ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES

SCHOOL OF BIO-MEDICINE AND LABORATORY SCIENCE

DEPARTMENT OF MICROBIOLOGY, IMMUNOLOGY AND PARASITOLOGY

INFECTIOUS AND TROPICAL DISEASES MASTER'S PROGRAM

HEPATOCELLULAR CARCINOMA AMONG CHRONIC HEPATITIS B PATIENTS AT SAINT PAUL'S HOSPITAL MILLENNIUM MEDICAL COLLEGE

ADDIS ABABA, ETHIOPIA

By

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Addis Ababa University

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Hepatocellular Carcinoma among Chronic Hepatitis B patients at Saint Paul's Hospital Millennium Medical Collage Addis Ababa, Ethiopia

By: Bethelhem Hailu Gebregziabher

A Thesis Submitted to the Department of Microbiology, Immunology, and Parasitology, College of Health Sciences, Addis Ababa University, in Partial Fulfillment of the Requirement for the Degree of Master of Science in Tropical and Infection Diseases

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Statement of the Author

I declare that this thesis is my work and all sources of materials used for this thesis have been duly cited and acknowledged. This thesis is submitted in partial fulfilment of requirements for masters of Science degree from Aklilu Lemma Institute of Health Research, Addis Ababa University. I declare that this thesis is not submitted to any other institution for the award of any academic degree, diploma or certificate.

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List of Abbreviation and Acronyms

AFP – Alpha Feto-Protein

AHR – Adjusted Hazard Ratio

ALT – Alanine Transaminase

AOR – Adjusted Odds Ratio

APRI – AST to Platelet Ratio Index

AST – Aspartate Transaminase

BMI – Body Mass Index

CHB – Chronic Hepatitis B

CHR – Crude Hazard Ratio

CI – Confidence Interval

Cm- Centimetre

CT scan - Computed Tomography scan

DNA – Deoxyribo-Nucleic Acid

DM- Diabetes Mellitus

EASL – European Association for the Study of Liver

FS – Fibro Scan

GLOBOCAN- Global Cancer

HBeAg– Hepatitis B envelope Antigen

HBsAg – Hepatitis B surface Antigen

HBV – Hepatitis B Virus

HCC – Hepatocellular Carcinoma

HCV – Hepatitis C Virus

HDV- Hepatitis D Virus

HIV – Human Immunodeficiency Virus

IQR – Inter Quartile Range

IRB - Institutional Review Board

IRR – Incidence Rate Ratio

IU – International Unit

Kg – Kilogram

ML – Millilitre

MRI – Magnetic Resonance Imaging

MTCT – Mother to Child Transmission

NAFLD- Non-Alcoholic Fatty Liver Disease

NASH – Non Alcoholic Steato-Hepatitis

NIAAA – National Institute on Alcohol Abuse and Alcoholism

ng – Nano gram

OHI – Occult Hepatitis Infection

SEER – Surveillance Epidemiology and End Result

SPHMMC- Saint Paul's Hospital Millennium Medical College

US – Ultrasound

X^2 - Chi ^{2}

WCRF – World Cancer Research Fund

WHO – World Health Organization

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Abstract

Background: Liver cancer is the third leading cause of cancer death and the sixth most commonly diagnosed cancer globally. Hepatocellular carcinoma (HCC) accounts for up to 90% of liver cancer cases and chronic hepatitis B virus infection is one of the primary etiological factor. HCC presents a significant global health burden, particularly in regions with high (>8%) hepatitis B virus prevalence like Ethiopia.

Objective: To estimate the incidence rate of HCC and associated factors among chronic hepatitis B patients enrolled in a longitudinal follow-up study at Saint Paul's Hospital Millennium Medical College in Addis Ababa, Ethiopia since 2015 GC.

Method: This study was nested in a prospective cohort of chronic hepatitis B patients. Data was extracted from patient records which includes demographic, clinical, radiologic and laboratory variables. The primary outcome was the development of HCC confirmed on two imaging studies. Incidence rate was calculated per 1000 person years. To identify factors associated with HCC Univariable Cox proportional hazard regression was used followed by Firth's penalized multivariable regression, to address limited number of events and complete separation. Bootstrapping with 500 replication was then performed.

Result: Out of 1291 eligible CHB patients, 23 were diagnosed with HCC during the 10 year follow up, of which those diagnosed after 12 months of follow up were considered to be incidences(n=11). The incidence rate of HCC was 1.65 per 1000 person years (95%CI: 0.81-2.79) among all study participants and 7.07 per 1000 person years (95%CI: 3.91 – 12.76) among those with cirrhosis. Kaplan-Meier estimates showed cumulative HCC risk of 0.44% at 3 years, 0.86% at 5 years and 2.45% at 10 years. Firth's penalized cox regression followed by bootstrap with 500 replications showed both increasing age (AHR: 1.07 per year, 95% CI: 1.03 - 1.12, P=0.000) and cirrhosis (AHR: 52.83, 95% CI: 27.25 – 102.43, P=0.000) were significantly associated with HCC.

Conclusion: cirrhosis and increasing age were the strongest predictors of HCC in this 10 year cohort of CHB patients. Surveillance strategies especially targeting those with the strongest predictors are recommended to improve early detection.

Key words: Chronic Hepatitis B, Hepatitis B Virus, Hepatocellular carcinoma

1. INTRODUCTION

Estimates indicate that about one in five people will develop cancer during their lifetime, and roughly one in nine men and one in twelve women will die from it. Cancer not only hinders increases in life expectancy but also imposes significant societal and economic costs(Bray *et al.,* 2024).

In 2022, liver cancer claimed over 750,000 lives globally, making it the third leading cause of cancer death after lung and colorectal cancers and the sixth most commonly diagnosed cancer, with approximately 865,000 new cases. The mortality rate for liver cancer is particularly high among men, who are two to three times more likely to develop and die from the disease(Asafo-Agyei and Samant, 2024; Bray *et al.*, 2024).

Primary liver cancer mainly consists of hepatocellular carcinoma (HCC), accounting for 90% of cases. Chronic hepatitis B virus and chronic hepatitis C virus is associated with more than 70% of cases of hepatocellular carcinoma worldwide. Other risk factors aside from the fore mentioned include aflatoxin exposure, heavy alcohol use, obesity, type 2 diabetes, and smoking, with significant regional variations. In high-risk areas such as China and Eastern Africa, hepatitis B virus (HBV) and aflatoxin exposure are predominant risk factors(Asafo-Agyei and Samant, 2024; Llovet, 2022).

HCC is most common in Asia and sub-Saharan Africa, where HBV is widespread, leading to 20-35 cases per 100,000 people. In Southern Europe and North America, the incidence is moderate, with 10 cases per 100,000, while Northern and Western Europe have lower rates, under 5 cases per 100,000. In the United States, liver cancer has had the highest increase in mortality over the past 20 years, with 35,000 new cases annually. HCC is more common in men, with a male-to-

female ratio of 2.5:1, and its incidence increases with age, peaking at 65-70 years. In populations with HBV transmission from mother to child, such as Chinese and black African groups, the average age of onset is 40-50 years(Llovet, 2022).

Ethiopia, as part of the sub-Saharan African region, faces significant challenges in managing and controlling HBV and its complications, including HCC. The prevalence of HBV in Ethiopia is estimated to be around 9.4%, with regional variations as high as 28.8% in Afar and as low as 4.9% in Harari. Limited access to effective HBV vaccination, surveillance, and treatment exacerbates the issue, leading to high rates of HBV-related liver diseases and mortality(Weldemariam, 2020). In Ethiopia despite the introduction of HBV vaccination programs, challenges such as late administration of the birth dose and lack of comprehensive antenatal HBV screening persist. Consequently, many individuals are diagnosed with HBV and its complications at later stages, making management more complex and outcomes poorer(Mohammed *et al*., 2022).

St. Paul's Hospital in Addis Ababa, Ethiopia, serves a population with a high diversity (SPHMMC.,2024). At St. Paul Hospital Millennium Medical College, patients with CHB infection have been enrolled in a cohort study since 2015 GC(Desalegn, 2023). There is a lack of comprehensive data assessing the long-term outcomes of HCC among these patient groups over an1 extended period.

This research aims to assess HCC among CHB patients that have been followed at St. Paul's Hospital. By examining patient demographics, clinical presentations, treatment responses, and survival rates, this study seeks to provide insights into the effectiveness of current surveillance and management strategies identify areas for improvement. Understanding the outcome will help in bettering clinical practices and public health strategies, ultimately aiming to reduce the burden of HCC in Ethiopia and similar settings.

Furthermore, this study underscores the importance of comprehensive HBV management, from prevention through vaccination to effective treatment and surveillance, in mitigating the impact of HCC. Effective strategies, such as early vaccination, regular monitoring of HBV-infected individuals, and timely antiviral treatment, can significantly reduce the incidence of HCC. By identifying gaps in the current healthcare system and proposing evidence-based solutions, this research aims to enhance the quality of care for chronic HBV patients and improve their health outcomes.

