircle, if (Don't know); (Refuse)

6.	What is your marital status?	|__|(1) Single (2) Married (3) Widowed (4) Divorced (5) Other, specify_____ (6) Don't know (7) Refuse
7.	In which town do you live?	_____(text)
8.	Is your present residence rural or urban?	|__| (1) Rural (2) Urban (3) Don't know (4) Refuse
9.	How long have you lived in this residence?	|__|__|__| years
10.	What is your ethnic group?	|__| (1) Oromo (2) Harari (3) Somali (4) Amhara (5) Others, specify____ (6) Refuse
11.	What is your religion?	|__| (1) Islam (2) Christianity (3) Other, Specify ____ (4) None (5) Refuse
12.	What is your education level? (highest level achieved)	|__| (1) Illiterate (2) Primary (3) Junior middle school (4) Secondary (5) College/university and above (6) Don't know (7) Refuse
13.	What is your occupation?	|__| (1) Farmer (2) Unemployed (3) House wife (4) Student (5) Public (6) Day laborer (7) Servant (8) Other, specify_____.
14.	Have any of your first degree relatives (father, mother, brother, sister, son, daughter) had diagnosed with liver disease?	|__| (1) No (2) Yes (3) Don't know (4) Refuse
15.	Have you taken herbal medicine?	|__| (1) No (2) Yes (3) Don't know (4) Refuse **[If No, go to Q. 18]**
16.	Do you remember the name of herbal medicine? please specify	_____ (Text) Encircle, if (Don't know); (Refuse)
17.	For what disease/purpose did you take the herbal medicine?	_____ (Text) Encircle, if (Don't know); (Refuse)
18.	Height (to be measured at the time of interview)	**____** Cm
19.	Weight (to be taken at the time of interview)	_____Kg

Part 3: Tobacco use history

3.1. Cigarette smoking

20	Have you ever smoked at least 100 cigarettes in your lifetime?	|__| (1) No (2) Yes (3) Don't know (4) Refuse [If No, go to **Q. 26]**
21	On average, how many cigarettes/day do/did you smoke?	|__|

22	At what age did you start smoking cigarettes?	|__|__|__|
23	How many years have you smoked cigarettes? (Please make sure to exclude the in-between years of quitting)	|__|__|__|
24	Do you still smoke cigarettes now?	|__| (1) No (2) Yes (3) Don't know (4) Refuse
25	How long has it been since you stopped cigarette smoking? (only for past smokers [those who answer "**No" to Q. 24])**	|__|__|__|Years Encircle, if (Don't know); (Refuse)

3.2. Water pipe smoking

26	Have you smoked water pipe at least once a week for at least 1 year?	|__| (1) No (2) Yes (3) Don't know (4) Refuse **[If No, go to Q. 34]**
27	How many times per week do you smoke a water pipe?	|__|__|__| times/week
28	How much quantity of tobacco do/did you use each time?	|__|__|__| grams/session
29	On average, with how many people do/did you share the same pipe during a smoking session?	|__|__|__|
30	How many years have you smoked water pipes?	|__|__|__|
31	At what age did you start smoking water pipes?	|__|__|__|
32	Do you still smoke water pipes now?	|__| (1) No (2) Yes (3) Don't know (4) Refuse
33	How long has it been since you stopped water pipe smoking? (Only for past water pipe smokers **[If "No" to Q. 32])**	|__|__|__| Years; Encircle, if (Don't know); (Refuse)

3.3. Pipe smoking

34	Have you ever smoked at least 50 pipes in your lifetime?	|__| (1) No (2) Yes (3) Don't know (4) Refuse **[If No, go to Q. 40]**
35	On average, how many pipes of tobacco/week do/did you smoke?	|__|__|
36	How many years have you smoked a pipe?	|__|__|__|

37	At what age did you start smoking pipes?	|__|__|__|
38	Do you still smoke pipes now?	|__| (1) No (2) Yes (3) Don't know (4) Refuse
39	How long has it been since you stopped pipe smoking? (only for past pipe smokers **[If "No" to Q. 38])**	|__|__|__| Years; Encircle, if (Don't know); (Refuse)

3.4. Chewing Tobacco

40	Have you chewed tobacco for at least 1 year?	|__| (1) No (2) Yes (3) Don't know (4) Refuse **[If No, go to Q. 47]**
41	On average, how many times/week did/do you chew tobacco?	|__|__|
42	On average, how many grams of tobacco did/do you chew each time?	|__|__|__|
43	How many years have you chewed tobacco?	|__|__|__|
44	At what age did you start chewing tobacco?	|__|__|__|
45	Do you still chew tobacco now?	|__| (1) No (2) Yes (3) Don't know (4) Refuse
46	How long has it been since you stopped chewing tobacco? (only for past Tobacco chewers [**If "No" to Q. 45])**	|__|__|__| **years**; Encircle, if (Don't know); (Refuse)

Part 4. Khat chewing history

47	Have you ever chewed khat once a week, or more frequently, for at least one year?	|__| (1) No (2) Yes (3) Don't know (4) Refuse **[If No, go to Q. 55]**
48	At what age did you start chewing khat regularly, at least once a week?	|__|__|__| years
49	On average, how many days per week do/did you use khat?	|__| Please specify number of days Encircle, If, (don't know); (Refuse)
50	What is/was the name or type of khat you chew regularly	----------(text)
51	On average, how much quantity of khat do/did you use each time? **(Use visual scale)**	|__|__|__| grams/session

52	Other than water, what extras did/do you use when you chew khat?	Cigarette |__| (1) No (2) Yes (3) Don't know (4) Refuse Hoja |__| (1) No (2) Yes (3) Don't know (4) Refuse Peanut |__| (1) No (2) Yes (3) Don't know (4) Refuse Kola |__| (1) No (2) Yes (3) Don't know (4) Refuse Tea |__| (1) No (2) Yes (3) Don't know (4) Refuse Coffee |__| (1) No (2) Yes (3) Don't know (4) Refuse Other, specify____ (1) No (2) Yes (3) Don't know (4) Refuse
53	Are you chewing khat regularly these days?	|__| (1) No (2) Yes (3) Don't know (4) Refuse
54	How long has it been since you stopped khat chewing? **(only for past chewers [those who answer "No" to Q. 53)**	|__|__|__| Encircle, If, (don't know); (Refuse)

Part 5. Alcohol drinking history

55	Have you ever drunk alcohol (*Tela, Tej, Areke,* beer, hard liquor) at least once per week for more than six months?	|__| (1) No (2) Yes (3) Don't know (4) Refuse **[If No, go to Q. 70]**
56	At what age did you begin drinking alcohol?	|__|__|
57	On average, how many days in a week do you drink alcohol?	|__|__| Please specify number of days. Encircle, If, (don't know); (Refuse)
58	What type of alcohol do you usually drink?	Beer |__| (1) No (2) Yes (3) Don't know (4) Refuse Tela |__| (1) No (2) Yes (3) Don't know (4) Refuse Wine |__| (1) No (2) Yes (3) Don't know (4) Refuse Tej |__| (1) No (2) Yes (3) Don't know (4) Refuse Hard Liquor |__| (1) No (2) Yes (3) Don't know (4) Refuse Areke |__| (1) No (2) Yes (3) Don't know (4) Refuse
59	On average, how many drinks (bottles of beer, glasses of tela, wine or liquor) do you have on a typical day when you are drinking? **(Please show picture of alcohol beverages)**	Beer |__|__| Encircle, If, (don't know); (Refuse) Tela |__|__| Encircle, If, (don't know); (Refuse) Wine |__|__| Encircle, If, (don't know); (Refuse) Tej |__|__| Encircle, If, (don't know); (Refuse) Hard Liquor |__|__| Encircle, If, (don't know); (Refuse) Areke |__|__| Encircle, If, (don't know); (Refuse)
60	How often do you have six or more drinks (using the previously mentioned measurement containers) on one occasion?	|__| (1) Never (2) Less than monthly (3) Monthly (4) Weekly (5) Daily (6) Don't know (7) Refuse
61	How often during the last year have you found that you were not able to stop drinking once you had started?	|__| (1) Never (2) Less than monthly (3) Monthly (4) Weekly (5) Daily (6) Don't know (7) Refuse

62	How often during the last year have you failed to do what was normally expected of you because of drinking?	|__| (1) Never (2) Less than monthly (3) Monthly (4) Weekly (5) Daily (6) Don't know (7) Refuse
63	How often during the last year have you needed a first drink in the morning to get yourself going after a heavy drinking session?	|__| (1) Never (2) Less than monthly (3) Monthly (4) Weekly (5) Daily (6) Don't know (7) Refuse
64	How often during the last year have you had a feeling of guilt or remorse after drinking?	|__| (1) Never (2) Less than monthly (3) Monthly (4) Weekly (5) Daily (6) Don't know (7) Refuse
65	How often during the last year have you been unable to remember what happened the night before because of your drinking?	|__| (1) Never (2) Less than monthly (3) Monthly (4) Weekly (5) Daily (6) Don't know (7) Refuse
66	Have you or someone else been injured because of your drinking?	|__| (1) Never (2) Less than monthly (3) Monthly (4) Weekly (5) Daily (6) Don't know (7) Refuse
67	Has a relative, friend, doctor or other health care worker been concerned about your drinking or suggested you cut down?	|__| (1) Never (2) Less than monthly (3) Monthly (4) Weekly (5) Daily (6) Don't know (7) Refuse
68	Do you still drink alcohol now?	|__| (1) No (2) Yes (3) Don't know (4) Refuse
69	How many years has it been since you stopped drinking? (**Only for past drinkers [If "No" to Q. 68])**	|__|__| years

Part 6. Dietary history

Please recall your diet history **one year ago** when responding to the following questions.

