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A CLINICAL EPIDEMIOLOGICAL STUDY ON END-STAGE LIVER DISEASE IN GHANA

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A Clinical Epidemiological Study on End-stage Liver Disease in Ghana

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By

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To my loved ones

Nyame nhyira mo nyinaa

ABSTRACT

End-stage liver disease (ESLD), including advanced liver cirrhosis and hepatocellular carcinoma (HCC), is the final stage of long-standing injury to the liver due to risk factors such as chronic viral hepatitis and alcoholic liver disease. There is a high disease burden and mortality globally, especially in sub-Saharan African (SSA) countries such as Ghana, where the primary cause of HCC and cirrhosis is infection with hepatitis B virus (HBV). To overcome the problem of ESLD in Ghana and SSA, epidemiological data on patient characteristics, challenges with diagnosis and management and mortality estimates are necessary so that well-directed and appropriate policies can be developed. This thesis, therefore, aimed to determine the clinical epidemiological profile of liver cirrhosis and HCC patients in Ghana and to explore diagnostic and management practices associated with the care of patients.

In **Study I**, we described the clinical characteristics of ESLD from liver cirrhosis and HCC in Ghana and evaluated the performance of the aspartate aminotransferase (AST) - platelet ratio index (APRI) score and alpha fetoprotein (AFP) in a cross-sectional study involving 141 HCC, 216 cirrhosis and 218 chronic HBV patients. We found a median age at diagnosis of 44 years, with most patients presenting at an advanced stage of disease. APRI cut-off of 2 had sensitivity of 45.4% and specificity of 95% in the diagnosis of cirrhosis, whilst a cut-off of 1 had sensitivity of 75.9% and specificity of 89%. The AUC of AFP of 0.88 indicated the utility of this test in the surveillance of HCC in Ghana.

Study II evaluated the in-hospital testing of HBV infection and burden of disease in Ghana by reviewing hospital-based data from 136,068 laboratory register entries, 165,213 blood bank register entries, and 83,920 delivery register entries in 22 healthcare institutions. We found that HBsAg RDT testing was widely available in government hospitals, however, HBV serological profile and DNA testing were mostly limited to teaching hospitals. The crude national seroprevalence was 8.40% ((95% CI 8.25-8.57%), whilst the pooled estimate was 11.39% (95% CI 10.43-12.35). Seroprevalence in children <5 years was 1.87% (95% CI 1.07-3.27). Our study indicated that Ghana remains a country with high endemicity and limitations in the full complement of testing for HBV infection.

In **Study III**, we explored the opinions and practices of cirrhosis patients and health workers on the nutritional management of cirrhosis through a qualitative study. We found that patients and health workers felt dietary recommendations for patients were frequently addressed but could be significantly improved. Additionally, we found that local guidelines were not available for nutritional assessment and management in the opinion of study participants. Participants believed these to be important and necessary in managing cirrhosis patients in Ghana.

Study IV assessed the proportion of liver-related deaths from liver cirrhosis and HCC, and their known risk factors in Ghana, and determined clinical factors associated with mortality. We found that 8.8% of deaths between 2018 -2020 in adults aged 18 years and above were

due to liver-related causes. The proportion of liver-related deaths associated with HBV infection was 48.76%, HCV infection was 10.0%, and alcohol was 7.01%. Predictors of in-patient mortality in cirrhotic patients were elevated WBC (OR = 1.14 95% CI: 1.00 -1.30) and the revised model for end-stage liver disease with incorporation of sodium (MELD-Na) score (OR = 1.24 95% CI: 1.01-1.54). For HCC patients, female sex (OR=3.74 95% CI: 1.09-12.81) and hepatic encephalopathy (grade 1) were associated with higher mortality (OR = 5.66 95% CI: 1.10-29.2).

In conclusion, this thesis presented the current landscape of end-stage liver disease, clinical epidemiology, diagnosis, and management in Ghana. It enhanced knowledge of the burden of viral hepatitis-related to liver cirrhosis and liver cancer. Finally, it shed light on factors associated with in-hospital mortality in Ghana.

LIST OF SCIENTIFIC PAPERS

I. Nartey YA, Awuku YA, Agyei-Nkansah A, Duah A, Bampoh SA, Ayawin J, Asibey SO, Björkström NK, Ye W, Afihene MY, Roberts LR. Ambulatory end-stage liver disease in Ghana; patient profile and utility of alpha fetoprotein and aspartate aminotransferase: platelet ratio index. BMC gastroenterology. 2020 Dec;20(1):1-1.

II. Nartey YA, Okine R, Seake-Kwawu A, Senya K, Duah A, Owusu-Ofori A, Bampoh SA, Plymoth A, Roberts LR, Bockarie AS, Awuku YA, Obiri-Yeboah D. Hepatitis B Virus testing patterns in Ghana; A nationwide cross-sectional review of in-hospital testing and epidemiologic burden of disease from 2016 – 2021 (manuscript).

III. Nartey YA, Asem M, Agyei-Nkansah A, Awuku YA, Setorglo J, Duah A, Bampoh S, Ayawin J, Asibey SO, Ye W, Afihene MY. Nutritional management of cirrhosis patients: A qualitative study exploring perceptions of patients and health workers in Ghana. Clinical Nutrition ESPEN. 2019 Dec 1;34:18-22.

IV. Nartey YA, Antwi SO, Awuku YA, Bockarie AS, Plymoth AP, Roberts LR. Mortality burden due to liver cirrhosis and hepatocellular carcinoma in Ghana; Prevalence of risk factors and predictors of poor in-hospital survival (manuscript)

LIST OF RELATED WORK

The following work was published during the PhD education but is outside the scope of this thesis:

The Polaris Observatory HCV Collaborators. Global Change in Hepatitis C Virus Prevalence and Cascade of Care between 2015 and 2020: A Modeling Study. The Lancet Gastroenterology & hepatology

Olakunde, B. O., Adeyinka, D. A., Olakunde, O. A., Uthman, O. A., Bada, F. O., Nartey, Y. A., ... & Ezeanolue, E. E. (2021). A systematic review and meta-analysis of the prevalence of hepatitis B virus infection among pregnant women in Nigeria. PloS one, 16(10), e0259218.

Caulfield, A., Plymoth, A., Nartey, Y. A., & Mölsted-Alvesson, H. (2020). The 6-star doctor? Physicians' communication of poor prognosis to patients and their families in Cape Coast, Ghana. BMJ global health, 5(6), e002334.

Groussin, M., Poyet, M., Sistiaga, A., Kearney, S. M., Moniz, K., Noel, M., ...Nartey Y.A. ... & Alm, E. J. (2021). Elevated rates of horizontal gene transfer in the industrialised human microbiome. Cell, 184(8), 2053-2067.

Hambridge, T., Nartey, Y., Duah, A., & Plymoth, A. (2019). Hepatitis B mother-to-child transmission in the Eastern Region of Ghana: a cross-sectional pilot study. The Pan African Medical Journal, 33.