1.1 Statement of the Problem

Hepatocellular carcinoma represents a significant global health challenge as one of the most common and deadly forms of liver cancer. HBV infection is a primary (60%) etiologic factor for HCC, contributing to a substantial portion of liver cancer cases worldwide. Antiviral therapies have been developed to manage chronic HBV infection, aiming to reduce viral load, liver inflammation, and down the road the risk of HCC development. However, the long-term effectiveness of these treatments in preventing HCC and place and mode of surveillance strategies have not been studied, particularly in resource-limited settings(Anugwom *et al.,* 2021; Lampertico *et al.,* 2017; WHO, 2023).

1.2 Significance of the Study

This study aimed to address the existing knowledge gap in our country by evaluating the incidence and associated factors of HCC among chronic HBV patients at St. Paul Hospital Millennium Medical College. Understanding the outcome is crucial for developing informed clinical guidelines and improving management and surveillance strategies for CHB patients, ultimately contributing to the national and global effort to reduce the burden of HBV and HCC.

1.3 Objectives

1.3.1 General Objective

To assess hepatocellular carcinoma among chronic hepatitis B patients enrolled in a cohort study at St. Paul's Hospital Millennium Medical College since 2015 GC.

1.3.2 Specific Objectives

- To determine the incidence rate of HCC among chronic hepatitis B patients enrolled in the cohort study.
- To identify risk factors associated with the development of HCC in chronic hepatitis B patients.

2. LITERATURE REVIEW

HCC is a primary liver tumor, making up over 90% of liver cancers. The five-year survival rate for HCC is 18%, making it second only to pancreatic cancer in terms of poor prognosis. The main risk factor for HCC is cirrhosis, which involves chronic liver damage from inflammation and fibrosis. About 85% of those diagnosed with HCC have cirrhosis. Cirrhosis is caused by chronic HBV or hepatitis C virus (HCV) infections, alcohol abuse, metabolic syndrome, or hemochromatosis linked to HFE1 gene mutations. About one-third of cirrhotic patients, who make up 1% of the global population, will develop HCC. HCC occurs in with an annual incidence rate of 2%-4% among those with cirrhosis without chronic hepatitis virus infection but studies show that cirrhotic patients with hepatitis B or C have a 3-8% annual risk of developing HCC. HCC is less common in cirrhosis due to alcohol, nonalcoholic steatohepatitis (NASH), alpha-1 antitrypsin deficiency, autoimmune hepatitis, Wilson's disease, and cholestatic liver disorders, with a 1-3% annual incidence(Asafo-Agyei and Samant, 2024; Llovet, 2022).

About 60 % of HCC cases in Asia and Africa are attributed to HBV, whereas it is responsible in 20% of the cases in the Western world. In patients with HBV, risk factors for developing HCC include a family history of the disease, HBeAg seropositivity, high viral load, and genotype C. Chronic treatment with effective antiviral HBV therapies can help manage these risks(Llovet, 2022; Philips *et al*., 2021).

Hepatitis B is a significant global health issue. Among the WHO regions the highest infection rates are seen in the Western Pacific Region, with 116 million chronically infected people, and African Region, with 81 million infected individuals. In other regions the numbers are also substantial ranging from 60 to 5 million. Alarmingly, about 90% of the world's undiagnosed and untreated hepatitis B cases are concentrated in the Western Pacific, African, and South-East Asia Regions(WHO., 2023).

Infants born to HBeAg-positive mothers have more than 90% risk of contracting HBV at birth if they are not vaccinated. It has been evidenced that administering a birth dose of the hepatitis B vaccine which is then followed by two additional doses, can reduce the prevalence of chronic HBV by about 90% in infants born to HBeAg-positive mothers and nearly eliminate it in those born to HBeAg-negative mothers (MacLachlan and Cowie, 2015; Nelson *et al.*, 2016).

In areas with high HBV prevalence, 70-95% of people show past or present signs of infection (Hou *et al*., 2005). Regions like North Africa, the Middle East, parts of eastern and southern Europe, parts of Latin America, and South Asia are considered intermediate prevalence areas. In the fore mentioned areas the virus is mainly spread through perinatal and horizontal transmission. These regions represent 40% of the global population, and vaccination has notably reduced HBV prevalence, especially in Europe. About 12% of the world's population lives in low prevalence areas, which include Australia, Northern and Western Europe, Japan, North America, and some South American countries. In the mentioned regions, most infections occur in adolescents and adults through injection drug use, sexual contact, and other bloodborne transmission methods. Eastern Europe and North America, despite being low prevalence areas, have a high number of injection drug users diagnosed with chronic hepatitis B. In these low prevalence countries the majority of pattients with chronic hepatitis B were born in areas where the disease is endemic. When assessing the burden of chronic hepatitis B, it's crucial to consider global migration (MacLachlan and Cowie, 2015). In Europe, HBV infection rates are higher among migrants (5%) and asylum seekers (10%) compared to the general population (1%)(Kim *et al.*, 2021).

A population-based study in Guangdong province, China, observed a significant drop in HBsAg prevalence, from 16.67% in 1992 to 11.10% in 2006, thanks to an integrated vaccination program. However, a 2015 study showed that China still has a high overall prevalence of 8.76%. While the vaccination program has reduced prevalence among children, the age group with the highest prevalence has shifted to those between 23 and 59 years old.(Zeng *et al*., 2016).

Liver cancer rates have been declining in many high-risk countries in East and Southeast Asia since the late 1970s and in Japan and China since the 1990s due to decreased prevalence of viral hepatitis infections and reduced exposure to aflatoxin. HBV vaccination, introduced in East Asia in the early 1980s, significantly lowered HBV infection rates and HCC incidence. However, in countries like Thailand, where HCC accounts for less than 30% of liver cancer cases, overall liver cancer rates continue to rise despite declines in HCC. Similarly, formerly low-risk countries, particularly in Europe and North America, are experiencing increasing incidence rates(Bray *et al*., 2024).

Hepatitis B is a widespread virus in Africa, with a chronic prevalence rate of 6.1%. In 2019, the continent saw an estimated 990,000 and 80,000 new infections and deaths respectively related to hepatitis B. Coinfection with HIV and hepatitis D virus in patients with hepatitis B-related liver disease affects approximately 1.9 million and 1.6 million people, respectively. In the WHO Africa region, only about 1.8% of those infected know their status, and 110,000 have received treatment. Sub-Saharan African countries has the highest age-standardized death rate from cirrhosis. In this region HCC was found to be the second leading cause of cancer death in men and the third in women in 2020. HCC due to hepatitis typically occurs at a median age of 42 years (Spearman and I. Andersson, 2023). Deaths due to hepatitis B-related HCC and cirrhosis account for 2% of annual deaths in Africa. Genotype A is the predominant genotype in Southern, Eastern, and Central parts of Africa, while genotype D is most common genotype in countries located in Northern Africa, and genotype E is found in Western and Central Africa (Kramvis and C. Kew, 2007).

In Africa, HCC is a significant healthcare challenge. The 2020 GLOBOCAN report estimates there were 70,542 new cases and 66,944 deaths from HCC in Africa. However, the actual number of HCC-related deaths is likely higher than reported. In sub-Saharan Africa, HCC tends to occur at a younger age than in East Asia or the Western world, partly because HBV-related HCC develops earlier than HCC from other liver diseases. According to a study conducted in seven African countries found that the median age for HCC onset in sub-Saharan Africa is 46 and variation was seen in Egypt with age of onset of 58 years. This variation may be due to several factors, including birthplace, early HBV infection, viral integration, exposure to carcinogens, and specific HBV

genotypes or sub-genotypes. Vaccine-related changes and population mobility are listed as a reason for the uniform risk of HBV related HCC across sub-Saharan Africa. The spread of HIV and the resulting HBV-HIV co-infection has also influenced the epidemiology, with newer studies indicating high HCC incidence in eastern and southern Africa(Anugwom *et al*., 2021).

Ethiopia is considered one of the countries where hepatitis B virus is endemic. Despite this, there is no effective nationwide plan for surveillance, diagnosis, investigation, prevention, and control of viral hepatitis. The prevalence of hepatitis B varies by region(Mohammed *et al*., 2022). Nationally, the prevalence is 9.4%, with the highest rate in Afar Regional State at 28.8% seroprevalence and the lowest in Harari Regional State at 4.9% (Weldemariam, 2020). A study conducted at Dessie Referral and Kemise General Hospitals in Northeastern Ethiopia found an HBV prevalence of 27.4%(Mohammed *et al*., 2022).

A cohort study in Gondar found that among anti-Hepatitis B core positive HIV patients, 19% had occult hepatitis B infection. In eastern Ethiopia, OHI prevalence was 5.6% in HIV-negative individuals and 6% in HIV-positive individuals with isolated anti-Hepatitis B core antigen (Ayana *et al*., 2020; Patel *et al*., 2020). Another study among adults in Southwest Ethiopia reported a hepatitis B surface antigen seroprevalence of 9%, strongly associated with gum and body tattooing and contact with jaundiced individuals (Belay *et al*., 2020). Among pregnant women, HBV infection rates range from 2.3% in southern Ethiopia to 7.9% at Gambella Hospital(Alemu *et al*., 2020). A study in Ethiopia examining mother-to-child transmission (MTCT) of hepatitis B found that 10.1% of children born to mothers with chronic hepatitis B showed evidence of active HBV infection. High viral load and positive HBeAg status were identified as risk factors for MTCT. However, HBeAg was not a reliable predictor of viral load. Among women of childbearing age with chronic hepatitis B, the risk of MTCT was 14.3%, meaning approximately one in seven women face this risk(Johannessen *et al*., 2021).