70	What was the staple food you used to eat? (regularly taken food)	|__| (1) Rice (2) Teff Enjera (3) Corn bread (4) Wheat bread (5) Sorghum Enjera (6) Pasta (7) other (specify)_____ (8) Don't know (9) Refuse
71	What is the source of your dietary staple?	|__| (1) Home grown (2) From the market (3) Don't know (4) Refuse
72	Do you store the dietary grain underground? (if the source of the dietary grain is home grown)	|__| (1) No (2) Yes (3) Don't know (4) Refuse
73	Have you ever consume peanut once a week, or more frequently, for at least one year?	|__| (1) No (2) Yes (3) Don't know (4) Refuse [**If No, go to Q. 76]**
74	What form of peanut do/did you	**Roasted** |__| (1) No (2) Yes (3) Don't know (4)

	consume?	Refuse
		Butter |__| (1) No (2) Yes (3) Don't know (4) Refuse
		Other form, specify————————
75	How often do/did you consume this form of peanut?	**Roasted** |__| (1) Never (2) Seldom (3) 1-3 per month (4) 1-2 per week (5) 3-4 per week (6) Daily (7) Don't know (8) Refuse
		Butter |__| (1) Never (2) Seldom (3) 1-3 per month (4) 1-2 per week (5) 3-4 per week (6) Daily (7) Don't know (8) Refuse
		Other form, specify_____

Instruction –Dear participant please take few moment to memorize the foods you ate one year ago and I will say the food items and you will tell me how frequently did you consume it?

Ser.	**Food items**	**Frequency** (Mark with an 'X' the frequency with which he/she consumed the food recorded)							
		Daily	3-4 per week	1-2 per week	1-3 per month	Seldom	Never	Don't Know	Refuse
76.	**Meat (beef, camel, goat, sheep)**	|__|	|__|	|__|	|__|	|__|	|__|	|__|	|__|
77.	**Chicken**	|__|	|__|	|__|	|__|	|__|	|__|	|__|	|__|
78.	**Fish**	|__|	|__|	|__|	|__|	|__|	|__|	|__|	|__|
79.	**Milk or other dairy products**	|__|	|__|	|__|	|__|	|__|	|__|	|__|	|__|
80.	**Eggs** (from chicken)	|__|	|__|	|__|	|__|	|__|	|__|	|__|	|__|
81.	**Bread**								
82.	**Pasta** (spaghetti, maccheroni, etc)	|__|	|__|	|__|	|__|	|__|	|__|	|__|	|__|
83.	**Rice**	|__|	|__|	|__|	|__|	|__|	|__|	|__|	|__|
84.	Beans	|__|	|__|	|__|	|__|	|__|	|__|	|__|	|__|
85.	Lentils	|__|	|__|	|__|	|__|	|__|	|__|	|__|	|__|
86.	**Green leafy vegetables**								
	Cabbage	|__|	|__|	|__|	|__|	|__|	|__|	|__|	|__|
	Lettuce	|__|	|__|	|__|	|__|	|__|	|__|	|__|	|__|
	Kosta	|__|	|__|	|__|	|__|	|__|	|__|	|__|	|__|
87.	**Other vegetables**								
	Tomato	|__|	|__|	|__|	|__|	|__|	|__|	|__|	|__|
	Fosoliya	|__|	|__|	|__|	|__|	|__|	|__|	|__|	|__|
	Pumkin	|__|	|__|	|__|	|__|	|__|	|__|	|__|	|__|
	Carrot	|__|	|__|	|__|	|__|	|__|	|__|	|__|	|__|

	Potato	|__|	|__|	|__|	|__|	|__|	|__|	|__|	|__|
	Onion	|__|	|__|	|__|	|__|	|__|	|__|	|__|	|__|
	Green pepper	|__|	|__|	|__|	|__|	|__|	|__|	|__|	|__|
88.	**Fruits**								
	Avocado	|__|	|__|	|__|	|__|	|__|	|__|	|__|	|__|
	Orange	|__|	|__|	|__|	|__|	|__|	|__|	|__|	|__|
	Banana	|__|	|__|	|__|	|__|	|__|	|__|	|__|	|__|
	Mango	|__|	|__|	|__|	|__|	|__|	|__|	|__|	|__|
	Pineapple	|__|	|__|	|__|	|__|	|__|	|__|	|__|	|__|
	Papaya	|__|	|__|	|__|	|__|	|__|	|__|	|__|	|__|
	Lemon	|__|	|__|	|__|	|__|	|__|	|__|	|__|	|__|

END of the questionnaire: Thank you very much for the time you have given us!

Name of interviewer: .....

Date of interview completed (dd/mm/yyyy) |__|__|/|__|__|/|__|__|__|__|

	BEER/LAGER/CIDER - ½ pint (285 ml) - 1 pint (570 ml) - 1 small bottle/1 small can (330 ml) - 1 large bottle/1 large can (500 ml)
	WINE (include Babycham, Champagne) - 1 small glass (125 ml) - 1 large glass (250 ml) - 1 bottle = 6 small glasses (750 ml)
	APERITIFS, PRE DINNER DRINKS (vermouth, martini, cinzano, dubonnet, pastis, etc.) - 1 single measure (25 ml) - 1 double measure (50 ml) - 1 bottle = 28 single measures (700 ml)
	FORTIFIED WINE (sherry, port, other fortified or tonic wine) - 1 small glass (50 ml)
	SPIRITS/LIQUORS - 1 single measure (25 ml) - 1 larger measure (35 ml) - 1 double measure (50 ml) - 1 bottle = 28 single measures (700 ml)

Figure: Alcoholic beverages (Industrial made)

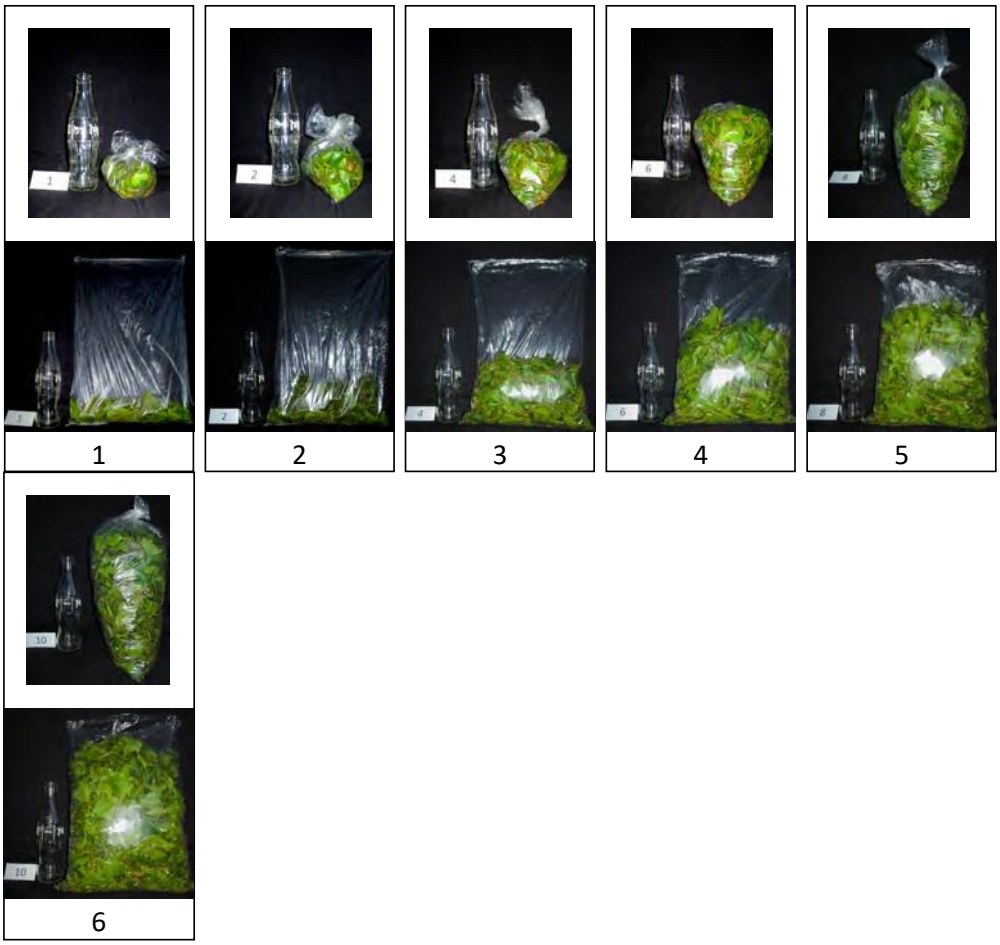

Figure: Visual analogue scale used to quantify the khat usage. (1) 100 grams; (2) 200 grams; (3) 400 grams; (4) 600 grams; (5) 800 grams; (6) 1000 grams.

Questionnaire (Afann Oromo version)

Yuniversitii Addis Abaabaati kollejjii fayyaa mana barnoota faarmasiitiin unkaa odeffannoo fayyaa tiruutiin (kan akka fayyadama tamboo, dhugaati, jimaa fi akkata nyaata) walqabaate qopha'ee. Qorannoon kun kayyoon isa dhibaa afilaatoksinii dhibee tiruu iraati qabuu qorachuuf bahaa itiyoopiyaa kessati adeemsifama.

Galmaa

Qorannoo kan irratti hirmachuuf ulaaga waligaltee kan malatessu kessanif galatoma. Kayyoo qorannoo kana galmaan ga'uuf, walanamtoota garagaraa hirmachisna. Qorannoo kana kan jalqabnuu gaffilee adda addaa dhima dhunfaa, fayyadama tamboo, dhugaati, jimaa fi akkata nyaata ilaalchisee gafachuun ta'a. Dabalataniis dherina fi ulfina kessan herregna. Odeffannoo kennitan iciitiin akka eegamu nan mirkanessa. Bu'aa fi fayidaan qorannoo kana ummataf qabu dhugumma deebii ykn odeffannoo keessaniin kan murta'uu ta'a. Kanafu gaffiin isinii hin galee yoo jiraate, gafachuun adda basadha. Sa'aati barbadanittii gaffii fi deebii kan addan kutuu ykn lagachuu ni dandessuu.

Gaffii qabduu Eyyee ☐ Lakkii ☐

[Yoo hirmatan gaffii dabalata qabate, gaffatan ibsa dabalata ni kenna]

Gaffii dabalata hin qabdaan taanan, haa jalqabnu.