Yang, J.D., Mohamed, E.A., Aziz, A.O.A., Shousha, H.I., Hashem, M.B., Nabeel, M.M., Abdelmaksoud, A.H., Elbaz, T.M., Afihene, M.Y., Duduyemi, B.M. Nartey Y… and Ayawin, J.P., 2017. Characteristics, management, and outcomes of patients with hepatocellular carcinoma in Africa: a multicountry observational study from the Africa Liver Cancer Consortium. The Lancet Gastroenterology & Hepatology, 2(2), pp.103-111.

CONTENTS

LIST OF ABBREVIATIONS

AFP	Alpha fetoprotein
AFP-L3	Lens culinaris agglutinin reactive AFP
ALT	Alanine aminotransferase
APRI	Aspartate aminotransferase - platelet ratio index
AST	Aspartate aminotransferase
BCLC	Barcelona Clinic Liver Cancer
cAg	Core antigen
CHAG	Christian Health Association of Ghana
CHPS	Community based Health Planning and Services
DCP	Des gamma carboxyprothrombin
DNA	Deoxyribonucleic acid
ELISA	Enzyme-linked immunosorbent assay
ESLD	End-stage liver disease
FIB-4	Fibrosis-4
GALAD	Gender, age, AFP-L3, AFP, DCP
GHS	Ghana Health Service
GLOBOCAN	Global Cancer Observatory
HBV	Hepatitis B Virus
HBcAb	Hepatitis B core antibody
HBeAb	Hepatitis B e antibody
HBeAg	Hepatitis B e antigen
HBsAb	Hepatitis B surface antibody
HBsAg	Hepatitis B surface antigen
HCC	Hepatocellular carcinoma
HCV	Hepatitis C Virus
HEAT	Hepatitis Evaluations to Amplify Testing and Treatment
LMIC	Low- and middle- income country
MELD	Model for End-stage Liver Disease
MELD-Na	Model for End-stage Liver Disease-Sodium
MOH	Ministry of Health

NAFLD	Non-alcoholic fatty liver disease
NASH	Non-alcoholic steatohepatitis
NHIS	National Health Insurance Scheme
PCR	Polymerase chain reaction
RNA	Ribonucleic acid
SSA	Sub-Saharan Africa
TACE	Transarterial chemo-embolisation
WHO	World Health Organisation

1 INTRODUCTION

Some of the first published articles on liver disease in Ghana were in the 1950s by authors such as G.M. Edington, who noted that liver enlargement was common in the population and that the number of liver cirrhosis and liver cancer cases were high [1]. As it was then and remains to date, liver disease represents a significant problem not only in Ghana but worldwide [2, 3]. It poses numerous challenges for physicians who manage the condition. Liver disease may be acute or chronic, where chronic liver disease is defined as continued and progressive hepatic injury for more than six months [4]. With long-standing disease and the appearance of specific clinical phenomena, the term end-stage liver disease is used.

End-stage liver disease (ESLD) is a term that comprises advanced cirrhosis, decompensated liver cirrhosis and liver cancer [5]. Liver cirrhosis is the end-stage of progressive liver fibrosis due to continuous insult and injury to the liver from various causes [6, 7]. Cirrhotic patients may have compensated or decompensated states of disease. When a patient has decompensated cirrhosis, there is a history or presence of ascites, jaundice, hepatic encephalopathy or variceal haemorrhage [8]. On the other hand, liver cancer, most commonly primary hepatocellular carcinoma (HCC), is a liver malignancy that arises due to tumour development and growth resulting from related risk factors [9]. It may occur as a result or consequence of cirrhosis in 80-90% of cases or develop de novo (in the absence of cirrhosis) in 10-20% of cases.[9]

Obtaining an accurate picture of the global extent of end-stage liver disease is challenging because of various factors. Firstly, there is a significant overlap between terminologies and diagnoses, leading to the failure to categorise patients accurately. This is true for both incidence and prevalence data, even more so for mortality data [10-12]. Additionally, early stages of disease may go undiagnosed for many years, particularly for hepatocellular carcinoma, especially in settings where active and functional surveillance systems do not exist [11]. Lastly, in resource-limited settings, health-seeking behaviours, alternate therapy such as spiritual or herbal remedies, and a lack of diagnostic capabilities in terms of infrastructural and human resources prevent accurate identification and recording of data for research and public health intervention [3, 13]. Therefore, it is necessary to conduct epidemiological studies on liver cirrhosis and HCC in regions where such data are lacking to better understand disease burden and management challenges, with the goal of developing policies and strategies to reduce disease burden.

2 LITERATURE REVIEW

2.1 EPIDEMIOLOGY

2.1.1 Incidence and Prevalence

The morbidity from ESLD is increasing globally, and the trend is expected to continue in the coming years [10-12]. The global prevalence of cirrhosis is 4.5% - 9.5%. This figure is related to the prevalence of risk factors such as hepatitis B, hepatitis C and alcoholic hepatitis, which vary across different countries and continents [13]. In sub-Saharan Africa, the pooled prevalence of liver cirrhosis in patients with chronic hepatitis B infection is 4.1% in the general population and in primary health centres and 12.7% in tertiary referral centres [14]. In Sweden, where the incidence is among the lowest globally due to a relatively lower burden of chronic viral hepatitis and alcoholic liver disease, the crude annual incidence rate of cirrhosis is 14.1 per 100,000 population [15]. The incidence in West African countries, such as The Gambia, is as high as 30-50 per 100,000 population [3]. The prevalence of decompensated cirrhosis is harder to ascertain but is thought to occur in a quarter of cases of cirrhosis every year [16]. A study in Uganda found the prevalence of decompensation to be 17.6% among inpatients with cirrhosis, whilst another study among European patients found the probability of decompensation to be 23% five years after cirrhosis diagnosis [17, 18].

Approximately 83% of new cases of HCC are from low- and middle-income countries (LMICs), demonstrating the unequal distribution of HCC worldwide [19]. The global age-standardised incidence of HCC is 7.3 cases per 100,000 [20]. GLOBOCAN 2018 data shows that the estimated age-standardised incidence rate of HCC in Africa is 8.4 per 100,000 population, compared with 5.1 per 100,000 in Europe. Within sub-Saharan Africa, the age-standardised incidence rates vary from 4.8 per 100,000 person-years in East Africa to 8.3 per 100,000 person-years in West Africa [21]. The prevalence of HCC is between 4.3 per 100,000 in Latin America and the Caribbean to 10.9 per 100,000 in Asia [22]. Worldwide, HCC was the 5th commonest cancer in males and 9th in females in 2020 [23]. HCC is the second and third most common cancer in sub-Saharan Africa in men and women, respectively [24]. Overall, HCC was the second leading cause of cancer in Ghana in 2020, responsible for 24.9% of new cancer cases. In the same year, it was the commonest cause of cancer in males and the fourth commonest in females [25].