A study conducted across multiple centers in Ethiopia, involving 369 hepatocellular carcinoma patients, revealed that 71% of them tested positive for HBV. Among these, 29% had previously

undergone antiviral therapy. The study found that patients with untreated HBV were 3.5 times more likely to succumb to HCC compared to those who had received treatment for HBV(Abza *et al.,* 2022).

People with chronic hepatitis B need specific surveillance for liver cancer mainly HCC even if they don't have cirrhosis. Guidelines suggest monitoring certain groups closely: Asian and black males over 40, Asian females over 50, African/North African blacks with hepatitis B over 20, those co-infected with hepatitis D, and individuals with a first-degree relative with HCC. High viral DNA levels of HBV increase the risk and also worsen the prognosis of HCC. Active HBV replication is believed to promote cancer through direct and indirect mechanisms, so antiviral treatments can reduce this risk. Alanine aminotransferase (ALT) levels, indicating liver injury, combined with factors like age and infection duration, help identify high-risk HBV carriers. Additional risk factors include environmental exposures such as alcohol, smoking, and the toxin aflatoxin, as well as having a family history of HCC(Harris *et al*., 2019).

The current method for monitoring HCC in patients with cirrhosis involves performing an ultrasound every six months. This approach, which has an overall sensitivity of 84% for detecting HCC and 47% for early-stage HCC, was based on a Chinese study involving 18,000 patients with liver disease caused by HBV. However, this protocol might not be as effective for cirrhosis caused by other factors or for patients with multiple causes of cirrhosis(Philips *et al*., 2021). Checking for liver cancer every six months instead of once a year helps catch very early-stage tumors more often and reduces the number of advanced cases. This approach allows for more effective treatments and leads to better outcomes for patients(Santi *et al*., 2010).

Ultrasound (US) is a low-cost, noninvasive method for monitoring liver cancer that doesn't expose patients to radiation. However, its effectiveness in detecting HCC in a cirrhotic liver can be hampered by factors like liver texture abnormalities, obesity, and the quality and experience of the ultrasonographer. A meta-analysis revealed that US has a 94% sensitivity for detecting HCC at any stage, but only 63% for early-stage tumors. Adding alpha-fetoprotein (AFP) measurements

didn't significantly improve sensitivity. Performing US every six months rather than doing it annually has increased early-stage HCC detection sensitivity to 70%. In patients with Child-Pugh classes A and B cirrhosis, combining AFP with US raised early-stage HCC detection sensitivity from 32% to 65%. One study found that 20% of US exams for HCC surveillance were inadequate, especially in obese patients or those with alcohol-related cirrhosis or non-alcoholic fatty liver disease (NAFLD), likely due to subcutaneous fat and liver steatosis affecting US visualization. In a comparative study done in 163 patients with cirrhosis that were randomly assigned to biannual US or annual triphasic CT, Biannual US proved more sensitive (71.4%) than CT (66.7%) and was also less costly. CT, while not cost-effective, carries risks of radiation exposure and kidney damage. MRI is the most sensitive imaging method for HCC, but its high cost and limited availability restrict its use. A recent South Korean study with 407 patients found MRI with liver-specific contrast more effective than US, especially for very early-stage HCC, detecting 84.8% of cases compared to 27.3% with US. However, this study's findings might not apply broadly, as most patients had HBV-related cirrhosis and low average BMI. CT or MRI can be used for patients with inadequate US results or in those at high risk to have inadequate US visualization due to various underlining conditions (Dănilă and Sporea, 2014; Harris *et al*., 2019; Kim *et al.*, 2017).

The primary groups for HCC screening in Africa are individuals with HBV infection and those with established cirrhosis. Due to the younger age at which HCC often occurs in Africa, the target age group for screening is broader, increasing the potential costs of screening programs. All populations in Africa are at high risk for HBV-associated HCC, but areas with high aflatoxin exposure should be prioritized, especially where pre-harvest interventions to reduce aflatoxin in grains haven't been implemented. Regions like certain parts of Kenya and The Gambia are known for high aflatoxin levels, though comprehensive studies are lacking for many areas(Anugwom *et al.*, 2021).

Combining biomarkers with radiological methods has proven more effective for HCC surveillance than using either approach alone. Specifically, using alpha-fetoprotein (AFP) along with ultrasound enhances early HCC detection, with a sensitivity of 63% and a specificity of 84%. Monitoring trends in AFP levels over time, rather than relying on a single AFP measurement,

provides better early HCC detection. Even if AFP levels are below 20 ng/mL, a consistent increase warrants close monitoring. The standard AFP cut-off of 20 ng/mL for HCC detection was established based on patients with viral hepatitis. Adjusting AFP cut-offs based on the cause of liver disease can improve specificity: a higher cut-off of 59 ng/mL for viral hepatitis-related cirrhosis and a lower cut-off of 11 ng/mL for non-viral cirrhosis(Philips *et al.,* 2021).

There are no continental bodies providing surveillance program recommendations in Africa, leading to local adaptations of global guidelines based on cost and feasibility. Ultrasound and AFP level measurement are the preferred HCC screening methods. Advances in ultrasound technology and portable ultrasound machines offer opportunities for surveillance programs, but distributing these machines and training personnel requires significant effort and international cooperation(Anugwom *et al*., 2021).

Despite the significant impact of HCC in Africa, managing the condition has been challenging. Limited treatment options and reliance on palliative care result in higher mortality rates compared to other regions. Consequently, there's a strong focus on preventing HCC by addressing viral hepatitis, with early treatment and vaccination being key strategies. The WHO's global hepatitis strategy targets a 90% reduction in new hepatitis infections and a 65% decrease in deaths by 2030. By the end of 2022, 190 member states had nationally introduced the HBV vaccine, achieving 84% global coverage for the three-dose regimen. However, vaccination schedules vary, with many countries administering the first dose at six weeks instead of within 24 hours of birth, which fails to protect infants from mother-to-child transmission. Currently, 113 member states provide a single dose of the hepatitis B vaccine to newborns within their first 24 hours, with global coverage at 45%. This rate ranges from 80% to 18% in the WHO Western Pacific and African Regions respectively. A major hurdle in controlling HBV in Africa is the lack of birth dose vaccinations in many countries. However, given the poor antenatal HBV screening coverage in sub-Saharan Africa, introducing a birth dose vaccine could significantly improve HBV prevention. Nonetheless, implementing a birth dose vaccine presents challenges, including higher costs and the need for cold-chain logistics, which are significant obstacles across the continent(Anugwom *et al.*, 2021; Bray *et al.*, 2024).

In conclusion, HCC represents a critical global health challenge, particularly in regions with high rates of hepatitis B virus (HBV) infections such as Africa and Asia(Asafo-Agyei and Samant, 2024; Llovet, 2022). Effective antiviral therapies can mitigate the risk of HCC in HBV patients, highlighting the importance of early and ongoing treatment (Llovet, 2022; Philips et al., 2021). Despite global vaccination efforts reducing HBV prevalence in many regions, significant challenges remain, particularly in high-prevalence areas where perinatal transmission is common (WHO, 2023). Surveillance and early detection of HCC are critical for improving outcomes, with ultrasound and biomarkers like alpha-fetoprotein (AFP) being standard approaches, although their effectiveness can be limited by patient factors such as obesity and liver texture (Dănilă and Sporea, 2014; Harris et al., 2019). Enhanced imaging techniques offer improved sensitivity but are often cost-prohibitive and less accessible (Kim et al., 2017). In Africa, the high prevalence of HBV and associated liver cancer underscores the urgent need for improved surveillance and vaccination strategies. The WHO's global hepatitis strategy aims to dramatically reduce new infections and deaths by 2030, but achieving these targets requires addressing significant logistical and economic challenges, particularly in implementing birth dose vaccinations to prevent mother-to-child transmission (Anugwom et al., 2021; Bray et al., 2024). Ultimately, combating HCC necessitates a comprehensive approach that includes effective antiviral treatments, widespread vaccination, especially at birth, and robust surveillance systems to detect and manage the disease at its earliest stages. Addressing these multifaceted challenges is essential to reducing the global burden of HCC and improving survival rates for those affected by this challenging disease

3. METHODS

3.1 Study Area

The study was conducted at St. Paul's Hospital Millennium Medical College (SPHMMC).. SPHMMC is a tertiary referral and teaching hospital located in Addis Ababa, Ethiopia, providing a wide range of healthcare services and serving a diverse patient population from both urban and rural areas. St. Paul's Millennium Medical College was officially established by a decree from the Council of Ministers in 2010. However, its roots go back further, with the medical school opening its doors in 2007 and the hospital being founded in 1968 by Emperor Haile Selassie. The institution operates under the governance of a board within the Federal Ministry of Health. Notably, the College pioneered Ethiopia's first integrated modular and hybrid problem-based curriculum for undergraduate medical education. It is now expanding its offerings to include postgraduate programs and a broader range of undergraduate courses. It has an inpatient capacity of over 700 beds and is estimated to handle an average of 1200 emergency and outpatient visits daily (SPHMMC.,2024).