Kutaa 1: Odeffannoo jalqaba

Gafatan ossoo gaffii fi deebii hin jalqabiin kutaa 1ffa guutuu qaba.

1.	Lakk. qorannoo	|__|__|__|
2.	Haala hirmaata	(1) Keesii (2) Kontirolii
3.	Guyyaa gaffii fi deebii	|__|__|/|__|__|/|__||__||__||__| (Guya/Baati/ wagga)
4.	Saala	|__| (1) Dhiira (2) Dhalaa

Kutaa 2: odeffannoo dhunfaa

5.	Umuriin kee meeqa?	|__|__|__| wagga
6.	Haali ga'eela kee?	|__|(1)Hin fuune (2) kan fudhe (3)kan jala du'ee (4) kan wal hike (5)kan biro_____ (6)hin beeku (7)debisu hin barbadu
7.	Magala kam jiraata?	_____ (Barressi)
8.	Magala moo badiya jiraata?	|__|(1)badiya (2) magala (3) hin beeku (4)debisu hin barbadu
9.	Bakka amma jirtuu, ammata meeqa jiraate?	|__|__|__| wagga
10.	Qomoon kee maalii?	|__| (1) Oromo (2) Harari (3) Somaalee (4) Amara (5) kan biro_____ (6) Debisu hin barbadu
11.	Amantaan kee maalii?	|__| (1) Muslima (2) Kiristaana (3) kan biro_____ (4) Hin qabu (5) Debisu hin barbadu
12.	Sadarkaa barnoota?	|__| (1) Hin barannee (2) Sad 1^{ffa} (3) 4-8ffa (4) sad 2^{ffa} (5) Kollejjii/yuunivarsitii (6) hin beeku (7) debisu hin barbadu
13.	Dalagaan kee maalii?	|__| (1)Qoote bulaa (2)Dalaga hin qabuu (3)Hadha warra (4)Barata (5)Kan motumma (6)dafqaan bulaa (7)Kessumesituu (8)kan biro_____
14.	Firootan kee dhihoo kessa (Hadha, Abba, obolessa, obolettii, daa'imaa), dhukkuba tiruu kan qabuu jira?	|__| (1) Lakkii (2) Eyyee (3) Hin beeku (4) Debisu hin barbadu

15.	Qoricha aadaa fudhate beekta?	|__|(1) Lakkii (2) Eeyyee (3) hin bekuu (4) Debisuu diduu **[Lakkii yota'e, garaa G. 18]**
16.	Maqaa qoricha aadaa atii fudhate ni yadata? Maloo ibsii	_____ (baressi)… Hin beeku ykn Debisu hin barbadu (itti marsii)
17.	Dhibee maliif qoricha arman olii fudhate?	_____ (baressi)… Hin beeku ykn Debisu hin barbadu (itti marsii)
18.	Dherina (yeroo gaffii fi deebii kan fudhatamu)	____ Cm
19.	Ulfatina (yeroo gaffii fi deebii kan fudhatamu)	_____Kg

Kutaa 3: seenaa fayyadama Tamboo

3.1. Sijaraa xuuxu

20	Umurii kee guutuu sijaraa xuuxee bekta (yoo xiqqate sijaraa 100)?	|__| (1) Lakkii (2) Eeyyee (3) hin bekuu (4) Debisuu diduu **[Lakkii yota'e, garaa G. 26]**
21	Averejiitin sijaraa meeqa guyyati xuuxa turtee ykn xuuxa jirta?	|__|
22	Umurii kee meeqati sijaraa xuuxu jalqabdee?	|__|__|__|
23	Ammata meeqa sijaraa xuuxe?*(yoo gidduuti addan kutteta ta'ee, ammata gidduti addan kutee irra hirrisi*)	|__|__|__|
24	Ammas sijaraa ni xuuxa?	|__| (1) Lakkii (2) Eeyyee (3) hin bekuu (4) Debisuu diduu
25	Siigaaraa Xuuxxu efa dhabidee/dhifite yeroo meqaa sita'e (Namoota xuuxxu dhabanifi) **[yoo G 24 ffaaf debini lakki ta'e]**	|__|__|__|wagaa; Yoo deebini hin bekuu/debisuu diidani irrati marisi

3.2. Gaaffilee shiishaa xuuxxu

26	Yoxiqaatee yeroo toko torbantokoo kesaati wagaa tokofi **s**hiishaa fayaadamitee/xuuxxe bekitaa?	|__| (1) Lakkii (2) Eeyyee (3) hin bekuu (4) Debisuu diduu **[Lakkii yota'e, garaa G. 34]**
27	Torbbani tokko kesatti shiishaa yeroo meqaa xuuxxaa?	|__|__|__| yeroo/toribaan kesatii
28	Yeroo tokko kesatti baayyinaan tamboo hangam fayyadamtaa/xuuxxaa?	|__|__|__| giraaman yeroo took kesatti
29	Giddugalaan name meqa wajiini shiishaa tokko qodataa/qodate bekitaa yeroo xuuxxuu?	|__|__|__|
30	Wagaa meqaaffi shiishaa xuuxxe?	|__|__|__|
31	Umurii meqaatti shiishaa xuuxxu jaliqabide?	|__|__|__|

32	hanigaa amaatti shiishaa xuuxxa?	|__| (1) Lakkii (2) Eeyyee (3) hin bekuu (4) Debisuu diduu
33	Shiishaa Xuuxxu efa dhabidee yeroo meqaa sita'e (Namoota xuuxxu dhabanifi) **[yoo G 32 ffaaf debini lakki ta'e]**	|__|__|__| wagaa

3.3. Gaaffilee pipaa xuuxxu

34	Yeroo xuuxxu jalabidee kate pipaa 50 xuuxxe bekitaa?	|__| (1) Lakkii (2) Eeyyee (3) hin bekuu (4) Debisuu diduu **[Lakkii yota'e, garaa G. 40]**
35	Giddugalaani taniboo pipaa meqaa torribaani kesatti xuuxxa?	|__|__|
36	Wagaa meqaafi pipaa xuuxxe?	|__|__|__|
37	Pipaa xuuxxu umirii meqaatti jaliqabide?	|__|__|__|
38	hanigaa amaatti pipaa xuuxxa?	|__| (1) Lakkii (2) Eeyyee (3) hin bekuu (4) Debisuu diduu
39	Pipaa Xuuxxu efa dhabidee/dhisite yeroo meqaa sita'e (Namoota xuuxxu dhabanifi) **[yoo G 38 ffaa debini lakki ta'e]**	|__|__|__| wagaa; Yoo deebini hin bekuu/debisuu diidani irrati marisi

3.4. Gaaffilee Tamiboo fuudhachuu/alafachu

40	Yooxiqaate wagaa tokkofi tamiboo fudhatee/ alafate bekitaa?	|__| (1) Lakkii (2) Eeyyee (3) hin bekuu (4) Debisuu diduu **[Lakkii yota'e, garaa G. 47]**
41	Giddugalaani toribaan tokko kessatti yeroo meqaa tamiboo fudhataa/ alafataa /fudhatee/ alafate bikitaa?	|__|__|
42	Giddugalaani yeroo tokko kessatti tamiboo giraamaa meqaa fudhataa alafataa /fudhatee/ alafate bikitaa?	|__|__|__|
43	Wagaa meqaafi taniboo fudhatte/ alafataa?	|__|__|__|
44	Tamiboo fudhachuu/ alafachu umirii meqaati jaliqabide?	|__|__|__|
45	Yeroo amaa kanaa tamiboo fudhataa/ alafataa?	|__| (1) Lakkii (2) Eeyyee (3) hin bekuu (4) Debisuu diduu
46	Tamiboo fudhachu efa dhabidee yeroo meqaa sita'e **(Namoota xuuxxu dhabanifi) [yoo G 45 ffaa debini lakki ta'e]**	|__|__|__| Wagaa; Yoo deebini hin bekuu/debisuu diidani irrati marisi

Kutaa 4. Seenaa jimaa qamayuu

47	Yoxiqaatee torbantokoo kesaati yeroo toko wagaa tokofi Water pipe xuuxxe bekitaa?	|__| (1) Lakkii (2) Eeyyee (3) hin bekuu (4) Debisuu diduu **[Lakkii yota'e, garaa G. 55]**
48	Jimaa yeroo hundaa yookin yooxiqaate toriboonitti yeroo tokko qamayuu umirii meqaatti jaliqabide?	|__|__|__|
49	Giddugalaani toribaan tokko kessatti yeroo meqaa jimaa qamaata/qamaate bikitaa?	|__| guyyaa , guyyaa lakkofisaan ibsi Yoo deebini hin bekuu/debisuu diidani irrati marisi
50	Maqaani jimaa atti yeroo hundaa qamaatu/qamaate bekitu maali jedhaama?	----------(baressi)
51	Giddugalaani yeroo tokko kessatti jimaa giraamaa meqaa qamaata/ qamaate bikitaa? (ijaani laalli talimaami)	|__|__|__| giraam/Yeroo tokko keesaa
52	Yeroo jimaa qamaatu bishaanni alaa wanitaa biraa maali fudhataa/ fudhatee bekitaa?	Tamiboo/sigaaraa|__| 1) Lakkii (2) Eeyyee (3) hin bekuu (4) Debisuu diduu Hojaa |__| (1)Lakkii (2) Eeyyee (3) hin bekuu (4) Debisuu diduu lawisaa|__|(1)Lakkii (2)Eeyyee(3) hin bekuu (4)Debisuu diduu Koolaa|__| (1)Lakkii (2) Eeyyee (3)hin bekuu (4) Debisuu diduu Shaayii |__| (1)Lakkii (2)Eeyyee (3)hin bekuu (4)Debisuu diduu bunaa |__| (1)Lakkii (2)Eeyyee (3)hin bekuu (4) Debisuu diduu ka biraa (adda baasi)_____
53	Yeroo amaa kanaa jimaa qamaataa?	|__| (1) Lakkii (2) Eeyyee (3) hin bekuu (4) Debisuu diiduu
54	Jimaa qamaayu efaa dhabidee yeroo meqaa sita'e **(Namoota jimaa qamayu dhabanifi) [yoo G 53 ffaaf debini lakki ta'e]**	|__|__|__| Yoo deebini hin bekuu/debisuu diidani irrati marisi