2.1.2 Mortality

Roughly 2 million people die each year because of liver disease. Half of these deaths are due to cirrhosis, and the other half result from liver cancer and viral hepatitis [10]. Globally, the age-standardised mortality varies by world region (**Figure 1**). The age-standardised mortality rate from HCC in Europe in 2018 was 4.4 per 100,000 population, compared with 8.4 per 100,000 in Africa. Ghana has the fourth highest age-standardised mortality rate from HCC compared with other African countries, and 3% of deaths were a result of viral hepatitis when medical causes of mortality were reviewed [22, 26]. The median survival for HCC patients is 10.9 months in Egypt and just 2.5 months in other African countries such as Ghana, Nigeria, Uganda, and Sudan [27]. This poor survival in countries like Ghana is likely related to the

advanced stage at presentation, lack of adequate surveillance of at-risk patients, and lack of targeted therapies however, more studies in this area are warranted.

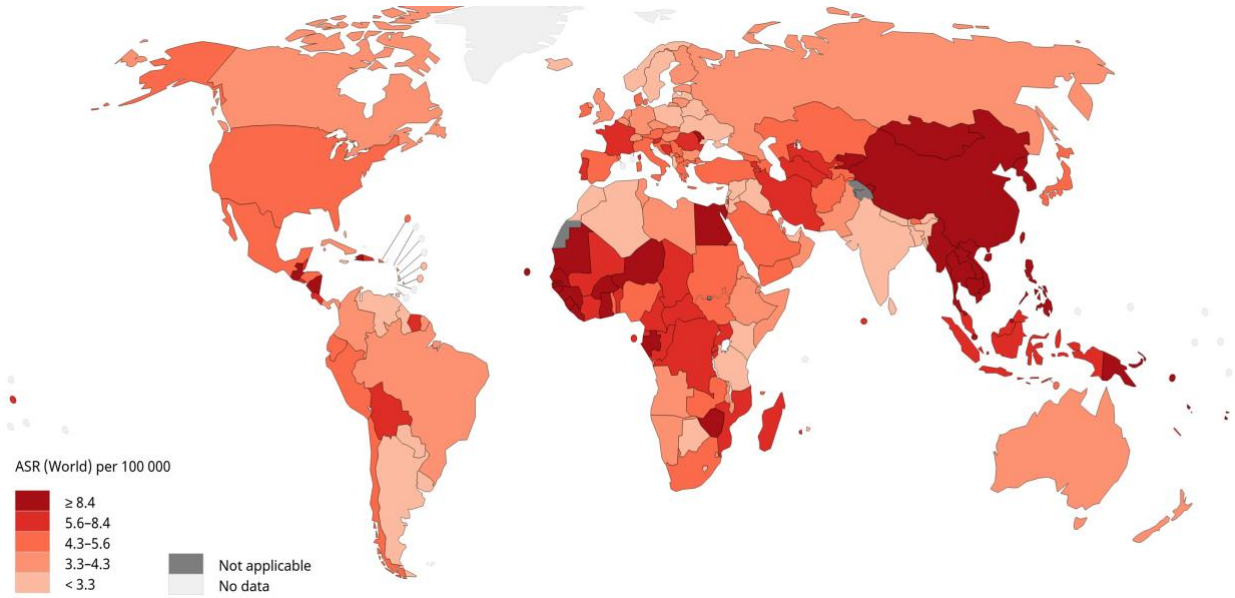

Figure 1. Age-standardised (world) mortality rates of liver cancer for both sexes, in all ages. Source: (http://gco.iarc.fr/today). Reproduced with permission

2.2 PATHOPHYSIOLOGY

2.2.1 Liver Cirrhosis

The hallmark of cirrhosis is the development of hepatic fibrosis, formation of regenerative nodules and distortion of the liver architecture. It is a relatively slow and initially reversible process that may take 10-30 years to develop, depending on the cause of disease [28]. Continued necroinflammation and hepatic stellate cell activation lead to fibrosis, the formation of new blood vessels and loss of the normal liver parenchyma [29]. These architectural changes lead to increased blood flow resistance in the portal circulation, giving rise to an elevation in the portal vein blood pressure (portal hypertension), which contributes to decompensation through complications such as ascites and the development of varices [30]. These changes also lead to impairment in hepatic function, resulting in an inability of the liver to perform synthetic, metabolic, storage or excretory functions. This contributes to the development of jaundice, ascites, hepatic encephalopathy and variceal bleeding [31].

2.2.2 Hepatocellular Carcinoma

The development of HCC is related to the presence of cirrhosis in most cases and stems from the inflammation, fibrosis, and distorted hepatic architecture that results from cirrhosis [32]. Additionally, the risk factors which induce the cascade to fibrosis and cirrhosis alter hepatocytes, leading to dysplasia of these cells and the formation of dysplastic foci and dysplastic nodules [33]. Risk factors play a significant role in hepatocarcinogenesis, and the

presence of co-factors such as smoking or alcohol increases the likelihood of HCC induction [33]. Irrespective of the initial risk factors, the pathogenesis of HCC takes a similar final course, mediated by alterations in key signalling pathways, gene mutations and epigenetic modifications which potentiate tumour development [32].

2.3 AETIOLOGY AND RISK FACTORS OF END-STAGE LIVER DISEASE

The aetiology of ESLD demonstrates geographical variation. This is demonstrated in **Figure 2,** which shows the aetiology of HCC in different world regions based on data from previous studies [27, 34]. These differences are related to socioeconomic, environmental, and genetic factors. There have been limited studies in Ghana to enumerate the proportion of ESLD attributable to known risk factors. Therefore, it is essential to understand these dynamics to develop policies to tackle the disease burden.