3.2 Study Design

This study was nested within a prospective cohort of chronic hepatitis B patients at St. Paul's Hospital Millennium Medical College who have been in an ongoing follow up since 2015 GC. This nested retrospective cohort study examined the baseline and ongoing risk factors that are associated with the subsequent development of HCC.

3.3 Source Population

Individuals diagnosed to have been HBsAg positive for at least 6 months.

3.4 Study Population

The study population consists of CHB patients who have been enrolled in a cohort study at St. Paul's Hospital Millennium Medical College since 2015 GC and fulfil the inclusion criteria.

3.4.2 Inclusion Criteria

- Participants who have been part of the ongoing cohort study at St. Paul's Hospital Millennium Medical College since the year 2015 GC.
- Participants with available follow-up data

3.4.3 Exclusion criteria

- 12 Participants that were without complete follow up data were excluded.

3.5 Sample Size Determination

A formal sample size calculation was not performed for this study because it utilized the entire available cohort from a longstanding prospective study of CHB patients. All eligible participants were included in the analysis.

Since HCC is a relatively rare outcome and all incidents were captured this study represents a complete case analysis of the original source cohort rather than a sample drawn from larger population.

The statistical inference and effect estimation were based on the total number of observed events and not on a predefined sample size.

3.6 Data Collection

The data was collected from Red Cap software, Epi-data and from patient hard copy charts. Review and extraction of historical data from patient medical records, including baseline characteristics, clinical details, and past follow-up information up to the current point was done.

3.7 variables

3.7.1 Dependent variables:

- The development HCC, confirmed through imaging (ultrasound, CT, MRI) (Bruix & Sherman, 2011).

3.7.2 Independent variables:

- Male gender
- Age
- Cirrhosis
- HBV Viral load
- HBeAg
- HCV co-infection
- HDV co-infection
- Alcohol Drinking
- Khat chewing
- BMI
- APRI
- Fibroscan result
- DM and dyslipidaemia comorbidity

3.8 Operational Definition

- HCC: Primary liver malignancy diagnosed based on two imaging studies the first one being an ultrasound and the next one advanced imaging CT or MRI. AFP measurement was used as a supportive diagnostic tool(Galle *et a*l., 2018; Marrero *et al.*, 2018).

- Cirrhosis: Defined by the presence of at least one of the following(Berzigotti *et al*., 2021):
    - clinical evidence of decompensation (e.g. ascites, hepatic encephalopathy, variceal bleeding)
    - liver stiffness measurement >9.9 kPa
    - APRI score >1
    - suggestive imaging findings(nodular surface, blunted edge, portal hypertension and porto-systemic collaterals)
    - Clinical features such as icteric sclera were noted but not used alone to define cirrhosis

- HBV viral load: HBV DNA level measured in IU/mL categorized into the following groups which have different implication on risk of HCC(WHO guideline.,2024: Lampertico *et al*., 2017).
    - <2000 IU/mL
    - 2000 – 20,000 IU/mL
    - >20,000 IU/mL

- FibroScan (transient elastography): it assesses liver stiffness which is measured in Kilopascals (KPa). The below categorized as below(Liguori *et al*., 2025; MSKCC.,2025;):
    - ≤7KPa
    - 7.1-12.5 KPa
    - ≥12.5KPa

- APRI: a noninvasive method to assess liver fibrosis that is calculated by dividing patents AST by the Upper normal limit of AST (40 U/L was used) then multiplying it by 100 and dividing it by platelet count(Liguori *et al*., 2025).
    - ≤0.5
    - 0.5-1
    - ≥1

- BMI: calculated as weight in Kg over height in meter squared. It was categorized in the following groups(WHO,.2025):
    - <18.5 Kg/m²
    - 18.5 – 24.9 Kg/m²
    - ≥ 25 Kg/m²

- Ascites: presence of free intra-peritoneal fluid detected on imaging studies or physical examination(Runyon, 2009).

- Liver steatosis: defined by imaging findings on Ultrasound, CT an MRI (Radiopaedia.,2025.).

- Alcohol misuse: participants that have a history of drinking four or more drinks on any day or eight or more per week and five or more drinks on any day or 15 or more per week in women and men respectively(NIAAA.,2025).

- Khat use: participants that were actively chewing khat at the time of enrollment despite the amount(Mihretu *et al.*, 2017).

3.9 Statistical Analysis

The statistical analysis were structured and applied to address both the primary and secondary outcome measures comprehensively. STATA Version 14 application was used and the following statistical methods were utilized:

3.9.1 Descriptive Statistics

To summarize baseline characteristics of the cohort. Mean, median, standard deviation, and ranges for continuous variables (e.g., age, liver function tests) and frequencies and percentages for categorical variables (e.g., sex, presence of cirrhosis) was used.

3.9.2 Incidence Rate Calculation

Incidence rate was calculated by dividing number of new HCC cases by total person year. The person year was calculated by subtracting the end date from start date. The last date of data collection was used as the end date for currently active participants and the last date of follow up was used as an end date for those diagnosed with HCC, which are lost to follow up and withdrew from follow up. The date of death was used as an end date for those who have died.

3.9.3 Survival Analysis

Used to evaluate the time to an event of HCC patients.

- Kaplan-Meier Survival Analysis was used to estimate probabilities or risk of developing HCC at different of time.
- Log-Rank Test was performed to compare the risk of developing HCC between different groups in the cohort.

3.9.4 Cox Proportional Hazards Regression

To assess the predictors of HCC development cox proportional hazards was conducted. Uni-variable cox proportional hazards was performed and then variables were selected based on mainly clinical relevance and statistical significance (P value <0.05) to be included in the multivariable model in order to observe independent predictors of HCC development.

Firth's penalized cox regression was used to address and mitigate the small number of events and complete separation. Two variables were chosen to respect the rule of 10. Following this to assess the internal stability and increase precision bootstrap resampling with 500 replications was performed.

3.10 Ethical Considerations

- Informed Consent: All participants provided informed written consent at the time of enrolment, with procedures approved by the Institutional Review Board (IRB) of St. Paul Hospital Millennium Medical College and also a national ethical clearance has been obtained for the cohort.

- Confidentiality: Data was anonymous and securely stored, ensuring participant confidentiality.

-Each participant was identified only with their identification number which will keep them anonymous and maintain their confidentiality.

- Ethical Approval: This study was reviewed and approved by the institutional review committee of Addis Ababa University Aklilu Lemma Health Research Institute.

4. RESULTS

4.1 Descriptive statistics

A total of 1291 CHB patients were eligible to be included in this study from the 1303 total participants that were enrolled in the cohort study. The summarized baseline characteristics of the study participants as well as the follow up data are listed in Table 1.

Table 1 Demographic, Medical History and Clinical Characteristics of All Study Participants

Variables		Total study participants(N=1294)	
		Diagnosed with HCC	Not Diagnosed with HCC
Sex	Females	6(26.1%)	521(41.1%)
	Males	17(73.9%)	747(58.9%)
	Total	23(100%)	1268(100%)
Cirrhosis	Yes	23(100%)	298(23.5%)
	No	0(0%)	970(76.5%)
	Total	22(100%)	1268(100%)
Age	18-25	0(0%)	283(22.3%)
	26-35	6(26.1%)	539(42.5%)
	36-45	6(26.1%)	281(22.1%)
	≥46	11(47.8%)	165(13.1%)
	Total	23(100%)	1268(100%)
Initial HBV viral load (IU/mL)	<2000	5(21.7%)	722(57.4%)
	2000 - 20,000	2(8.7%)	254(20.2%)
	>20,000	16(69.6%)	282(22.4%)
	Total	23(100%)	1258(100%)
HBV E-antigen	Negative	15(68.2%)	1002(88.1%)
	Positive	7(31.8%)	135(11.9%)
	Total	22(100%)	1137(100%)
Anti HCV	Negative	20(100%)	1060(97.3%)
	Positive	0(0%)	29(2.7%)
	Total	20(100%)	1089(100%)
Anti HDV	Negative	21(95.4%)	1226(98.5%)
	Positive	1(4.6%)	18(1.5%)
	Total	22(100%)	1244(100%)

Initial Fibro-scan result (kPa)	≤7	0(0%)	868(69.8%)
	7.1-12.5	1(4.6%)	205(16.5%)
	>12.5	21(95.4%)	170(13.7%)
	Total	22(100%)	1243(100%)
Initial APRI score	≤0.5	10(45.4%)	1077(86.2%)
	0.5-1.0	6(27.3%)	97(7.7%)
	>1.0	6(27.3%)	76(6.1%)
	Total	22(100%)	1250(100%)
BMI	<18.5	5(23.8%)	162(13.9%)
	18.5 – 24.9	11(52.4%)	681(58.6%)
	≥25	5(23.8%)	319(27.5%)
	Total	21(100%)	1162(100%)
DM and dyslipidemia	No	21(91.3%)	1241(97.9%)
	Yes	2(8.7%)	27(2.1%)
	Total	23(100%)	1268(100%)
Family history of liver cancer	No	22(95.6%)	1209(95.4%)
	Yes	1(4.4%)	59(4.6%)
	Total	22(100%)	1268(100%)
Alcohol misuse	No	23(100%)	1215(95.8%)
	Yes	0(0%)	53(4.2%)
	Total	23(100%)	1268(100%)
Khat chewing	No	21(91.3%)	1064(83.9%)
	Yes	2(8.7%)	116(9.2%)
	Stopped	0(0%)	88(6.9%)
	Total	23(100%)	1268(100%)
Years diagnosed to have CHB before cohort	<5	21(91.3%)	1131(89.8%)
	5.10	2(8.7%)	86(6.8%)
	>10	0(0%)	43(3.4%)
	Total	23(100%)	1260(100%)
Last HBV viral load (IU/mL)	<2000	10(90.9%)	837(78.7%)
	2000 - 20,000	1(9.1%)	166(15.6%)
	>20,000	0(0%)	61(5.7%)
	Total	11(100%)	1064(100%)
Last Fibro-scan result (kPa)	≤7	0(0%)	881(84.7%)
	7.1-12.5	4(40%)	93(8.9%)
	>12.5	6(60%)	66(6.4%)
	Total	10(100%)	1040(100%)
Last APRI score	≤0.5	6(50%)	1008(89.7%)
	0.5-1.0	4(33.3%)	72(6.4%)
	>1.0	2(16.7%)	44(3.9%)
	Total	12(100%)	1124(100%)
Imaging studies	No	0(0%)	519(40.9%)
	Yes	23(100%)	749(59.1%)
	Total	22(100%)	1268(100%)