Kutaa 5. Seenaa dhuugaatti/alkoolii dhuuguu

55	Yooxiiqaate torribaanitti yeroo tokko ji'a ja'affi olii dhugaatti/alkoolii (akka farisoo, daadhi arake , biiraa, dhugaatti jabaa) dhugidee bekitaa?	|__| (1) Lakkii (2) Eeyyee (3) hin bekuu (4) Debisuu diduu **[Lakkii yota'e, garaa G. 70]**
56	Dhugaatti/alkoolii dhuguu umirii meqaatti jaliqabide?	|__|__|
57	Giddugalaani torribaan tokko kessatti guyyaa meqaa dhugaatti/alkoolii dhugidaa?	|__|__| guyyaa lakkofisaan ibsi Yoo deebini hin bekuu/debisuu diidani irrati marisi
58	Yeroo baayyee dhugaatti/alkoolii	Biiraa |__| (1) Lakkii (2) Eeyyee (3) hin bekuu (4) Debisuu diduu

	akaamiitii dhugidaa?	farisoo |__| (1) Lakkii (2) Eeyyee (3) hin bekuu (4) Debisuu diduu Wayinaa |__| (1) Lakkii (2) Eeyyee (3) hin bekuu (4) Debisuu diduu Daadhi |__| (1) Lakkii (2) Eeyyee (3) hin bekuu (4) Debisuu diduu Dhugaatti jebaa |__| (1) Lakkii (2) Eeyyee (3) hin bekuu (4) Debisuu diduu Arake |__| 1) Lakkii (2) Eeyyee (3) hin bekuu (4) Debisuu diduu	
59	Giduugalatti yeroo tokko kessatti dhugaatti/alkoolii amammi (xariimusaa biiraattini, xaasaa farisoottini, xarimusaa dhugaatti/alkoolii jabaadhan) dhuugidaa yoo baayeeni dhuugaa jetti? (Dhifaamaa wajinii fakki dhugaatichaa/alkooliichaa nuutti agarisiisii)	Biraa |__|__| Yoo deebini hin bekuu/debisuu diidani irrati marisi farisoo |__|__| Yoo deebini hin bekuu/debisuu diidani irrati marisi Wayinaa |__|__| Yoo deebini hin bekuu/debisuu diidani irrati marisi Daadhi |__|__| Yoo deebini hin bekuu/debisuu diidani irrati marisi Dhugaatti jebaa |__|__| Yoo deebini hin bekuu/debisuu diidani irrati marisi Arake |__|__| Yoo deebini hin bekuu/debisuu diidani irrati marisi	
60	Haga meeqa dhugaati ja'a fi ja'a ol al-tokkoti fudhate beekta?	|__| (1)Tasa (2) Baatii 1 gadiin (3) baatii baatiin (4)torbee torbeen (5)Guyya guyyan (6) Hin beeku (7) debisu hin barbadu	
61	Wagga darbee kessa, akka ati dhugatii dhaabu hin dandenyee yaade beekta?	|__| (1)Tasa (2) Baatii 1 gadiin(3) baatii baatiin (4) torbee torbeen (5)Guyya guyyan (6) Hin beeku (7) debisu hin barbadu	
62	Wagga darbee kessa, dhugati irran kan ka'ee, waan dalaguu qabdu osoo hin dalagiin haftee?	|__| (1)Tasa (2)Baatii 1 gadiin (3) baatii baatiin (4) torbee torbeen (5)Guyya guyyan (6) Hin beeku (7) debisu hin barbadu	
63	Wagga darbee kessa, sababa galgala dhugati baayee dhugdeef, ganaman dalaga dalaguuf dhugatii barbade bekta?	|__| (1) Tasa (2) Baatii 1 gadiin (3) baatii baatiin (4) torbee torbeen (5)Guyya guyyan (6) Hin beeku (7) debisu hin barbadu	
64	Wagga darbee kessa, egga dhugati dhugdee booda yeroo meeqa gabbiin sitii dhaga'ame beeka?	|__| (1) Tasa (2) Baatii 1 gadiin (3) baatii baatiin (4) torbee torbeen (5)Guyya guyyan (6) Hin beeku (7) debisu hin barbadu	
65	Wagga darbee kessa, egga dhugati dhugdee booda yeroo meeqa waan galgala umamee irranfate beekta?	|__| (1) Tasa (2) Baatii 1 gadiin (3) baatii baatiin (4) torbee torbeen (5)Guyya guyyan (6) Hin beeku (7) debisu hin barbadu	
66	Sababa dhugatitiin balaan sira ykn nama bira irrati gahee beeka?	|__| (1) Tasa (2) Baatii 1 gadiin (3) baatii baatiin (4) torbee torbeen (5)Guyya guyyan (6) Hin beeku (7) debisu hin barbadu	
67	Hiriyyan, maatiin ykn doktorii akka dhugaati dhabduu ykn hiristuu siti himanii beeku?	|__| (1) Tasa (2) Baatii 1 gadiin (3) baatii baatiin (4) torbee torbeen (5)Guyya guyyan (6) Hin beeku (7) debisu hin barbadu	

68	Ammas dhugaati ni dhugdaa?	|__| (1) Lakkii (2) Eeyyee (3) hin bekuu (4) Debisuu diiduu
69	Egga dhugaati dhabdee ammata meeqa si ta'ee? **(gaffiin kun yoo deebiin 68 ffa lakkii yoo ta'e)**	Ammata |__|__|

Kutaa 6: Akkata Nyaata

Yeroo akkata nyaata deebsituu, seenaa wagga tokko yaadadhu deebisi.

70	Nyaatni yeroo baayee nyaatu maalii?	|__| (1) Ruuzii (2) biddena xaafii (3) daaboo boqollo (4) daaboo qammadii (5) biddena bishinga (6) pastaa (7) kanbiro_____ (8) hin beeku (9) debisu hin barbadu
71	Maddii nyaata yeroo baayee itti fayyadamtuu maalii?	|__| (1) nan qote (2) bittatiin (3) hin beeku (4) debisu hin barbadu
72	Yoo maddii nyaata kee qotuun yoo ta'e, qalaba oomishtee lafa jala awwaltee kesse beekta?	|__| (1) Lakkii 2. Eyyee 3. Hin beeku 4. Debisu hin barbadu
73	Torbeeti yoo xiqqate al takka loozii nyaate beekta? (wagga tokkof)	|__| (1) Lakkii 2. Eyyee 3. Hin beeku 4. Debisu hin barbadu **[Lakkii yota'e, garaa G. 76]**
74	Loozii akkami fayyadama turtee?	**Akkayii** |__| (1) Lakkii (2) Eyyee (3) Hin beeku (4) Debisu hin barbadu **Dhadhaa loozii** |__| (1) Lakkii (2) Eyyee 3. Hin beeku (4) Debisu hin barbadu **Kan biro** _____
75	Yeroo hammamiif loozii fudhacha turtee?	**Akkayii** |__|(1)Tasa (2) Baatii 1 gadiin(3) baatii baatiin (4) torbee torbeen (5)Guyya guyyan (6) Hin beeku (7) debisu hin barbadu **Dhadhaa loozii**|__|(1) Tasa (2) Darbee darbee (3) Baatii 1 gadiin(4) 1-2 torbee (5) 3-4 torbeen (6) Guyya guyyan (7) Hin beeku (8) debisu hin barbadu **Kan biro** _____

Ajaja: hirmata keenya yeroo murasa fudhaati, wagga darbee kessa nyaata nyaate yaadadhu, ani gosa nyaata sin gafadha atiimo hamama akka fudhate nati himi

Ser.	Gosa nyaata	**Deddeebii**							
		Guyya guyyan	3-4 torbeen	1-2 torbeen	1-3 baatiin	Darbee darbee	Tasa	Hin beeku	Debisu hin barbadu
76.	**Foon (horii, gala, re'ee, holaa)**	|__|	|__|	|__|	|__|	|__|	|__|	|__|	|__|
77.	**Foon lukkuu**	|__|	|__|	|__|	|__|	|__|	|__|	|__|	|__|
78.	**Foon qurxummii**	|__|	|__|	|__|	|__|	|__|	|__|	|__|	|__|
79.	**Annanii fi bu'aa annanii**	|__|	|__|	|__|	|__|	|__|	|__|	|__|	|__|
80.	**kilee (**kan lukkuu**)**	|__|	|__|	|__|	|__|	|__|	|__|	|__|	|__|
81.	**Daaboo**								
82.	**Pastaa(makaroni)**	|__|	|__|	|__|	|__|	|__|	|__|	|__|	|__|
83.	**Ruuzii**	|__|	|__|	|__|	|__|	|__|	|__|	|__|	|__|
84.	**baqeela**	|__|	|__|	|__|	|__|	|__|	|__|	|__|	|__|
85.	**Misira**	|__|	|__|	|__|	|__|	|__|	|__|	|__|	|__|
86.	**Kuduraa baala magariisa**								
	Raafuu	|__|	|__|	|__|	|__|	|__|	|__|	|__|	|__|
	Salaxaa	|__|	|__|	|__|	|__|	|__|	|__|	|__|	|__|
	Qoosxaa	|__|	|__|	|__|	|__|	|__|	|__|	|__|	|__|
87.	**Kuduraa ka biraa**								
	Timatima	|__|	|__|	|__|	|__|	|__|	|__|	|__|	|__|
	Foosoliyaa	|__|	|__|	|__|	|__|	|__|	|__|	|__|	|__|
	Buqqee	|__|	|__|	|__|	|__|	|__|	|__|	|__|	|__|
	karrottii	|__|	|__|	|__|	|__|	|__|	|__|	|__|	|__|

	Dinicha	|__|	|__|	|__|	|__|	|__|	|__|	|__|	|__|
	Qullubbii	|__|	|__|	|__|	|__|	|__|	|__|	|__|	|__|
	Chorqaa	|__|	|__|	|__|	|__|	|__|	|__|	|__|	|__|
88.	**Muduraa**								
	Abukaadoo	|__|	|__|	|__|	|__|	|__|	|__|	|__|	|__|
	Burtukaan	|__|	|__|	|__|	|__|	|__|	|__|	|__|	|__|
	Muuzaa	|__|	|__|	|__|	|__|	|__|	|__|	|__|	|__|
	Maangoo	|__|	|__|	|__|	|__|	|__|	|__|	|__|	|__|
	Ananasii	|__|	|__|	|__|	|__|	|__|	|__|	|__|	|__|
	Papayati	|__|	|__|	|__|	|__|	|__|	|__|	|__|	|__|
	Loomii	|__|	|__|	|__|	|__|	|__|	|__|	|__|	|__|

Gaafii fi deebiin keenya Goolabame!!!
Turtii kessaniif baay'ee galatoma!!!