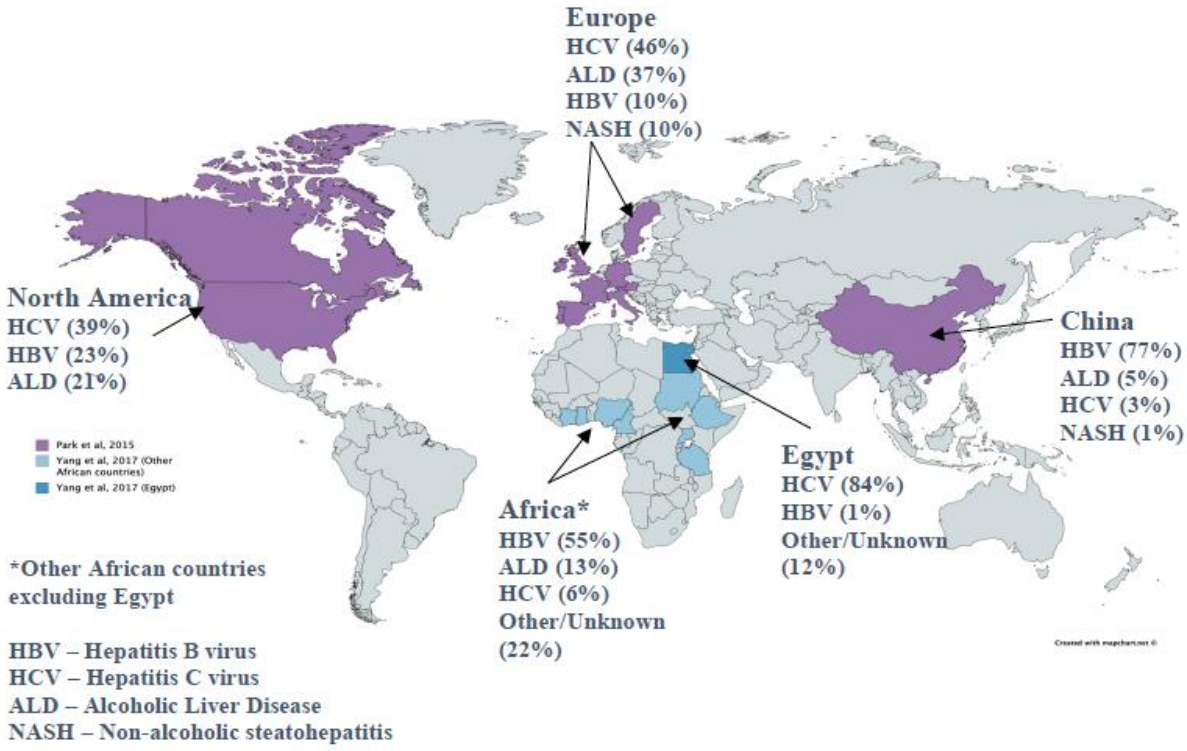

Figure 2. Aetiology of HCC in different world regions, adapted from references [21, 28]. Map created using: https://www.mapchart.net/africa.html.

2.3.1 Chronic viral hepatitis

Hepatitis B virus (HBV) and Hepatitis C virus (HCV) are the predominant risk factors for ESLD due to chronic viral hepatitis [10]. The prevalence of HBV is highest in LMICs and contributes to the high prevalence of ESLD in these regions [35]. Globally, HBV prevalence was 3.5% in 2016, but in Ghana, 8.4 - 12.3% of the population are Hepatitis B surface antigen (HBsAg) positive [36-38]. This high endemicity in Ghana, as in many other countries with a similar burden, is multifactorial. A significant proportion of infections are acquired

through mother to child transmission either perinatally or in early childhood, and 95% of infants who acquire the infection through these means remain chronically infected, thereby putting them at risk of developing ESLD [37]. Although there are existing guidelines on the management of HBV positive pregnant women and their babies, studies have suggested that the knowledge and practice of health workers may be inadequate and that training is critical if the burden of chronic HBV infection is to improve [39-41]. Moreover, birth-dose vaccination of neonates born to HBV positive pregnant women has still not been incorporated into the free vaccination schedules in several sub-Saharan African countries, including Ghana, and this may be another source of chronic HBV infection [42].

HCV infection is responsible for 400,000 deaths each year, mostly due to HCC and cirrhosis. In Europe, the prevalence of infection is 1.8%, whilst parts of the Mediterranean report a prevalence of up to 2.3% [10]. Cirrhosis and HCC progress after 20-40 years of chronic infection [43]. Diagnosis and treatment require measurement of HCV RNA and genotyping. These are costly and difficult for patients in sub-Saharan African countries to afford [3]. The high burden of disease and lack of treatment of infected persons for both HBV and HCV is believed to be related to several factors. These include inadequate screening, difficulty in carrying out diagnostic tests, poor follow-up and inability to afford treatment. Studies in Ghana and other sub-Saharan African countries are needed to identify how these factors contribute to diagnosing and managing ESLD in the region.

2.3.2 Alcohol

Alcohol is a significant risk factor for liver disease and is one of the most common causes of cirrhosis and HCC in Europe and North America [44]. According to the WHO, alcohol consumption is increasing at an alarming rate, and chronic alcohol consumption is reported to cause almost 2 million deaths each year [44, 45]. In Ghana, a qualitative study described that both men and women are increasing the amount of consumption of a local gin known as ‘akpeteshie', which contains an alcohol volume of 40-50% [46]. There is a synergistic relationship between alcohol and other risk factors for ESLD, including chronic viral hepatitis and obesity. Studies have shown that the risk of mortality increases more than two-fold in patients with hepatitis C and that the degree of liver damage doubles in an obese person compared to a non-obese person [12, 47, 48]. The proportion of ESLD attributable to alcoholic liver disease in Ghana is unknown. This knowledge is crucial in framing preventive and treatment policies to reduce the burden of ESLD in Ghana.

2.3.3 Non-alcoholic fatty liver disease

Non-alcoholic fatty liver disease (NAFLD) is the hepatic manifestation of metabolic syndrome. It is believed that soon, it will emerge as one of the leading causes of ESLD worldwide. Its primary risk factors are Diabetes Mellitus and obesity. As the global burden of these conditions rises, it is probable that the prevalence of NAFLD and the development of ESLD will also rise [49]. The worldwide prevalence of NAFLD is almost 25%, and there is geographic variability in this figure, with those in the Middle East and South America most heavily affected [50]. NAFLD can develop into Non-alcoholic steatohepatitis (NASH) and

subsequently cirrhosis. Up to a quarter of patients with NASH develop cirrhosis, which can then lead to HCC. It is also possible for individuals to develop HCC from NASH without liver cirrhosis [51]. Little is known about the disease burden in sub-Saharan Africa. However, the prevalence of obesity and Diabetes mellitus is steadily increasing in the region, in part due to urbanisation and an increase in sedentary lifestyles [52].

2.3.4 Other clinical conditions

Conditions such as haemochromatosis, Wilson's disease, alpha 1 anti-trypsin deficiency and autoimmune hepatitis are also associated with ESLD [28, 29]. The increasing incidence of some of these conditions, such as autoimmune hepatitis, is related to increased awareness and diagnostic techniques [10]. Disease progression to cirrhosis and ESLD can be slowed down or halted by early diagnosis and treatment [10, 29].

2.3.5 Environmental agents

One of the most common environmental agents associated with end-stage liver disease is dietary aflatoxin, a naturally occurring toxic product of the fungi *Aspergillus flavus* and *Aspergillus parasiticus* [53]. Dietary aflatoxins may be found in foods such as maise and peanuts, which have been contaminated and are classed as a human carcinogen by the WHO [54]. In regions of the world such as Europe and North America, exposure to aflatoxin is, on average, 1 ng per kg body weight (ng/kg bw) per day however, in developing countries, especially those in sub-Saharan Africa, the exposure may be as high as 100 ng/kg bw per day [54]. There is a synergistic relationship between aflatoxin and chronic viral hepatitis, especially HBV, in inducing cirrhosis and hepatocarcinogenesis [53]. A study in The Gambia reported a 2.8-fold higher odds of cirrhosis in individuals with greater lifetime consumption of peanuts and a 3.8-fold higher odds of cirrhosis in individuals with a known aflatoxin-associated mutation in their plasma [55]. A systematic review by Liu et al. involving studies conducted in China, Taiwan and sub-Saharan African countries found that the odds ratio of HCC was 6.37 in those exposed to aflatoxin compared to those unexposed and that the combined effect of aflatoxin and HBV exposure accounted for an 11.3-fold higher odds of HCC [56].