At baseline, median age of participants was 31 (IQR= 26- 40) and 59% are males. The median baseline viral load was 1248 IU/mL (IQR= 240 –14231) with 12.2% of them being E- antigen positive. The baseline the tested liver enzymes showed a median ALT of 25 U/L (IQR= 18.5 – 37), median AST of 25 (IQR= 20 – 34) and median platelet count of 272 (IQR= 220 – 323). The median liver elasticity (fibro-scan result) was 5.8 KPa (IQR= 4.6 – 8.1). A small proportion of the participants had co-infection with HCV (2.6%) and HDV (1.5%). 29(2.2%) of the participants had metabolic abnormalities such as DM and dyslipidemia and the median BMI was 22.4 Kg/m² (IQR= 19.6 – 25.3). 361(28%) of the participants were alcohol drinkers upon enrollment while 169(13%) had history of alcohol drinking but have stopped prior to being enrolled in the study and specifically 46 (3.6%) of the participants had history of alcohol misuse. Majority (84%) of the participants do not have history of khat use.

The median observation time was 5.4 years (IQR= 1.2 - 9.4). On follow up the last measured HBV viral load of the participants had a median of 218 IU/mL (IQR= 11- 1360) while the last measured fibroscan result of participants had a median of 5 KPa (IQR= 4.1 – 6.3). APRI score that was calculated by using baseline data that yielded a median score of 0.2 (IQR= 0.2 – 0.4) and the APRI score that was calculated by using the last follow up data of the participants had median score of 0.2 (IQR= 0.2 – 0.3).

Out of the total 1291 participants, 772(60%) of them have had imaging studies done at different point of time in the 10 year follow up period which gives us a total of 2289 imaging studies that were observed for this study. The median number of imaging study per participant was 2 (IQR= 1 - 4). With minimum of 1 and a maximum of 16 imaging study per a study participant. Among the 772 individuals who have had imaging studies done 59(7.6%) of them have had advanced imaging studies like tri-phasic CT scan (n=45) or MRI (n=14), 126(16.4%) had cirrhosis, 190(25%) had fatty liver and 57(7.2%) had mass that is not HCC.

Table 2 Demographic, Radiologic and laboratory Characteristics of HCC Diagnosed Participants

HCC positive cases	Enrollment date	End of follow up date	Age at the time of HCC diagnosis	Imaging Modalities	Mass size (cm)	Number	AFP	Cirrhosis
1	04/03/2015	16/03/2015	57	CT scan	5.7*5.3	Multiple		Yes
2	06/03/2015	28/05/2015	35	CT scan	3*3	One	>400 IU/mL	Yes
3	24/03/2015	30/07/2015	27	CT scan	7*8	One		Yes
4	25/05/2015	27/05/2015	56	CT scan	2.7*2.7	One	14.8 IU/mL	Yes
5	16/06/2015	16/06/2015	40	CT scan	-	Multiple		Yes
6	20/07/2015	27/07/2015	31	CT scan	-	Multiple	33.5 IU/mL	Yes
7	04/09/2015	18/09/2015	31	MRI	-	Multiple	>400 IU/mL	Yes
8	15/09/2015	15/09/2015	26	MRI	7*4	One	24 IU/mL	Yes
9	23/10/215	19/12/2015	40	MRI	-	Multiple	>400 IU/mL	Yes
10	05/03/2015	13/06/2024	49	MRI	-	Multiple	1000 ng/mL	Yes
11	25/03/2015	18/03/2019	54	CT scan	12*7	One	-	Yes
12	21/04/2015	23/04/2021	46	CT scan	3.8*2	Two	35.87 IU/mL	Yes
13	27/04/2015	29/08/2017	43	CT scan	-	Multiple	-	Yes
14	01/06/2015	28/11/2017	52	CT scan	10.8*8.4	Multiple	>400 IU/mL	Yes
15	13/07/2015	26/12/2016	49	MRI	-	Multiple	>400 IU/mL	Yes
16	15/07/2015	29/05/2020	69	CT scan	3.2 * 3	One	>300 ng/mL	Yes
17	07/09/2015	19/01/2016	51	CT scan	6.5*4.4	One	453 IU/mL	Yes
18	15/9/2015	29/12/2016	66	MRI	6 * 6.3	Multiple	5.33 IU/mL	Yes
19	18/9/2015	19/12/2019	41	CT scan	12*7	One	6.7 IU/mL	Yes
20	06/11/2015	03/11/2021	38	CT scan	-	Multiple	73.3 ng/mL	Yes
21	01/12/2015	14/01/2016	60	CT scan	3*3	One	>400 IU/mL	Yes
22	05/01/2016	24/02/2016	55	MRI	-	Multiple	>400 IU/mL	Yes
23	23/04/2015	16/05/2024	64	MRI	10.8	One	85509 ng/mL	Yes

Among the total study participants 321(25%) had fulfilled the criteria to be categorized under the cirrhosis group. Out of the total 1291 study population 23 of them were diagnosed to have HCC with in the 10 years of follow-up period.

Among the 23 HCC cases majority (74%) were males and aged above 46 years (48%) at the time of enrollment and the median age at the time of diagnosis was 49 (IQR=38 – 56) years. All had advanced imaging studies with 65.2% of them having tri-phasic CT scan and 34.8% had MRI. On the imaging studies the Majority (56.5%) had more than one Mass. 43.5% of them had AFP >400 IU/mL. All the 23 of those diagnosed with HCC had cirrhosis (Table 2).

4.2 Incidence Rate

Among the total of 23 participants that were diagnosed with HCC 12 of them were diagnosed within the first 12 months of follow up and were presumed to be present already at enrolment. Hence 11 of them were new cases that were included in the calculation of incidence rate.

Over the 10 year follow up period of 6,668 person years the overall incidence rate was 1.65 cases per 1000 person years (95%CI: 0.91 - 2.98). In those with cirrhosis the incidence rate was 7.07 cases per 1000 person year (95%CI: 3.91 - 12.76), whereas no HCC cases were observed among non-cirrhotic individuals.

During the total follow-up period, all new HCC cases (n=11) occurred among patients with cirrhosis and no cases were observed among non-cirrhotic patients. Due to the absence of events in the non-cirrhotic group, the incidence rate ratio (IRR) for HCC could not be estimated.

4.3 Kaplan-Meier Analysis and Log Rank Test

Using Kaplan-Meier estimates, the cumulative risk of HCC was 0.44% (95%CI: 0.17% – 1.18%) at 3 years, 0.86% (95%CI: 0.41% – 1.80%) at 5 years and 2.45% (95%CI: 1.02% – 5.78%) at 10 years.

Table 3 Log Rank Test Results

Variable		HCC Event observed	Events expected	chi²	P value
Sex	Female	5	4.72	0.03	0.866
	Male	6	6.28		
Age	18 – 25	0	2.04	15.66	0.001
	26 – 35	1	4.58		
	36 – 45	4	2.60		
	≥46	6	1.78		
Cirrhosis	No	0	8.34	34.7	<0.001
	Yes	11	2.66		
Initial HBV Viral Load (IU/mL)	< 2000	2	6.14	22.46	<0.001
	2000 – 20000	0	2.39		
	>20000	9	2.47		
Initial Fibro-scan result (kPa)	< 7	0	7.65	77.15	<0.001
	7 – 12.5	0	1.97		
	> 12.5	11	1.37		
BMI	< 18.5	1	1.34	0.23	0.891
	18.5 – 24.9	6	6.32		

	≥ 25	4	3.34		
Years diagnosed to have CHB before cohort	<5	9	9.66	1.84	0.399
	5 – 10	2	0.90		
	>10	0	0.44		
Dyslipidemia and DM	No	10	10.75	2.34	0.126
	Yes	1	0.25		
Alcohol Misuse	No	11	10.71	0.29	0.587
	Yes	0	0.29		
Khat use	No	11	9.56	1.65	0.438
	Yes	0	0.85		
	Stopped	0	0.59		
HBV E antigen	No	8	9.71	2.56	0.110
	Yes	3	1.29		
Anti HCV	No	11	10.75	0.26	0.618
	Yes	0	0.25		
Anti HDV	No	11	10.82	0.18	<0.001
	Yes	0	0.18		
Follow up	Follow Up	0	7.82	27.38	<0.001
	On Antiviral	11	3.18		
Family history of liver cancer	No	10	10.32	0.17	0.684
	Yes	1	0.68		

As presented in Figure 1 and Figure 2, respectively, the Kaplan-Meier estimates stratified by cirrhosis (Log rank X^2= 34.7, P<0.001) and initial viral load (Log rank X^2= 22.5, P<0.001) those with cirrhosis and viral load > 20,000 IU/mL have a significantly increased probability of developing HCC compared to those without cirrhosis and lower viral load count respectively.