Maqaa gafata: _____

Guyyaa gaafii fi deebii: _____

Questionnaire (Amharic version)

በ አዲስ አበባ ዩኒቨርሲቲ የጤና ሳይንስ ኮሌጅ ፋርማሲ ት/ቤት የ ፋርማኮሎጂ እና ክሊኒካዊ ፋርማሲ ትምህርት ክፍል: በምስራቅ ኢትዮጲያ ውስጥ ላለው የ ጉበት በሽታ መንስኤ የ አፍላቶክሲን ሚናን ለመመርመር በሚደረገው ጥናት የማህበራዊ-ስነ-ሕዝብ፣ ትንባሆ የማጨስን ታሪክ፣ ጫት መጠቀምን፣ አልኮል የመጠጣትን ታሪክ፣ እና የባለፈው ዓመት የአመጋገብ ሁኔታን በተመለከተ መረጃዎችን ለመሰብሰብ ጥቅም ላይ የሚውል መጠይቅ::

መግቢያ

በ አዲስ አበባ ዩኒቨርሲቲ የጤና ሳይንስ ኮሌጅ ፋርማሲ ት/ቤት የ ፋርማኮሎጂ እና ክሊኒካዊ ፋርማሲ ትምህርት ክፍል: በምስራቅ ኢትዮጲያ ውስጥ ላለው የ ጉበት በሽታ መንስኤ የ አፍላቶክሲን ሚናን ለመመርመር በሚደረገው ጥናት ላይ ለመሳተፍ ፈቃደኛ መሆንዎን በፊርማዎ ስላረጋገጡልን እናመሰግናለን። የዚህን ጥናት ዓላማን ለማሳከት ሲባል በ ህይወት ፋና ስፔሊሻሊይዝድ ሆስፒታል የሚገኙ ህመምተኞችን ቃለ መጠይቅ የምናደርግ ይሆናል።

ቀደም ሲል እንደተነጋገርነው በመጀመሪያ ቃለ መጠይቁን የምናደርግ ሲሆን ጥያቄዎቹም የማህበራዊ-ስነ-ሕዝብ፣ ትንባሆ የማጨስን ታሪክ፣ ጫት መጠቀምን፣ አልኮል የመጠጣትን ታሪክ፣ እና የባለፈው ዓመት የአመጋገብ ሁኔታን የሚያጠቃልል ይሆናል። ደግሜ ላረጋግጥልዎ የምፈልገው ነገር ቢኖር ከቃለ መጠይቁ ላይ የሚገኙ ማናቸውም መረጃዎች በሙሉ ምስጥራዊነታቸው የሚጠበቅ ሲሆን በፅሁፍ ላይ የሚወጣው መረጃ ደግሞ በስምዎ ወይም ደግሞ በእርስዎ መገለጫ እንደማይሆን ነው።

የዚህ ጥናት ለሰው ልጆች የሚሰጠው ጥቅም እርስዎ በሚሰጡት ትክክለኛ ምላሽ ላይ የተመሰረተ ነው። ስለዚህ ግልፅ ያለሆነ ጥያቄ ካለዎት መጠየቅ እንዲያመናቱ እና በማንኛውም ጊዜ ቃለ መጠይቁን ማቋረጥ ወይንም መመለስ የማይፈልጉት ጥያቄ ካለ ምላሽ አለመስጠት ይችላሉ።

ግልፅ ያልሆነ ነገር ወይንም ጥያቄ አለዎት። |__| አዎ |__| የለኝም

(የጥናቱ ተሳታፉ ጥያቄ ካነሱ፤ ቃለ መጠይቅ አድራጊው ሰው ለጥያቄዎቹ ምላሽ ይሰጣል ወይም ትሰጣለች በተጨማሪም ተሳታፊዉ እርግጠኛ እንዲሆን ይደርጋል።)

ጥያቄ ከሌለዎት አሁን መጀመር እንችላለን?

ክፍል 1: መሰረታዊ መረጃ

ቃለ መጠይቅ አድራጊው እባክህ/ሽ ተሳታፊው በጥናቱ ለመሳተፍ ፈቃደኝነታቸውን ካረጋገጡ በኋላ: ቃለ መጠይቁን ከመጀመርዎ በፊት ይህንን ክፍል ይሙሉት።

1.	የጥናቱ ተሳታፊ መለያ ቁጥር	|__|__|__|
2.	የተሳትፎው አይነት	|__| (1) የጉበት በሽታ ያለባቸው ተሳታፉዎች (2) የ ጉበት በሽታ የሌለባቸው ጤናማ ተሳታፉዎች
3.	የቃለ-መጠይቁ ቀን፡	|__|__|/|__|__|/|__||__||__||__| (ቀን/ወር/አመት)
4.	ፆታ	|__| (1) ወንድ (2) ሴት

ክፍል 2: የማህበራዊ-ስነ-ሕዝብ

5.	እድሜ	|__|__|__| አመት

		እባክዎ የተሳታፊው ምላሽ ከሚከተሉት አንዱን ከሆነ ያስምሩበት፡ (አላውቅም፤ ለመመለስ ፈቃደኛ አይደሉም)
6.	የጋብቻ ሁኔታ	|__| (1) ያላገባ (2)ያገባ (3) ባል/ሚስት በሞት የተለያት/የተለየችው (4) የፈታ/የፈታች (5) ሌላ፥ ይግለጹ _____(6) አላውቅም (7) ለመመለስ ፈቃደኛ አይደሉም
7.	በአሁኑ ሰዓት የመኖሪያ ስፍራ ?	_____(በፅሁፍ)
8.	የሚኖሩበት ቦታ ገጠር ወይንስ ከተማ ነው?	|__| (1) ገጠር (2) ከተማ (3)አላውቅም (4) ለመመለስ ፈቃደኛ አይደሉም
9.	አሁን በሚኖሩበት አካባቢ ለስንት ጊዜ ኖረዋል?	|__|__|__|አመታት
10.	ብሔር	|__| (1) ኦሮሞ (2) ሐረሪ (3) ሶማሌ (4) አማራ (5)ሌላ, ይግለጹ _____ (6) ለመመለስ ፈቃደኛ አይደሉም
11.	ሃይማኖት	|__| (1) እስልምና (2) ክርስትና (3) ሌላ, ይግለጹ _____ (4) ምንም (5) ለመመለስ ፈቃደኛ አይደሉም
12.	የደረሱበት ከፍተኛ የትምህርት ደረጃ	|__| (1) ያልተማረ/ች (2) አንደኛ ደረጃን ያጠናቀቀ/ች (3) የመለስተኛ ደረጃን ያጠናቀቀ/ች (4) የሁለተኛ ደረጃን ያጠናቀቀ/ች (5) ኮሌጅ/ዩኒቨርሲቲ/ከዛ በላይ ያጠናቀቀ/ች (6) ፈቃደኛ አይደሉም
13.	የስራ ሁኔታ	|__| (1) አርሶ አደር (2) ስራ አጥ(3) የቤት እመቤት (4) ተማሪ (5) የሕዝብ አገልግሎት (6) የቀን ሰራተኛ (7) የቤት ሰራተኛ (8) ሌላ: ለይተው ይግለጹ _____. (9) ለመመለስ ፈቃደኛ አይደሉም
14.	ከእርስዎ የመጀመሪያ ደረጃ ዘመድ (አባት ፣ እናት ፣ ወንድም ፣ እህት ፣ ሴት እና ወንድ ልጅ) የ ጉበት በሽታ ያለበት ሰው አለ/ነበር?	|__| (1) አይደለም (2) አዎ (3)አላውቅም (4) ለመመለስ ፈቃደኛ አይደሉም
15.	ባሀላዊ የጽዋት መድሀኒት ከመታመመዎ በፊት ወስደው ነበር?	|__| (1) አይደለም (2) አዎ (3) አላውቅም (4) ለመመለስ ፈቃደኛ አይደሉም **(የተሳታፉው/ዋ ምላሽ "አይደለም" ከሆነ ወደ ጥያቄ ቁጥር 18 ይሂዱ)**
16.	ስሙ ምን ይባላል?	_____(በፅሁፍ) እባክዎ የተሳታፊው ምላሽ ከሚከተሉት አንዱን ከሆነ ያስምሩበት፡ (አላውቅም፤ ለመመለስ ፈቃደኛ አይደሉም)
17.	ለምን ህመም/አገልግሎት ነው የወሰዱት?	_____(በፅሁፍ) እባክዎ የተሳታፊው ምላሽ ከሚከተሉት አንዱን ከሆነ ያስምሩበት፡