2.4 DIAGNOSIS AND SURVEILLANCE

2.4.1 Clinical scoring systems

Clinical scoring systems are used to grade the severity of disease, decide treatment modalities, and predict the survival or risk of mortality of patients. One such system is the Child-Pugh or Child-Turcotte-Pugh score, which determines the severity of liver cirrhosis and predicts mortality [30]. It is based on laboratory tests which include serum albumin, serum total bilirubin and prothrombin time (PT) or international normalised ratio (INR), as well as the degree of hepatic encephalopathy and ascites [57]. Another scoring system used is the Model for End-stage Liver Disease (MELD), which comprises the INR, serum creatinine

and serum bilirubin. One of its primary uses is prioritising patients for liver transplant [58]. Staging of disease is essential for inpatient management, and it is thought that in sub-Saharan Africa, the high morbidity and mortality may be related to the late stage of presentation. In Ghana, few studies describe the stage at presentation or the clinical profile of these patients. Such studies are important because stakeholders are unlikely to become invested in addressing physicians' concerns until there is solid evidence of disease burden.

The Barcelona Clinic Liver Cancer (BCLC) staging system is a commonly used classification scheme for HCC that estimates patients' average survival time based on their stage of disease. It can also be used to decide which type of treatment may be suitable for a particular BCLC stage, for example, liver resection, transplantation, tumour ablation, transcatheter arterial chemoembolisation (TACE) or sorafenib [59]. This staging system has been prospectively validated and classifies patients as having very early (BCLC 0), early (BCLC A), intermediate (BCLC B), advanced (BCLC C), or terminal stage disease (BCLC D) based on the Child-Turcotte-Pugh score, the performance status using the Eastern Cooperative Oncology Group (ECOG) score, and finally the tumour characteristics [59]. In Ghana, one study reported that based on the BCLC stage, only 8% of patients were eligible for liver resection, transplant or tumour ablation [60]. This was a single centre study, limited by insufficient laboratory information such as INR and details on tumour characteristics. Therefore, it is necessary to perform studies that incorporate patients from multiple sites and with as much clinical information as possible to characterise the HCC burden in Ghana adequately, which can subsequently inform surveillance and treatment policies.

2.4.2 Non-invasive markers for cirrhosis and HCC

Traditionally, liver biopsy has been considered the gold standard for diagnosing cirrhosis. This invasive procedure may not be required to make the diagnosis, especially if blood tests and radiology are sufficient and if the procedure poses a significant risk to the patient [61]. Non-invasive tests for cirrhosis include transient elastography, which has been validated but is expensive and not readily available in developing countries [62]. More affordable blood tests such as the aspartate aminotransferase (AST) to platelet ratio index (APRI) as well as the fibrosis-4 (Fib-4) index also exist and can be used to assess the presence of cirrhosis in patients with viral hepatitis in order to decide when to initiate antiviral treatment [61]. The APRI score has been validated for both fibrosis and cirrhosis however, the validation involved only one sub-Saharan African country. Moreover, the cut-off score for APRI was based on the premise that other tests, such as HBV DNA, would be available to identify patients requiring HBV treatment [62]. It is necessary to assess the performance of non-invasive markers such as APRI in other populations, particularly in sub-Saharan African countries, because of the potential impact in helping clinicians screen at-risk patients for cirrhosis in order for early identification and treatment.

There are different international guidelines for HCC surveillance programs, and many of these have similar recommendations [63]. 6-monthly ultrasound with or without alpha-fetoprotein (AFP) in individuals with HBV, cirrhosis, a positive HCC family history or age greater than 40 years is the general recommendation for surveillance by the WHO [62]. It has been reported that AFP measurement is not sensitive or specific enough and that alternate

biomarkers such as lens culinaris agglutinin reactive AFP (AFP-L3), des gamma carboxyprothrombin (DCP), alpha-1-fucosidase, or models such as the GALAD scoring algorithm, which combines these biomarkers with gender and age, are better predictors of the presence of HCC [63, 64]. These biomarkers, including AFP, should be validated in Ghana because of the need to develop surveillance programs which are affordable and practical in the local context.

2.5 MANAGEMENT AND SURVIVAL

The components of ESLD management include nutritional care, pharmacological therapy, and invasive or surgical procedures such as orthotopic liver transplantation. Supportive and palliative care are also necessary for patients with terminal stage disease [65]. Management can also be approached by identifying and treating the underlying condition and preventing further complications [30]. Treatment of the underlying cause of decompensated cirrhosis can lead to a regression of hepatic damage and reversal of decompensation [28]. Marcellin et al. reported that antiviral therapy with tenofovir disoproxil fumarate led to histological improvement and regression of liver fibrosis among cirrhosis patients [66]. Preventing complications such as variceal bleeding decreases mortality associated with their presence [30].

Nutritional care among ESLD patients is important because 20-60% of these patients develop malnutrition due to various pathophysiological mechanisms [65]. Guidelines recommend adequate macro and micronutrient intake to reduce the prevalence of malnutrition and associated complications such as ascites and hepatic encephalopathy, but it is unclear whether these guidelines translate into clinical practice in many countries [67, 68]. Assessment of the nutritional care, since it forms a significant component of management, is therefore necessary.

Treatment received by end-stage liver disease patients, particularly those with HCC, varies worldwide. The BRIDGE study demonstrated these differences, which were thought to be related to health system design (e.g. nationalised healthcare versus more privatised healthcare) and resources available (e.g. resource-rich setting versus less resource-rich setting) [34]. The first multi-country, multi-centre study to determine treatment and survival patterns of HCC patients in sub-Saharan Africa demonstrated that almost no patients received treatment specific for HCC and that the median survival was 2.5 months for these patients however there was significant limitation in data collection, retrieval of records and patient follow-up in these countries [27].

2.6 STRUCTURE OF THE HEALTH SECTOR IN GHANA

The Ministry of Health is central to the structure and activities of the health sector in Ghana (**Figure 3**). A large proportion of government health facilities fall under the agency of the Ghana Health Service and include, in hierarchical order from lowest to highest, Community based Health Planning and Services (CHPS), health centres, district hospitals and regional hospitals. Teaching hospitals are autonomous institutions that operate independently of the Ghana Health Service and serve as tertiary referral centres that manage referred patients from

regional hospitals. Furthermore, quasi-government and faith-based institutions support Ghana Health Service facilities and may function at the level of district or regional hospitals, based on the services provided and specialist services available. Patients may finance their healthcare costs through subscription to the National Health Insurance Scheme (NHIS) or out-of-pocket payments. It is important to note that no government-based health facility offers 100% coverage of costs on the NHIS. Patients who have this form of health insurance may still be required to pay for additional costs for tests and treatment that fall outside of NHIS coverage.