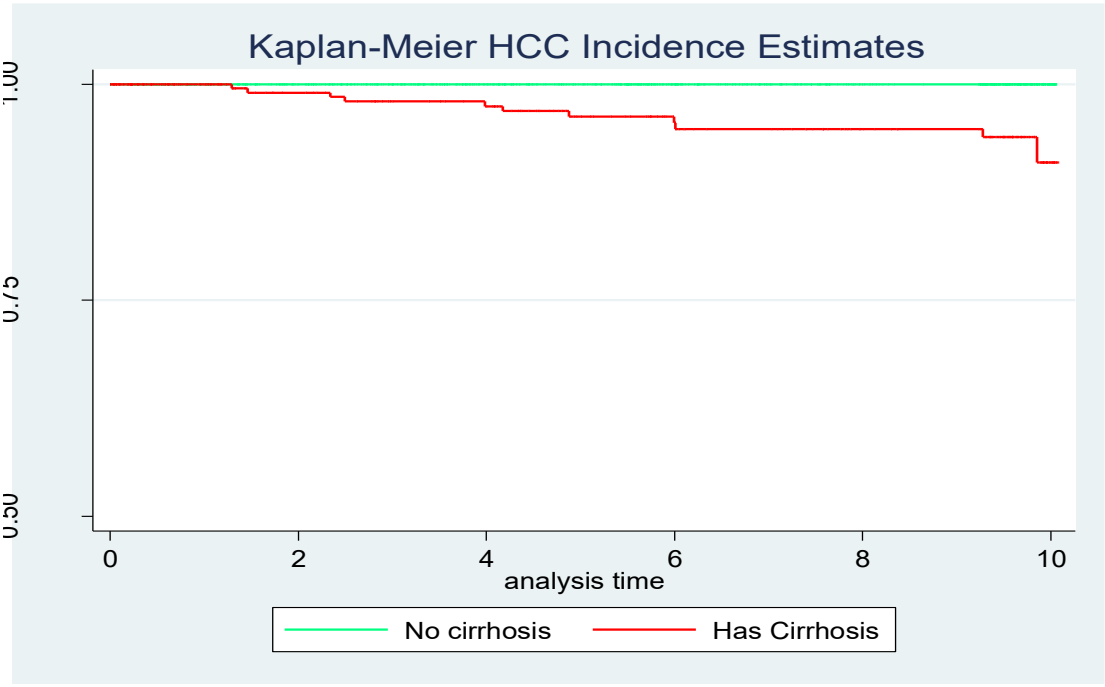

Figure 1 Kaplan-Meier estimates of HCC incidence in those with cirrhosis and without cirrhosis

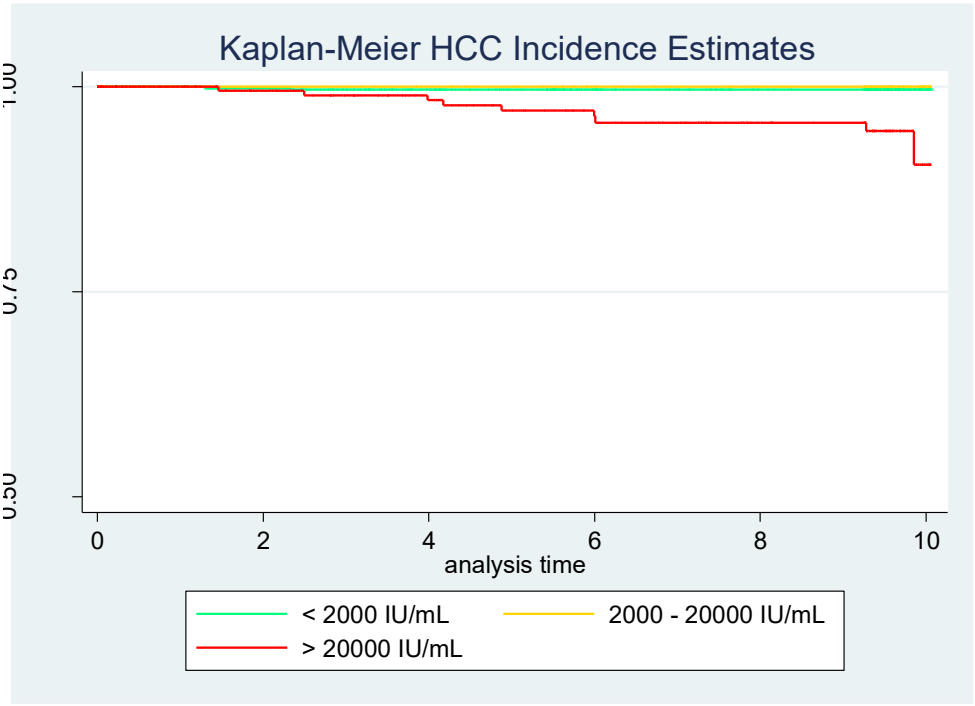

Figure 2 Kaplan-Meier estimates of HCC incidence in different HBV viral load groups

The probability of developing HCC among different age groups (Log rank X^2= 15.7, P= 0.001) is seen on Figure 3. Among the 11 new HCC cases 66% has age ≥ 46 years.

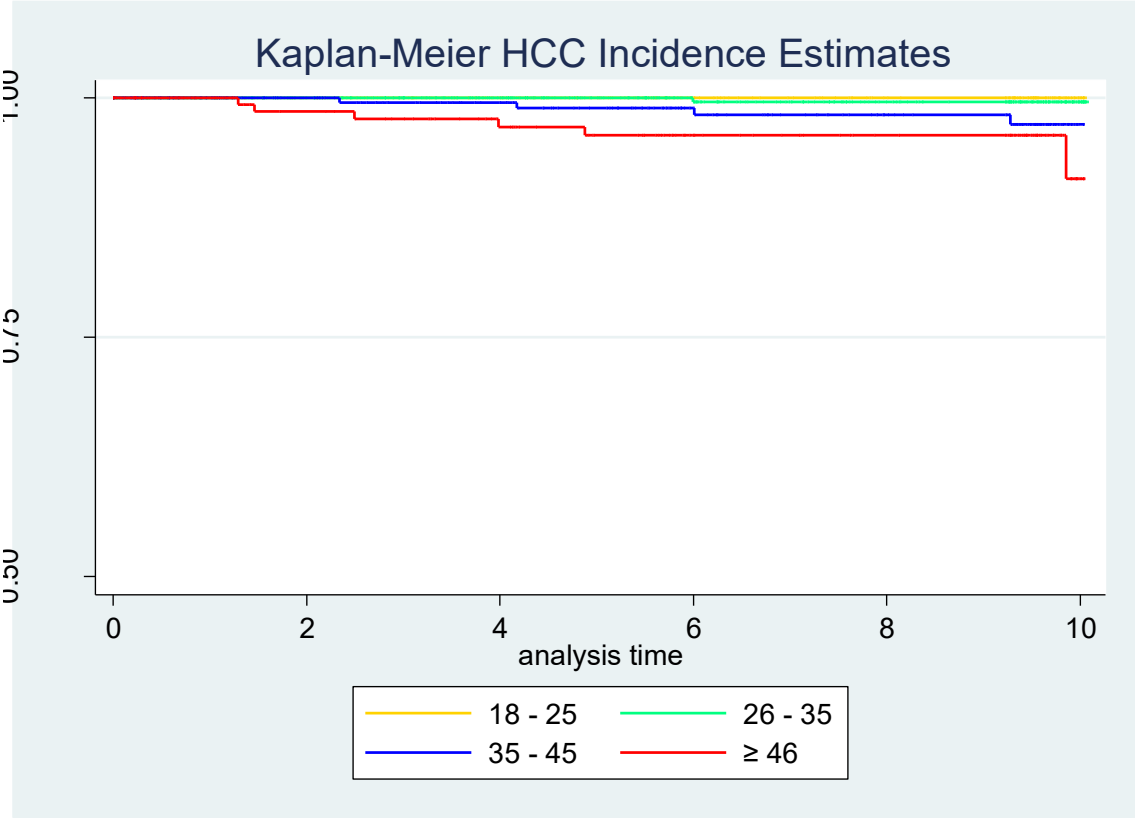

Figure 3 Kaplan-Meier HCC incidence estimate in different age group

Being on antiviral therapy was observed (Figure 4) to have a significant difference (Log rank X^2= 27.4, P value<0.001) in HCC estimation on the Kaplan-Meier curve. This observed difference is due to the baseline status of the study participants that landed them in the antiviral treatment group.