		(አላውቅም፤ ለመመለስ ፈቃደኛ አይደሉም)
18.	ቁመት	____ ሴ.ሜ
19.	ክብደት	_____ኪ.ግ

ክፍል 3: የትንባሆ መጠቀምን በተመለከተ

3. 1. ሲጋራ ማጨስን በተመለከተ

20.	በህይወትዎ ቢያንስ 100 ሲጋራዎችን አጭሰዋል?	|__|(1) አይደለም(2) አዎ (3) አላውቅም (4) ለመመለስ ፈቃደኛ አይደሉም **(የተሳታፉው/ዋ ምላሽ "አይደለም" ከሆነ ወደ ጥያቄ ቁጥር 26 ይሂዱ)**
21.	በአማካኝ በአንድ ቀን ውስጥ ምን ያህል ሲጋራ አጭሰው ነበር/ እያጨሱ ይገኛሉ?	|__|
22.	ሲጋራ ማጨስ የጀመሩት በስንት አመትዎ ነው?	|__|__|__| አመት
23.	ለምን ያህል አመት ሲጋራን አጭሰዋል? (በመካከል ያላጨሱበት ጊዜ ካለ እባክዎ ያንን ጊዜ በመቀነስ ይንገሩን)	|__|__|__| አመት
24.	በአሁኑ ሰዓት ሲጋራ እያጨሱ ይገኛለ?	|__| (1) አይደለም (2) አዎ (3) አላውቅም (4) ለመመለስ ፈቃደኛ አይደሉም
25.	ሲጋራ ማጨስ ካቆሙ ምን ያህል አመት ሆነዎት? (የቀድሞ ሲጋራ አጫሾችን ይመለከታል። **[ማለትም ለጥያቄ ቁጥር 24 አይደለም ብለው መልስ ለሰጡ**]	|__|__|__| አመት እባክዎ የተሳታፊው ምላሽ ከሚከተሉት አንዱን ከሆነ ያስምሩበት፡ (አላውቅም፤ ለመመለስ ፈቃደኛ አይደሉም)

3.2. ሺሻ መጠቀምን በተመለከተ

26.	ቢያንስ ለአንድ ዓመት ያህል በሳምንት ቢያንስ አንድ ጊዜ ሺሻን አጭሰዋል?	|__| (1) አይደለም (2) አዎ (3) አላውቅም (4) ለመመለስ ፈቃደኛ አይደሉም **(የተሳታፉው/ዋ ምላሽ "አይደለም" ከሆነ ወደ ጥያቄ ቁጥር 34 ይሂዱ)**
27.	በአማካኝ በአንድ ሳምንት ውስጥ ምን ያህል ጊዜ አጭሰው ነበር/ እያጨሱ ይገኛሉ?	|__|__|__| ጊዜ/በሳምንት
28.	በአንድ ሳምንት ውስጥ ምን ያህል ግራም ትንባሆ አጭሰው ነበር/ እያጨሱ ይገኛሉ?	|__|__|__| ግራም/በአንድ የማጨሻ ክፍለ ጊዜ
29.	በአማካኝ በአንድ የሺሻ ማጨሻ ክፍለ ጊዜ አንድ ሺሻ ማጨሻን ከስንት ሰው ጋር በጋራ ይጠቀማለ?	|__|__|__|
30.	ለምን ያህል ዓመት ሺሻን አጭሰዋል?	|__|__|__| አመት
31.	ሺሻን ማጨስ የጀመሩት በስንት ዓመትዎ ነው?	|__|__|__| አመት
32.	በአሁኑ ጊዜ ሺሻን ያጨሳሉ?	|__|(1) አይደለም (2) አዎ (3) አላውቅም (4) ለመመለስ ፈቃደኛ አይደሉም
33.	ሺሻ ማጨስ ካቆሙ ስንት ዓመት ሆነዎት? **(ለቀድሞ ሺሻ ተጠቃሚዎች ብቻ የሚጠየቅ ነው። (ለጥያቄ ቁጥር 32 ምላሻቸው አይደለም ለነበረ ተሳፉዎች**)	|__|__|__| አመት; እባክዎ የተሳታፊው ምላሽ ከሚከተሉት አንዱን ከሆነ ያስምሩበት፡ (አላውቅም፤ ለመመለስ ፈቃደኛ አይደሉም)

3.3. ፒፓ ማጨስን በተመለከተ

34.	በህይወትዎ ቢያንስ 50 ፒፓዎችን አጭሰዋል?	|__| (1) አይደለም (2) አዎ (3) አላውቅም (4) ለመመለስ ፈቃደኛ አይደሉም **(የተሳታፉው/ዋ ምላሽ "አይደለም" ከሆነ ወደ ጥያቄ ቁጥር 40 ይሂዱ)**
35.	በአማካይ በአንድ ሳምንት ውስጥ ምን ያህል ፒፓ አጭሰው ነበር/ እያጨሱ ይገኛሉ?	|__|__|
36.	ለምን ያህል አመት ፒፓን አጭሰዋል?	|__|__|__| አመት
37.	ፒፓ ማጨስ የጀመሩት በስንት አመትዎ ነው?	|__|__|__| አመት
38.	በአሁኑ ሰዓት ፒፓ እያጨሱ ይገኛሉ?	|__| (1) አይደለም (2) አዎ (3) አላውቅም (4) ለመመለስ ፈቃደኛ አይደሉም
39.	ፒፓ ማጨስ ካቆሙ ስንት ዓመት ሆነዎት? **(የቀድሞ ፒፓ አጫሾችን የሚመለከት ነው።። [ለጥያቄ ቁጥር 42 ምላሻቸው አይደለም ለነበረ ተሳፉዎች])**	|__|__|__| አመት; እባክዎ የተሳታፈው ምላሽ ከሚከተሉት አንዱን ከሆነ ያስምሩበት፡ (አላውቅም፤ ለመመለስ ፈቃደኛ አይደሉም)

3.4. ትንባሆን ማኝክን በተመለከ

40.	ቢያንስ ለአንድ ዓመት ያህል ትንባሆን አኝከዋል?	|__| (1) አይደለም (2) አዎ (3) አላውቅም (4) ለመመለስ ፈቃደኛ አይደሉም **(የተሳታፉው/ዋ ምላሽ "አይደለም" ከሆነ ወደ ጥያቄ ቁጥር 47 ይሂዱ)**
41.	በአመካኝ በአንድ ሳምንት ውስጥ ምን ያህል ጊዜ ትንባሆ አኝከዋል/ እያኘኩ ይገኛሉ?	|__|__|
42.	በአማካኝ ትንባሆ በሚጠቀሙበት ወቅት ምን ያህል ግራም ትንባሆ አኝከዋል/እያኘኩ ይገኛሉ?	|__|__|__|
43.	ለምን ያህል ዓመት ትንባሆን አኝከዋል?	|__|__|__| አመት
44.	ትንባሆ ማኝክ የጀመሩት በስንት ዓመትዎ ነው?	|__|__|__|አመት
45.	በአሁኑ ጊዜ ትንባሆ እያኘኩ ይገኛለ?	|__| (1) አይደለም (2) አዎ (3) አላውቅም (4) ለመመለስ ፈቃደኛ አይደሉም
46.	ትንባሆ ማኝክ ካቆሙ ስንት ዓመት ሆነዎት (**ለቀድሞ ትንባሆን በማኝክ ለተጠቀሙት ብቻ የሚጠየቅ ነው።። [ለጥያቄ ቁጥር 45 ምላሻቸው አይደለም ለነበረ ተሳፉዎች]**	|__|__|__| አመት; እባክዎ የተሳታፈው ምላሽ ከሚከተሉት አንዱን ከሆነ ያስምሩበት፡ (አላውቅም፤ ለመመለስ ፈቃደኛ አይደሉም)

ክፍል 4: ጫት መጠቀምን በተመለከተ

47.	በሳምንት አንድ ጊዜ ወይንም በጣም በተደጋጋሚ ቢያንስ ለአንድ አመት ጫት ተጠቅመዋል?	|__| (1) አይደለም (2) አዎ (3) አላውቅም (4) ለመመለስ ፈቃደኛ አይደሉም **(የተሳታፉው/ዋ ምላሽ "አይደለም" ከሆነ ወደ ጥያቄ ቁጥር 55 ይሂዱ)**
48.	ቢያንስ በሳምንት አንድ ጊዜ ጫት መጠቀም ማለትም በተከታታይነት የጀመሩት በስንት ዓመትዎ ነበር?	|__|__|__|አመት
49.	በአማካኝ በአንድ ሳምንት ውስጥ ምን ያህል ጊዜ ጫት ተጠቅመዋል/እየተጠቀሙ ይገኛሉ?	|__|የቀኑን ብዛት ይግለጹ ; እባክዎ የተሳታፈው ምላሽ ከሚከተሉት አንዱን ከሆነ ያስምሩበት፡ (አላውቅም፤ ለመመለስ ፈቃደኛ አይደሉም)
50.	አዘዉትረህ/ሽ የምትቅመው/ሚው ጫት ስሙ/አይነቱ ምን ይባላል ?	_____(በፅሁፍ)። እባክዎ የተሳታፈው ምላሽ ከሚከተሉት አንዱን ከሆነ (አላውቅም፤ ለመመለስ ፈቃደኛ አይደሉም)

51.	በአማካኝ በሚቅሙበት ግዜ ምን ያህል መጠን ጫት ይቅማሉ? **(ምስለ ሚዛን ይጠቀሙ)**	|__|__|__| ግራም/በአንድ መቃሚያ ክፍለ ጊዜ
52.	ጫት በሚቅሙበት ግዜ ከውሃ ውጪ ሌላ ምን ተጨማሪ ነገር ይጠቀማሉ?	ሲጋራ |__| (1) አይደለም (2) አዎ (3) አላውቅም (4) ፈቃደኛ አይደሉም አሻራ |__| (1) አይደለም (2) አዎ (3) አላውቅም (4) ፈቃደኛ አይደሉም ለውዝ |__| (1) አይደለም (2) አዎ (3) አላውቅም (4) ፈቃደኛ አይደሉም ኮካ |__| (1) አይደለም (2) አዎ (3) አላውቅም (4) ፈቃደኛ አይደሉም ሻይ |__| (1) አይደለም (2) አዎ (3) አላውቅም (4) ፈቃደኛ አይደሉም ቡና |__| (1) አይደለም (2) አዎ (3) አላውቅም (4) ፈቃደኛ አይደሉም ሌላ _____
53.	በአሁኑ ሰአት ጫት በተደጋጋሚ ይጠቀማሉ?	|__| (1)አይደለም(2)አዎ(3) አላውቅም(4) ለመመለስ ፈቃደኛ አይደሉም
54.	ጫት መቃም ካቆሙ ስንት ዓመት ሆነዎት? (ለቀድሞ ጫት ቃሚዎች ብቻ የሚጠየቅ **ነው።። [ለጥያቄ ቁጥር 53 ምላሻቸው አይደለም ለነበረ ተሳፉዎች]**	|__|__|__| አመት; እባክዎ የተሳታፊው ምላሽ ከሚከተሉት አንዱን ከሆነ ያስምሩበት፡ (አላውቅም) (ለመመለስ ፈቃደኛ አይደሉም)