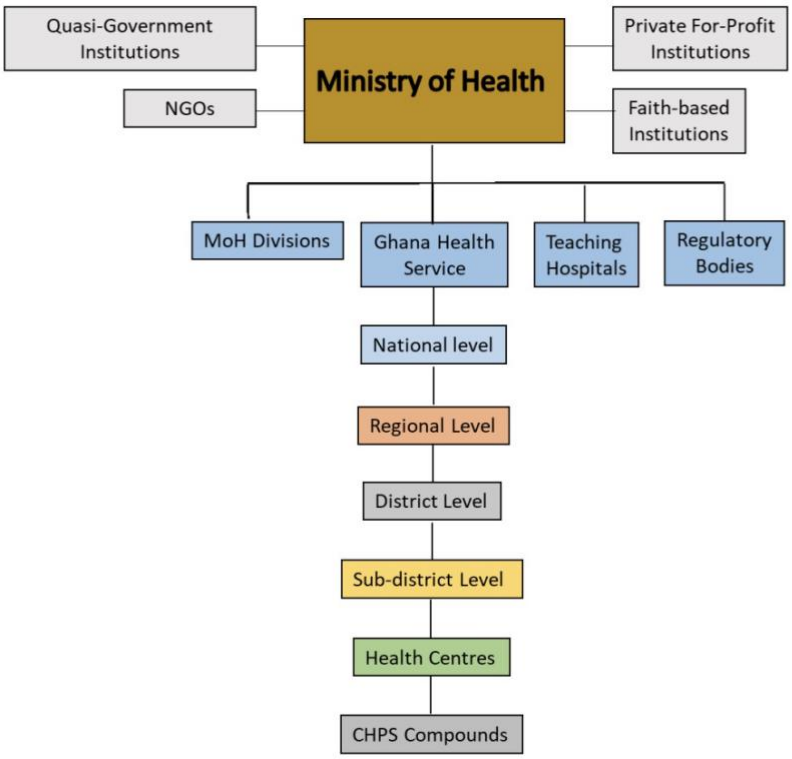

Figure 3. Structure of Health Sector in Ghana. Abbreviations: MOH – Ministry of Health, CHPS – Community Health Planning and Services NGOs – Non-Governmental Organisation. Source: https://www.afro.who.int/

3 RESEARCH AIMS

This thesis aims to improve the understanding of the epidemiology, diagnostic challenges, and management of end-stage liver disease in Ghana.

The specific study aims are:

- To describe clinical characteristics of end-stage liver disease patients in Ghana and assess the performance of aspartate aminotransferase (AST)—platelet ratio index (APRI) and alpha fetoprotein (AFP).
- To investigate the epidemiologic burden and challenges associated with the testing and treatment of hepatitis B infection in Ghana.
- To provide an improved understanding of the nutritional management of cirrhosis patients in Ghana.
- To determine the predictors of mortality in patients admitted with end-stage liver disease in Ghana.

4 MATERIALS AND METHODS

4.1 DATA SOURCES AND STUDY DESIGN

4.1.1 Study Base

Between January 2017 and December 2018, 725 cases and controls were recruited as part of the 'Ghana Gut and Saliva Microbiome in End-stage Liver Disease' study from three teaching hospitals in Ghana, namely the Korle Bu Teaching Hospital, Komfo-Anokye Teaching Hospital, and Cape Coast Teaching Hospital (**Figure 4**). These hospitals were chosen because they are tertiary level centres that hold weekly specialist hepatology and gastroenterology clinics and are the main points of referral for chronic and end-stage liver disease patients from the densely populated southern half of Ghana. Studies I and III of this thesis were derived based on work carried out for this study.

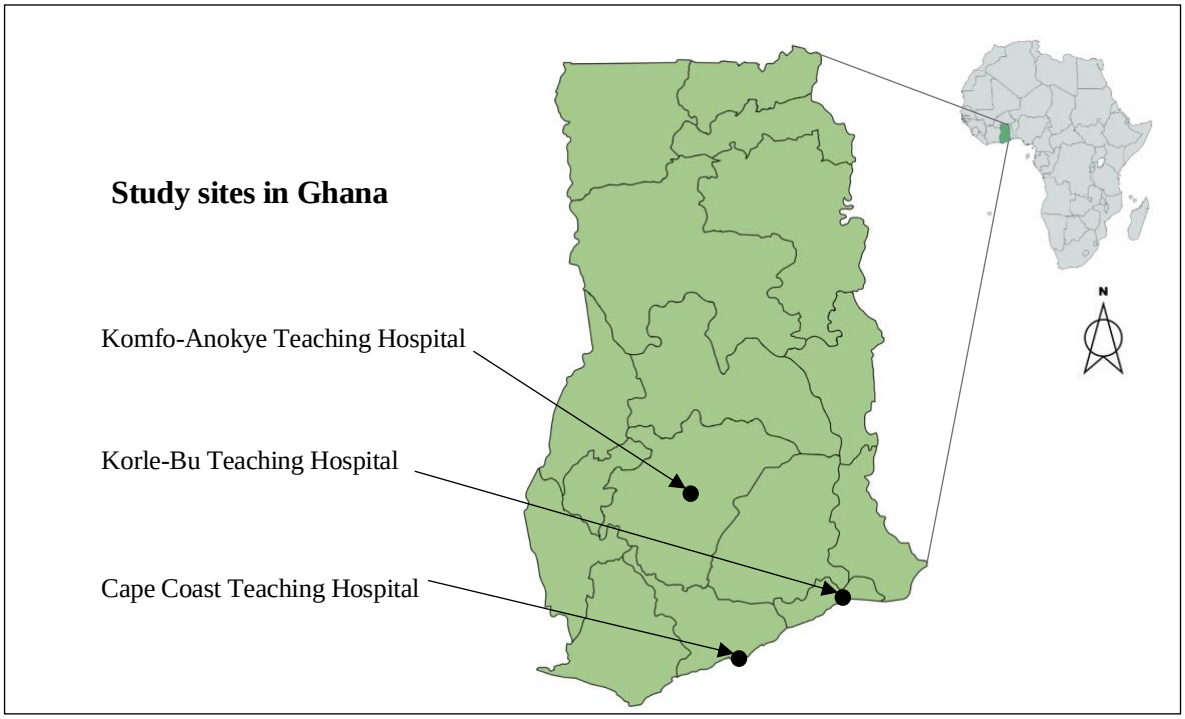

Figure 4. Map of Ghana showing study sites of the 'Ghana Gut and Saliva Microbiome in End-stage Liver Disease' Study. Map created using: https://www.mapchart.net/africa.html.

Between January 2021 and December 2021, the Hepatitis Evaluations to Amplify Testing and Treatment (HEAT) project was conducted in Ghana, where national and hospital-based laboratory, morbidity and mortality registers were reviewed to evaluate the epidemiologic and economic burden of and laboratory testing capacity for, Hepatitis B and C infection in Ghana. For the Ghana HEAT Project, individuals tested for HBV infection between January 2016 and January 2021 in selected facilities in 12 out of 16 administrative regions of Ghana, namely the Upper East, Upper West, Savannah, Northern, Bono, Bono East, Ashanti, Eastern, Western, Central, Greater Accra and Volta regions formed the study base (**Figure 5**). Studies II and IV of the thesis are based on work carried out for the HEAT project. An

overview of the study design and data sources used for each study in this thesis is summarised in Table 1.