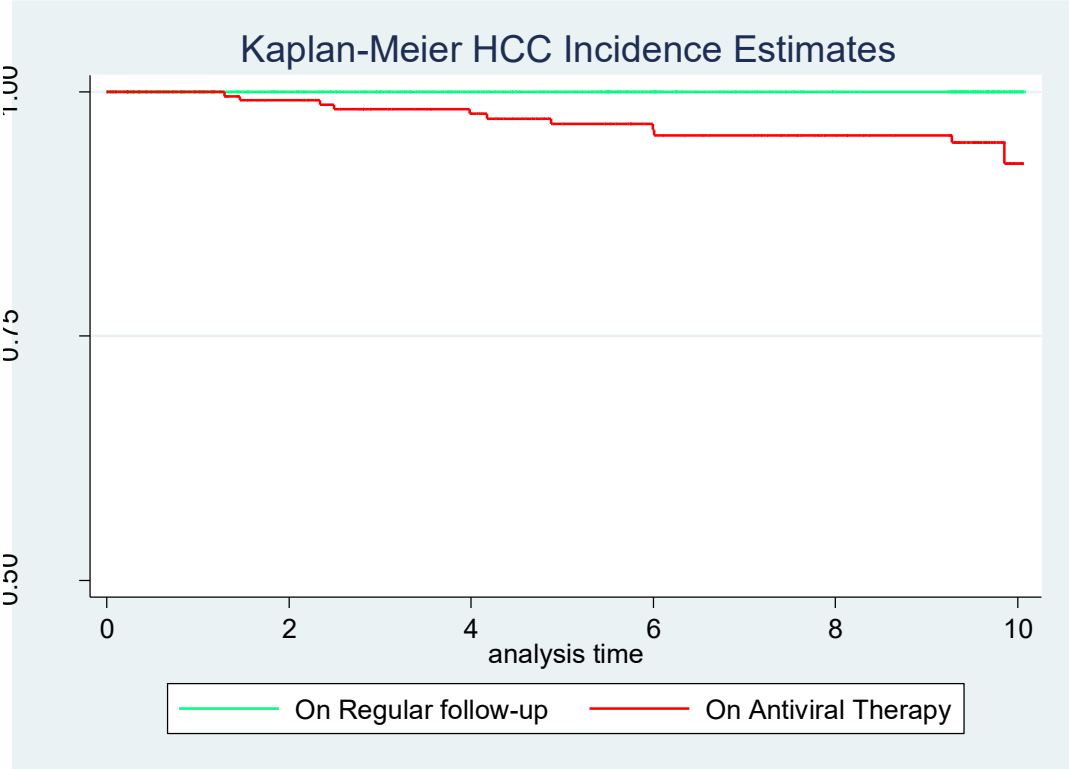

Figure 4 Kaplan-Meier HCC incidence estimates in those on antiviral therapy or on regular follow up

4.4 Cox Proportional Hazards Regression

The uni-variable cox regression analysis showed risk factors such as age (CHR=1.10, 95%CI: 1.05 – 1.16, P= 0.000) and initial viral load >20,000 (CHR=15.57, 95%CI: 3.36 – 72.11, P= 0.000) were statistically significant and eligible to be inserted in the multivariable cox regression. Cirrhosis had a very large CHR (4.0e+16) with P value of 1.00. This indicates complete separation. To address this Firth's penalized cox regression was used (Table 4).

Table 4 Univariable Cox Regression, Firth's Penalized Multivariable Cox Regression and Bootstrap Result

Variables		HCC status								
		Crude			Adjusted			Bootstrap Adjusted		
		HR	P value	95% CI	HR	P value	95% CI	HR	P value	95%CI
Age		**1.10**	**<0.001**	**1.05 – 1.16**	**1.07**	**0.002**	**1.03- 1.13**	**1.07**	**<0.001**	**1.03 – 1.12**
Sex		0.9	0.866	0.31- 4.0						
Cirrhosis		4.0e+16	1.000	0 - .	**52.83**	**0.006**	**3.06 - 912.2**	**52.83**	**<0.001**	**27.25 –102.43**
Alcohol Misuse		6.04e-16	1.000	0 - .						
DM and dyslipidemia		4.34	0.162	0.55 -34.01						
HBV E antigen		2.82	0.126	0.75 – 10.64						
Initial HBV viral load	<20,000	Ref								
	>20,000	**15.57**	**<0.001**	**3.36 -72.11**						
BMI	<18.5	Ref								
	18.5 – 24.9	1.27	0.826	0.15 -10.53						
	>25	1.60	0.675	0.18 -14.33						

Firth's penalized multivariable Cox regression model was used to assess factors that are independently associated with the development of HCC. Given the limited number of HCC events (n = 11), only two covariates were included in the final model to prevent overfitting. These were selected based on a combination of statistical significance in univariable analysis, clinical relevance, and avoidance of multicollinearity. The variables included were age and cirrhosis.

The Firth's penalized multivariable cox regression analysis showed that both age and cirrhosis remained statistically significant. Increasing age was significantly associated with a higher risk of developing HCC (Adjusted HR per 1-year increment: 1.07, 95% CI: 1.03 – 1.13, P value = 0.002). Cirrhosis was a strong predictor of HCC (Adjusted HR: 52.83, 95% CI: 3.06 – 912.2, P value = 0.006), indicating that participants with cirrhosis had 53 times higher risk of developing HCC compared to those without it(Table 4).

Bootstrap estimates based on 500 replication yielded a stable adjusted hazard ratios. For age (Bootstrap AHR per 1-year increment: 1.07, 95% CI: 1.03– 1.12, P value < 0.001) and for cirrhosis (Bootstrap AHR: 52.83, 95% CI: 27.25– 102.43, P value < 0.001) (Table 4).

5. DISCUSSION

This study, that was conducted on 1291 CHB patients that were on follow up for the past 10 years, provides a valuable look into the incidence and associated factors of HCC. The study showed incidence rate of 1.65 per 1000 person year (95%CI: 0.91 - 2.98) with 2.45% (95%CI: 1.02% - 5.78 %) cumulative risk of developing HCC at 10 years. Numerous factors associated with the development of HCC were assessed in the uni-variable cox-regression .several variables conventionally linked to HCC such as sex, hepatitis C and D, alcohol use, family history of liver cancer, diabetes mellitus (DM), dyslipidemia, and obesity did not reach statistical significance in the analysis of this study. Age (AHR=1.07, 95%CI: 1.03 – 1.12, P value<0.001) and cirrhosis (AHR=52.83, 95%CI: 27.25 – 102.43, P value<0.001) remained statistically significant in the multivariable Firth's penalized cox regression and after application of bootstrapping.

The result of this study showed that the incidence rate of HCC among CHB patients was 1.65 per 1000 person years (95%CI: 0.91 - 5.78) and in those with cirrhosis the incidence rate was 7.07 cases per 1000 person year (95%CI: 3.91 - 12.76). According to the REVEAL-HBV study the incidence rate of HCC in those with CHB ranged from 1.08 to 11.5 per 1000 person years in those with HBV load of <300 and ≥ million copies respectively (Chen *et al.,* 2006). Population based data from the databases in Taiwan and Hong Kong to identified 23851 patients with CHB that have been receiving antiviral treatment. Out of them 2.50% of them developed HCC and the cumulative incidence at 3 years was found to be 3.56% (95% CI 3.26% - 3.86%) (Hsu *et al.*, 2018). In a Taiwanese study that was conducted in men showed that the incidence rate of HCC was 3.24 per 1,000 person-years for those who were HBsAg positive (Yang *et al.*, 2002). In another study HCC incidence rate was 0.2 per 1000 person-years in those who are inactive carriers of HBV and 3 in with chronic HBV without cirrhosis (El-Serag, 2012; Fattovich *et al*., 2008). Despite the difficulty of finding similar published studies in sub-Saharan African countries, the incidence rate of this study is within the range of the incidence rates generated by studies from other regions.

In this study age remained an independent risk factor after penalized regression (Bootstrap AHR per 1-year increment: 1.07, 95% CI: 1.03 – 1.12, P value < 0.001). According to studies there is a Varity in the age of onset in different areas of the world. In this study the median age of HCC diagnosis was found to be 49(IQR=38 – 56). In alignment to this study the age range at which the diagnosis of HCC is made in most African and Asian countries is 30–60 years. Where as in areas in North America, and Europe the mean age of diagnosis is beyond the age of 60 (Park *et al*., 2015). According to a study conducted in 7 African countries the median age of HCC diagnosis was 45 and 32.5 to 37.5 for HCC due to HBV (Yang *et al.,* 2015) which is seen to be lower than this study's finding. According to the Surveillance, Epidemiology, and End Results (SEER) program, early onset of HCC was seen in USA among individuals that were born in sub-Saharan African countries. Among other areas being born in East Africa was strongly associated (AOR 3.5, 95% CI 1.5–6.8; P<0.01) with very early-onset of HCC (<40 years) (Yang *et al*., 2017a). The observed difference in age distribution of HCC in different areas is believed to be due to type of virus and timing of infection. When HBV is the causative agent, In Asian countries that have high incidence of HCC and Africa individuals develop HCC at earlier ages due to HBV infection is largely acquired by mother–child transmission and transmission among siblings of young ages respectively(El-Serag, 2012).