ክፍል 5: አልኮል የመጠጣት ታሪክ

55.	ቢያንስ በሳምንት አንድ ጊዜ ከስድስት ወራት በሊይ የአልኮል (ጠላ ፣ ጠጅ ፣ አረቄ ፣ ቢራ፣ እንደ ውስኪ ያሉ) መጠጦችን ጠጥተዋል?	|__| (1) አይደለም (2) አዎ (3) አላውቅም (4) ለመመለስ ፈቃደኛ አይደሉም **(የተሳታፉው/ዋ ምላሽ "አይደለም" ከሆነ ወደ ጥያቄ ቁጥር 70 ይሂዱ)**
56.	አልኮል መጠጣት የጀመሩት በስንት ዓመትዎ ነው?	|__|__| አመት
57.	በአማካይ በአንድ ሳምንት ውስጥ ምን ያህል ቀናት የአልኮል መጠጥ ይጠጣሉ?	|__| እባክዎ የቀኖቹን ብዛት ይፃፉ; እባክዎ የተሳታፊው ምላሽ ከሚከተሉት አንዱን ከሆነ ያስምሩበት፡ (አላውቅም፤ ለመመለስ ፈቃደኛ አይደሉም)
58.	በአብዛኛው ግዜ ምን አይነት የአልኮል መጠጥ?	ቢራ |__| (1) አይደለም (2) አዎ (3) አላውቅም (4) ፈቃደኛ አይደሉም ጠላ |__| (1) አይደለም (2) አዎ (3) አላውቅም (4) ፈቃደኛ አይደሉም ወይን |__| (1) አይደለም (2) አዎ (3) አላውቅም (4) ፈቃደኛ አይደሉም ጠጅ |__| (1) አይደለም (2) አዎ (3) አላውቅም (4) ፈቃደኛ አይደሉም ደረቅ አልኮል |__| (1) አይደለም (2) አዎ (3) አላውቅም (4) ፈቃደኛ አይደሉም አረቄ |__| (1) አይደለም (2) አዎ (3) አላውቅም (4) ፈቃደኛ አይደሉም
59.	የአልኮል መጠጥ በሚጠጡበት ቀን በአማካይ ምን ያህል (ቢራ [በጠሩሙስ]፣ ጠላ/ወይን /ውስኪ [በብርጭቆ]፣ አረቄ [በመለኪያ] ይጠጣሉ? **(እባክዎ የአልኮል መጠጫ እቃዎችን የሚያሳየውን ምስል ለተሳታፉው ያሳዩ!)**	ቢራ |__|__| ተሳታፊው የሚከተለውን ካሉ ያሰምሩቡት፡ (አላውቅም፤ ለመመለስ ፈቃደኛ አይደሉም) ጠላ |__|__| ተሳታፊው የሚከተለውን ካሉ ያሰምሩቡት፡ (አላውቅም፤ ለመመለስ ፈቃደኛ አይደሉም) ወይን |__|__| ተሳታፊው የሚከተለውን ካሉ ያሰምሩቡት፡ (አላውቅም፤ ለመመለስ ፈቃደኛ አይደሉም) ጠጅ |__|__| ተሳታፊው የሚከተለውን ካሉ ያሰምሩቡት፡ (አላውቅም፤ ለመመለስ ፈቃደኛ አይደሉም) ደረቅ አልኮል |__|__| ተሳታፊው የሚከተለውን ካሉ ያሰምሩቡት፡ (አላውቅም፤ ለመመለስ ፈቃደኛ አይደሉም) አረቄ |__|__| ተሳታፊው የሚከተለውን ካሉ ያሰምሩቡት፡ (አላውቅም፤ ለመመለስ ፈቃደኛ አይደሉም)

60.	በአንድ ጊዜ ስድስት ወይም ከዚያ በላይ የአልኮል መጠጥ (ቀደም ሲል በተገለጹት መለኪያዎች) በምን ያህል ጊዜ ይጠጣሉ?	|__| (1) በጭራሽ (2) አልፎ አልፎ (3) በየወሩ (4) በየሳምንቱ (5) በየቀኑ (6) አላውቅም (7) ለመመለስ ፈቃደኛ አይደሉም
61.	በባለፈው ዓመት አንዴ የአልኮል መጠጥ መጠጣት ከጀመርክ በኋላ ለማቆም ለምን ያህል ጊዜ ተቸገረህ ነበር?	|__| (1) በጭራሽ (2) አልፎ አልፎ (3) በየወሩ (4) በየሳምንቱ (5) በየቀኑ (6) አላውቅም (7) ለመመለስ ፈቃደኛ አይደሉም
62.	በባለፈው አመት የአልኮል መጠጥ በመጠጣትዎ ምክንያት መስራት የሚጠበቅብዎትን ተግባር ሳያከናውኑ ለምን ያህል ጊዜ ቀርተዋል?	|__| (1) በጭራሽ (2) አልፎ አልፎ (3) በየወሩ (4) በየሳምንቱ (5) በየቀኑ (6) አላውቅም (7) ለመመለስ ፈቃደኛ አይደሉም
63.	በባለፈው አመት ከመጠን በላይ የአልኮል መጠጥ ጠጥተው ከተኙ በሃላ ወደ ኖርማል ለመመለስ ለምን ያህል ጊዜ በጠዋት የአልኮል መጠጥ አስፈልጎት ያዉቃል?	|__| (1) በጭራሽ (2) አልፎ አልፎ (3) በየወሩ (4) በየሳምንቱ (5) በየቀኑ (6) አላውቅም (7) ለመመለስ ፈቃደኛ አይደሉም
64.	በባለፈው አመት ውስጥ የአልኮል መጠጥ ከጠጡ በኋላ የጥፋተኝነት ስሜት ወይም ጸጸት የደረሰዎት ለምን ያህል ጊዜ ነው?	|__| (1) በጭራሽ (2) አልፎ አልፎ (3) በየወሩ (4) በየሳምንቱ (5) በየቀኑ (6) አላውቅም (7) ለመመለስ ፈቃደኛ አይደሉም
65.	በባለፈው የአልኮል መጠጥ በመጠጣትዎ ምክንያት ባሳለፉት ምሽት ምን እንደ ተከሰተ ለማስታወስ ለምን ያህል ጊዜ አልቻሉም?	|__| (1) በጭራሽ (2) አልፎ አልፎ (3) በየወሩ (4) በየሳምንቱ (5) በየቀኑ (6) አላውቅም (7) ለመመለስ ፈቃደኛ አይደሉም
66.	በመጠጥዎ ምክንያት እርስዎ ወይንም ሌላ ሰው ጉዳት ደርሶባቸዋል?	|__| (1) በጭራሽ (2) አልፎ አልፎ (3) በየወሩ (4) በየሳምንቱ (5) በየቀኑ (6) አላውቅም (7) ለመመለስ ፈቃደኛ አይደሉም
67.	ዘመድዎ ፣ ጓደኛዎ ፣ ዶክተርዎ ወይም ሌላ የጤና ባለሙያ ስለ መጠጥ መጠታትዎ ያሳሰበው/ችው ነበር/ረች ወይም መጠጥ እንዲያቆሙ ሃሳብ ያቀረበልዎት/ችዎት ነበር/ረች?	|__| (1) በጭራሽ (2) አልፎ አልፎ (3) በየወሩ (4) በየሳምንቱ (5) በየቀኑ (6) አላውቅም (7) ለመመለስ ፈቃደኛ አይደሉም
68.	በአሁኑ ሰዓት የአልኮል መጠጥ ተጠቃሚ ነዎት?	|__| (1) አይደለም (2) አዎ (3) አላውቅም (4) ለመመለስ ፈቃደኛ አይደሉም
69.	አልኮል መጠጣት ካቆሙ ስንት ዓመት ሆነዎት? (ለቀድሞ የአልኮል ተጠቃሚዎች ብቻ የሚጠየቅ **(የተሳታፉው/ዋ ምላሽ ለጥያቄ ቁጥር 68 አይደለም ከሆነ)**	|__|__|

ክፍል 6: የባለፈው ዓመት የአመጋገብ ሁኔታን በተመለከተ
ለሚከተሉት ጥያቄዎች መልስ ሲሰጡ እባክዎ ከአንድ አመት በፊት የአመጋገብ ታሪክዎን ያስታውሱ።።

70	በመደበኛነት የሚበሉት ምግብ ምን ነበር?	| __ | (1) ሩዝ (2) ጤፍ እንጀራ (3) የበቆሎ ዳቦ (4) (3) የስንዴ ዳቦ (5) የማሽላ እንጀራ (6) ፓስታ (7) ሌላ (ዝርዝር ይግለጹ) _____ (8) አላወቅም (9) ለመመለስ ፈቃደኛ አይደሉም
71	የመደበኛ ምግብዎት ምንጩ ከየት ነው?	|__| (1) ከቤት (2) ከገበያው (3) አላውቅም (4) ለመመለስ ፈቃደኛ