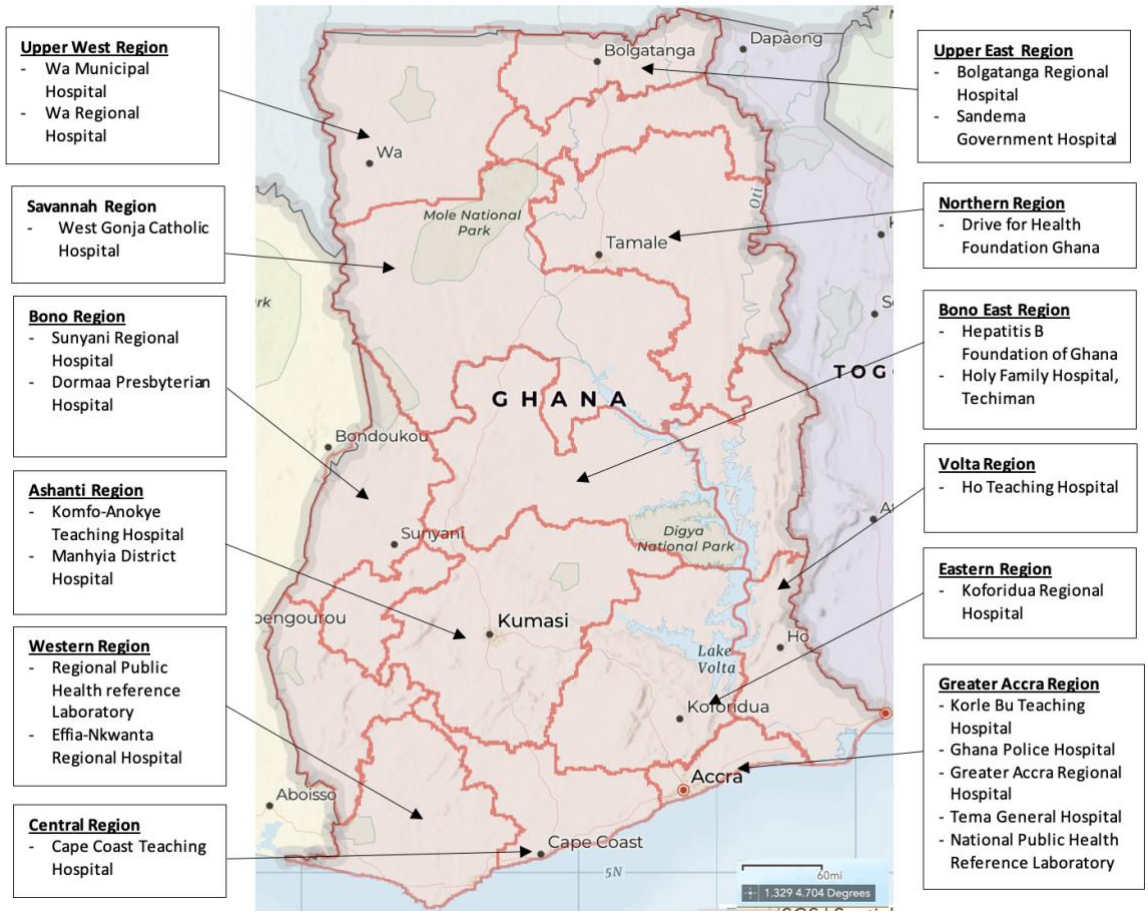

Figure 5. HEAT Project data collection sites. Map adapted from: https://arcg.is/15SS4X.

Table 1. Overview of study design and data sources used for this thesis

	Study Design	Study Population	Data Source
Study I	Cross-sectional study	Ambulatory end-stage liver disease patients	Patient questionnaires and medical records
Study II	Retrospective cross-sectional study	All patients investigated for hepatitis B infection	Hospital-based laboratory, medical and mortality registers
Study III	Qualitative study	Cirrhosis patients on admission and health care workers who manage cirrhosis patients	Patient and healthcare worker interviews
Study IV	Hospital-based cohort study	Cirrhosis and hepatocellular carcinoma patients admitted to referral centres .	Inpatient hospital-based medical records Mortality registers

4.1.2 Case recruitment

Patients referred to gastroenterologists or hepatologists at the Korle Bu, Komfo-Anokye or Cape Coast Teaching Hospital as a case of liver cirrhosis or hepatocellular carcinoma and in whom the diagnosis was subsequently confirmed based on clinical, serological, and radiological evidence were approached for recruitment for studies I and III. Patients who fulfilled the inclusion and exclusion criteria were asked to sign an informed consent form after the details of the study were fully explained by a research nurse and were subsequently enrolled. Participants were compensated for costs incurred for their clinic visits. The inclusion criteria were: 1) Ghanaian; 2) An adult aged above 18 years; and 3) Diagnosed with liver cirrhosis or primary HCC based on clinical, serological, and radiological evidence. Criteria for exclusion from the study were: 1) HIV positive status; 2) Use of any immunomodulatory medication. Participants were also required not to have been taking antibiotics at least one month prior to biological sample collection for fecal microbiome analysis for the 'Ghana Gut and Saliva Microbiome in End-stage Liver Disease' study. Patients recruited who had recently been given antibiotic therapy were called back after one month of being antibiotic-free for biological sample collection.

4.1.3 Control recruitment

Controls were recruited between January 2017 and December 2021. They were siblings of HCC or cirrhosis patients without a diagnosis or history of any cancer or liver cirrhosis, or in the case where siblings were not available, age- and sex-matched well patients with no history or diagnosis of cancer or liver cirrhosis, attending outpatient review.