In this study 77.3% of HCC cases were males which goes along with the established understanding. The higher prevalence of HBV infection among men than women is in part the reason behind the observed high male: female ratio of HCC (El-Serag, 2012). CHB infection is the primary cause of HCC in Eastern Asian countries and sub-Saharan African countries (Park *et al.,* 2015; Yang *et al.,* 2015). In this study cirrhosis was found to be among the independent associated factors for the development of HCC after firth's penalized multivariable cox regression (Bootstrap AHR: 52.83, 95% CI: 27.25– 102.43, P value< 0.001) and all the diagnosed HCC cases in this study were found to have cirrhosis. In a study conduct in the USA in CHB patients about 90% of those that developed HCC had cirrhosis. Interestingly, HCC in those without cirrhosis was statistically associated with being black (OR=6.78; 95% CI 2.05–22.4) and Asian (OR 11.6, 95% CI 2.63–50.8) (Chayanupatkul *et al*., 2017). In contrast to this research's findings it has been observed that in 30

to 50 percent of HBV associated HCC occur in the absence of cirrhosis in HBV endemic areas, such as Eastern Asia and most African countries (Yang *et al*., 2019, 2017b).

Similarly too previously conducted studies, in this study the initial vial load was statistically significant in the univariable cox regression (CHR 13.9 95%CI 2.9 – 65.4, P=0.001). In a Taiwanese study, that assessed the risk of HBV viral load elevation and its association with HCC, reported that the incidence of HCC increased in proportion to the serum level of HBV DNA. This association remained significant after adjusting for other risk factors (Chen *et al.,* 2009). In another study elevated serum viral DNA level specifically above 10,000 copies/mL was found to be an independent risk factor for the development of HCC (Chen et al., 2006). In another study the risk of HCC was found to be 5 fold even in inactive HBV carriers compared to individuals without the viral infection (Chen *et al*., 2010).

The majority (59%) of the study participants in this study were not alcohol consumers and it was not significantly associated with the development of HCC. But in previously conducted studies alcohol drinking has been associated with the development of HCC. According to the World Cancer Research Fund (WCRF) a 4% increment in risk of hepatic cancer development per 10g/day alcohol intake with a relative risk of 1.04(95% CI 1.02–1.06) (WCRF., 2015). In another study, the relative risk of mortality in males with HBV infection that have history of heavy alcohol drinking was 1.5 (95% CI = 1.2 - 2.0) compared to those without infection (Jee *et al*., 2004).

In summary, the findings of this study affirms the role of cirrhosis and increasing age in the development of HCC in CHB patients that have been established in the global data. Other established risk factors were not able to reach statistical significance in this study. This might reflect population specific characteristics.

6. STRENGTH AND LIMITATION

6.1 Strength

- The use of data generated from a prospective cohort study that has been going on for the past 10 years allows for more precise assessment of risk factors for the development of HCC in CHB patients.
- The use of Firth's penalized regression, considering the low number of HCC events and the observed complete separation, gives this study the statistical power and validity.
- This study had a comprehensive variable inclusion ranging from demographic, clinical, virology and metabolic variables.
- The findings of this study are valuable by addressing the knowledge gap observed in the subject matter due to lack of data and studies.

6.2 Limitations

- Even though the use of Firth's penalized regression reduced over fitting and bias with only 11 new cases the precision of the estimates might be limited.
- Generalizability of the study findings might be limited by the homogeneity of the cohort participants.

7. CONCLUSION AND RECOMMENDATION

7.1 conclusion

This study assessed the incidence and factors for the development of HCC among CHB patients over a 10 year follow up period. The incidence rate was 1.65 cases per 1000 person years. All HCC cases occurred in those with cirrhosis. Age and cirrhosis were the two independent predictors of HCC development in the Firth's penalized multivariable regression analysis. Other conventionally known risk factors like sex, co-infections and metabolic conditions did not show statistically significant association in this study. These findings provide a valuable data, which has been lacking in our country, on HCC risk among CHB patients.

7.2 Recommendation

- Regular screening with biannual ultrasound in CHB patients especially in those with cirrhosis and age beyond 49.
- Early diagnosis and initiation of treatment in individuals with CHB.
- Multicenter studies to see the risk of HCC development in CHB patients with larger number of cases and diverse population background.

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9 APPENDICES

9.1 Appendix A: Data Collection Tool

Patient Identification number----------

Baseline Information Collection Form

- To be filled from the enrollment visit record

1 Enrolment date----------

3 Age----------

3 Sex

Male ☐

Female ☐

4 Address ----------

5 Date of hepatitis B diagnosis (date of the first positive HBsAg) ----------

6 Reason for first HBsAg test

- Clinical suspicion (signs or symptoms of hepatitis or liver problem) ☐
- Routine screening ☐
- Other ----------

7 HIV status at enrolment ----------

8 Marital status

- Single ----------
- Married----------
- Widowed---------

9 Weight ----------

10 Height----------

11 History of liver cancer in first degree relatives

Yes ☐

No ☐

12 History of alcohol use

☐ 4-7 times per week ☐ 2-3 times per week ☐ 2-4 times per month

☐ <1 time per month ☐ Never ☐ Stopped

13 History of khat use

☐ 4-7 times per week ☐ 2-3 times per week ☐ 2-4 times per month

☐ <1 time per month ☐ Never ☐ Stopped

Activities Done Collection Form

- To be filled for follow up visit

Date ----------

1 Clinical Section

1.1 was physical examination documented yes ☐ No ☐

1.2 If yes,

- Signs of ascites yes ☐ No ☐
- Signs of cirrhosis yes ☐ No ☐
- Jaundice yes ☐ No ☐
- Hepatomegaly yes ☐ No ☐

2 Laboratory Section

2.1 HBsAg----------

2.2 HBeAg----------

2.3 HIV test----------

2.4 ALT----------

2.5 AST----------

2.6 ALP----------

2.7 Bilirubin (total) ----------

2.8 Platelet count ----------

2.9 HBV DNA (viral load) ----------

2.10 Creatinine----------

2.11 HDV antibody----------

2.12 AFP----------

3 Radiology section

3.1 Fibroscan performed yes ☐ No ☐

3.1.1 If yes,

- Median liver stiffness (KPa) ----------
- Fibroscan interquartile range (KPa) ----------
- Fibroscan success rate (%) ----------

3.2 Ultrasound performed yes ☐ No ☐

3.2.1 If yes,

- Presence of Cirrhosis yes ☐ No ☐
- Presence of ascites yes ☐ No ☐
- Presence of liver steatosis yes ☐ No ☐
- Presence of focal observation (tumor, mass, lesion or nodule) yes ☐ No ☐

If yes,

- Number of lesion/tumor/mass/nodule----------
- Largest lesion/tumor/mass/nodule (in cm) ----------
- Suspicious for HCC yes ☐ No ☐

3.3 CT scan performed yes ☐ No ☐

3.3.1 If yes,

- Is cirrhosis present yes ☐ No ☐

- Is ascites present yes ☐ No ☐
- Focal observations (tumor/ mass/ lesion/ nodule) yes ☐ No ☐

If yes,

- Number of lesion/tumor/mass/nodule----------
- Largest lesion/tumor/mass/nodule (in cm) ----------
- Suspicious for HCC yes ☐ No ☐

3.4 MRI performed yes ☐ No ☐

3.4.1 If yes,

- Presence of Cirrhosis yes ☐ No ☐
- Presence of ascites yes ☐ No ☐
- Presence of focal observation (tumor, mass, lesion or nodule) yes ☐ No ☐

If yes,

- Number of lesion/tumor/mass/nodule----------
- Largest lesion/tumor/mass/nodule (in cm) ----------
- Suspicious for HCC yes ☐ No ☐

4 Treatment status

4.1 on antiviral Drug treatment yes ☐ No ☐

4.2 If yes, name of the drug ----------

5 Outcome status

5.1 status

- Active ☐
- Died ☐
- Withdrew from cohort ☐
- Loss to follow up ☐
- Transferred ☐
- Exited due to seroconversion ☐

Remark----------

9.2 Appendix B: Ethical Clearance

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Aklilu Lemma Institute of Pathobiology Institutional Research Ethics Review Committee (ALIPB-IRERC)

Ethical Clearance Certificate

Ref. No.: ALIPB IRERC/152/2017/24
Date: December 09, 2024

Title of the project: "Hepatocellular carcinoma among chronic hepatitis b patients at Saint Paul's hospital Millennium Medical College Addis Ababa, Ethiopia"

PI: Bethelhem Hailu,
Recommendation of the ALIPB-IRERC
Dear Bethelhem,

The ALIPB-IRERC has reviewed your above mentioned Research Proposal and noted its merit. The IRERC would like to remind you as the PI to submit progress reports of the work every 6 months and the final report upon completion of the study. Furthermore, you are expected to notify the ALIPB-IRERC ahead of time any amendments or modifications in the protocol or premature suspension or termination of the study.

STATUS: Approved

Needs NRERB clearance: **Yes: ___ No: _x_**

IRERC Chairperson: Berhanu Erko, Prof.
Signature: Berhanu Erko

IRERC Secretary: Esayas Aklilu,PhD.
Signature:

Approval
Name: **Professor Mengistu Legesse, Director**
Signature:
Date: 9/12/2024

ADDIS ABABA UNIVERSITY
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Cc// IRERC office