		አይደሉም
72	በመደበኛነት የሚበሉትን የምግብ እህል መሬት ዉስጥ ያከማቻሉ? (**ምንጩ ከቤት ከሆነ)**	|__| (1) አይደለም (2) አዎ (3) አላውቅም (4) ለመመለስ ፈቃደኛ አይደሉም
73	ለውዝ/አቾሎኒ በሳምንት አንድ ጊዜ ወይም በተደጋጋሚ ጊዜ ቢያንስ ለአንድ አመት ወስደዋል?	|__| (1) አይደለም (2) አዎ (3) አላውቅም (4) ለመመለስ ፈቃደኛ አይደሉም **(የተሳታፉው/ዋ ምላሽ "አይደለም" ከሆነ ወደ ጥያቄ ቁጥር 81 ይሂዱ)**
74	በምን ዓይነት መልክ የተዘጋጀ አቾሎኒ ነው?	የተቆላ |__| (1) አይደለም (2) አዎ (3) አላውቅም (4) ፈቃደኛ አይደሉም ቅቤ |__| (1) አይደለም (2) አዎ (3) አላውቅም (4) ፈቃደኛ አይደሉም ሌላ, ይገለጽ_____
75	ይህን የአቾሎኒ አይነት ምን ያህል ግዜ ይመገቡት ነበር?	የተቆላ |__| (1) በጭራሽ (2) አልፎ አልፎ (3) በወር ከ 1-3 ግዜ (4) በሳምንትከ 1-2 ግዜ (5) በሳምንት ከ 3-4 ግዜ (6) በየቀኑ (7) አላውቅም (8) ፈቃደኛ አይደሉም ቅቤ |__|(1) በጭራሽ (2) አልፎ አልፎ (3) በወር ከ 1-3 ግዜ (4) በሳምንትከ 1-2 ግዜ (5) በሳምንት ከ 3-4 ግዜ (6) በየቀኑ (7) አላውቅም (8) ፈቃደኛ አይደሉም ሌላ, ይገለጽ_____

መመሪያ : የተከበሩ ተሳታፊ እባክዎትን ጥቂት ጊዜ ይውሰዱና ከአንድ አመት በፊት የተመገቡአቸውን ምግቦችን ለማስታወስ ይሞክሩ፡ ከዛም እኔ የምግቡን አየነት ስጠራልዎ እርስዎ ምን ያህል ድግግሞሽ እንደተመገቡ ይነግሩኛል?

ተ.ቁ	የምግብ ዝርዝር	ድግግሞሽ ('X' ምልክት ያድርጉ)							
		በየቀኑ	በሳምንት ከ 3-4 ግዜ	በሳምንትከ 1-2 ግዜ	በወር ከ 1-3 ግዜ	አልፎ አልፎ	በጭራሽ	አላ ውቅ ም	ፈቃደኛ አይደሉ ም
76.	**ስጋ (ስጋ, ግመል, ፍየል, በግ)**	|__|	|__|	|__|	|__|	|__|	|__|	|__|	|__|
77.	**ዶሮ**	|__|	|__|	|__|	|__|	|__|	|__|	|__|	|__|
78.	**አሳ**	|__|	|__|	|__|	|__|	|__|	|__|	|__|	|__|
79.	**ወተት እና የ ወተት ዉጤቶች**	|__|	|__|	|__|	|__|	|__|	|__|	|__|	|__|
80.	**የዶሮ እንቁላል**	|__|	|__|	|__|	|__|	|__|	|__|	|__|	|__|
81.	**ዳቦ**	|__|	|__|	|__|	|__|	|__|	|__|	|__|	|__|
82.	**ፓስታ (ስፓጌቲ ፣ ማካሮኒ ፣ ወዘተ)**	|__|	|__|	|__|	|__|	|__|	|__|	|__|	|__|
83.	**ሩዝ**	|__|	|__|	|__|	|__|	|__|	|__|	|__|	|__|
84.	**ባቄላ**	|__|	|__|	|__|	|__|	|__|	|__|	|__|	|__|
85.	**ምስር**	|__|	|__|	|__|	|__|	|__|	|__|	|__|	|__|
86.	**አረንጓዴ ቅጠላማ አትክልቶች**								
	ጎመን	|__|	|__|	|__|	|__|	|__|	|__|	|__|	|__|
	ሰላጣ	|__|	|__|	|__|	|__|	|__|	|__|	|__|	|__|
	ቆስጣ	|__|	|__|	|__|	|__|	|__|	|__|	|__|	|__|
87.	**ሌሎች አትክልቶች**								
	ቲማቲም	|__|	|__|	|__|	|__|	|__|	|__|	|__|	|__|
	ፎሶሊያ	|__|	|__|	|__|	|__|	|__|	|__|	|__|	|__|
	ዱባ	|__|	|__|	|__|	|__|	|__|	|__|	|__|	|__|
	ካሮት	|__|	|__|	|__|	|__|	|__|	|__|	|__|	|__|
	ድንች	|__|	|__|	|__|	|__|	|__|	|__|	|__|	|__|
	ቀይ ሽንኩርት	|__|	|__|	|__|	|__|	|__|	|__|	|__|	|__|
	ቃሪያ	|__|	|__|	|__|	|__|	|__|	|__|	|__|	|__|
88.	**ፍራፍሬዎች**								

	አቮካዶ	|__|	|__|	|__|	|__|	|__|	|__|	|__|	|__|
	ብርቱካን	|__|	|__|	|__|	|__|	|__|	|__|	|__|	|__|
	ሙዝ	|__|	|__|	|__|	|__|	|__|	|__|	|__|	|__|
	ማንጎ	|__|	|__|	|__|	|__|	|__|	|__|	|__|	|__|
	አናናስ	|__|	|__|	|__|	|__|	|__|	|__|	|__|	|__|
	ፓፓያ	|__|	|__|	|__|	|__|	|__|	|__|	|__|	|__|
	ሎሚ	|__|	|__|	|__|	|__|	|__|	|__|	|__|	|__|

የመጠይቁ መጨረሻ::

ስለሰጠኸን/ሽኝ ጊዜ በጣም አመሰግናለሁ!

ቃለ መጠይቁ ያደረገው ስም: ....

የቃለ መጠይቁ ቀን (ቀን / ወር / ዓመት) |__|__|/|__|__|/|__|__|__|__|

Appendix E: Checklists for abstraction of clinical, ultrasound and basic laboratory data

Checklist for abstraction of clinical data

Addis Ababa University, College of Health Sciences, School of Pharmacy, Department of Pharmacology and Clinical Pharmacy, checklist to be used for abstraction of clinical data of cases enrolled in the study that aims at evaluating the role of aflatoxins in the etiology of liver cirrhosis in Eastern Ethiopia.

1.	**Study Number**	|__|__|__|	
2.	**Status of study participant**	|__| (1) Case (2) Control	
3.	**Main complaints (Sign and symptoms)**		
	Jaundice	|__| (1) Yes (2) No	If yes, duration in weeks |____|
	Weight loss	|__| (1) Yes (2) No	If yes, duration in weeks |____|
	Pain	|__| (1) Yes (2) No	If yes, duration in weeks |____|
	Abdominal distension	|__| (1) Yes (2) No	If yes, duration in weeks |____|
	Fever	|__| (1) Yes (2) No	If yes, duration in weeks |____|
	Haematemesis	|__| (1) Yes (2) No	If yes, duration in weeks |____|
	Oedema	|__| (1) Yes (2) No	If yes, duration in weeks |____|
	Anorexia	|__| (1) Yes (2) No	If yes, duration in weeks |____|
	Other complaints (specify)		
4.	**Clinical findings**		
	Ascites	|__| (1) Yes (2) No	If yes, is it: |__| (1)Poorly controlled (moderate) (2)Easily controlled (mild)
	Splenomegaly	|__| (1) Yes (2) No	
	Jaundice	|__| (1) Yes (2) No	
	Caput medusae	|__| (1) Yes (2) No	
	Hepatic encephalopathy	|__| (1) Yes (2) No	If yes, is it: |__| (1) Mild (Grade 1 & 2) (2) Advanced (Grade 3 & 4)

Full Name of the Data Collector: _____ Signature: _____
Date_____

Checklist for Ultrasound Data Abstraction

Addis Ababa University, College of Health Sciences, School of Pharmacy, Department of Pharmacology and Clinical Pharmacy, checklist to be used for abstraction of data from **abdominal ultrasound result** of each participant (cases and controls) enrolled in the study that aims at evaluating the role of aflatoxins in the etiology of liver cirrhosis in Eastern Ethiopia.

1. Study Number	|__|__|__|	
2. Status of study participant	|__| (1) Case (2) Control	
3. Ultrasonographic changes		
Examination	**Result**	**Score**
Liver surface	1. Smooth surface (score=1) 2. Mild uneven surface or shallow, regular, waveform surface (rib induration is not considered) (score=2) 3. Undulant surface, including fine or coarse, irregular nodular surface (score=3)	|__|
Liver parenchyma (hepatic parenchyma away from the major vessel or bile duct)	1. The homogenous appearance of the parenchyma (score=1) 2. Heterogeneous with finely scattered hypoechoic & hyperechoic areas (score=2) 3. Coarse liver with irregular or patchy pattern (score=3)	|__|
Hepatic vessel	1. Smooth vessel wall (score=1) 2. Obscured or blurred vessel with a normal diameter (score=2) 3. Irregular and narrowed vessel (score=3)	|__|
Spleen size index (=oblique X diagonal diameter)	1. $<20cm^2$ (score=1) 2. $>20cm^2$ (score=2)	|__|
Total Score	**_____/11**	
Hepatic steatosis	1. Yes 2. No	
Space-occupying lesion	2. Present 2. Absent	
Any remark		

Full Name of the Radiologist: _____ Signature: _____
Date_____

Checklist for Basic Laboratory data collection

Addis Ababa University, College of Health Sciences, School of Pharmacy, Department of Pharmacology and Clinical Pharmacy, a checklist to be used for collection of **Basic laboratory data** from each participant (cases and controls) enrolled in the study that aims at evaluating the role of aflatoxins in the etiology of liver cirrhosis in Eastern Ethiopia.

1. Study Number	|__|__|__|	
2. Status of study participant	|__| (1) Case (2) Control	
Investigation	**Result**	**Remark**
3. Liver function and other tests		
ALT	_____U/L	
AST	_____U/L	
ALP	_____U/L	
Direct bilirubin	_____µmol/L	
Total bilirubin	_____µmol/L	
Albumin	_____g/L	
Creatinine	_____µmol/L	
Platelet count	_____10^9/L	
HBsAg	1. Positive 2. Negative	
Anti-HCV	1. Positive 2. Negative	

Full Name of the Lab technologist: _____ Signature: _____
Date_____