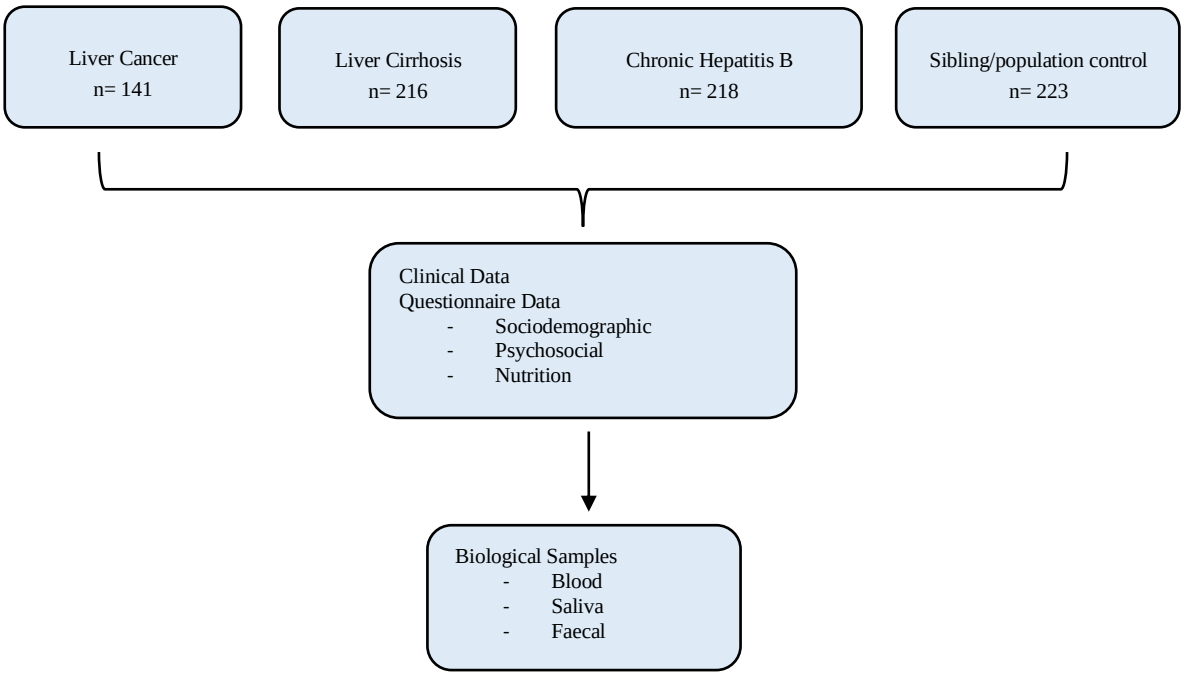

Figure 6. Study algorithm for recruitment of cases and controls in the 'Ghana Gut and Saliva Microbiome in End-stage Liver Disease' study

4.1.4 Hospital-based registers in Ghana

4.1.4.1 Laboratory registers

Paper-based and electronic registers for hospital-based, transfusion medicine, and public health reference laboratories were reviewed for data on patients tested for HBV between January 2016 - January 2021 across 12 out of 16 administrative regions in Ghana for studies II and IV (**Figure 5**). Data on the number and type of diagnostic tests performed, including rapid diagnostic tests (RDT), enzyme-linked immunosorbent assay (ELISA), polymerase chain reaction (PCR) and genotyping, was obtained.

4.1.4.2 Delivery ward registers

Paper-based delivery ward registers for parturient women who gave birth between January 2016 – January 2021 on labour wards of district, regional, faith-based and teaching hospitals were reviewed. Data on the number of parturient women tested for HBsAg at the time of the delivery and the result of the test were abstracted.

4.1.4.3 Morbidity registers and mortality registers

Inpatient and outpatient paper-based and electronic hospital-based registers for patients diagnosed with chronic liver disease, liver cirrhosis and HCC were reviewed. For inpatient records, data on age, sex, risk factors, date of admission, date of discharge or death, investigation results and treatment received were abstracted. Additionally, Medical Certificates of Cause of Death (MCCD) registers were reviewed for data on immediate, intermediate, and underlying conditions leading to death for deaths occurring between January 2018 and December 2021. Finally, the District Health Information Management System – 2 (DHIMS2) database of Ghana, which is a national database where morbidity and mortality data are entered from all Ghana Health Service facilities, was reviewed for crude number of deaths which occurred between January 2018 and December 2021.

4.2 DATA COLLECTION

For study I, the patient's medical records were reviewed, and relevant data related to comorbidities, personal and family history of liver disease or viral hepatitis and BMI were extracted. Additionally, clinical examination to determine the presence of ascites and hepatic encephalopathy were conducted by a clinician and results documented. Previously performed serological testing, including liver biochemistry, serum albumin, serum creatinine and INR, were recorded, and the Child-Pugh grade of the liver disease patient was also determined. Questionnaire data were obtained from participants in the form of an interview with a trained research assistant or physician. These included sociodemographic data on participants' age, gender, marital status, household income and education status.

For studies II and IV, field visits to study sites were conducted by the PhD student to perform data extraction from laboratory, transfusion medicine, delivery ward, morbidity, and mortality registers. Data on age, sex, type of facility, administrative region and year of testing

were extracted from registers reporting HBsAg, HBV serological profile and HBV DNA results. Data on primary immediate, intermediate, and underlying cause of death were extracted from MCCD registers into a study database. Field work lasted for 2-3 days at each study site.

For study III, in-depth interviews were conducted in English or a local dialect among patients with cirrhosis and healthcare workers to determine the experiences and views on the nutritional management of cirrhosis in Ghana. Interviews were conducted by a Ghanaian Masters student from the Department of Global Health, Karolinska Institute. Interviews lasted approximately 30-40 minutes and were recorded with the permission of study participants.

4.3 STATISTICAL ANALYSIS

In **study I**, we used the two-sample t-test and ANOVA to compare differences in means of continuous variables and the Mann-Whitney and Kruskal Wallis tests for differences in medians. The Pearson chi-square test was used for categorical variables. We performed uni- and multivariable logistic regression to investigate the association between clinical variables and outcomes of interest, including diagnosis and disease stage, and to determine sensitivities and specificities. AUROC analysis was used for the assessment of APRI score and AFP. A p-value of <0.05 was considered statistically significant.

Study II described the mean with standard deviation and median with interquartile ranges for patient characteristics. Furthermore, we calculated the proportion of patients testing positive for various HBV tests for various age-groups, sex and administrative region. We obtained pooled estimates by weighting regional data based on sample populations in each region. We used multivariable logistic regression to determine factors associated with positive HBV test results. A p-value of <0.05 was considered statistically significant.

In **study III**, recorded interviews were transcribed and coded into themes and sub-themes. Analysis of data obtained was by content analysis [69].

Study IV described mean with standard deviation and median with interquartile ranges and frequencies (percentages) for patient characteristics, risk factors, investigation results, and disease severity. Differences were examined using the student's t-test for continuous variables and chi-square test for categorical variables. We used multivariable logistic regression to determine predictors of mortality. The Kaplan-Meier method was used for survival analysis, using the log-rank test to assess survival probabilities between groups. A p-value of <0.05 was considered statistically significant.

4.4 ETHICAL CONSIDERATIONS

The ethical considerations in all four studies are imbibed in the tenets of good research ethics, based on the World Medical Association 2013 Declaration of Helsinki [70]. Ethical issues regarding studies included in this thesis were carefully considered, with thoughtful reflection on how to minimise harm and respect the integrity of all individuals included.

Ethical approval for all the studies was obtained from the University of Cape Coast Ethical Review Committee, Korle Bu Teaching Hospital Institutional Review Board, Komfo-Anokye Teaching Hospital Ethical Review Board, and the Ghana Health Service Ethics Review Committee in Ghana and was granted before data collection began.

In studies I and III, where patients were recruited into the study, the major considerations were obtaining informed consent, protecting the confidentiality of all study participants, minimising risk, doing no harm to the patient, respecting autonomy, respecting the right to withdraw at any time, and sharing of research data between different institutions.

Informed consent was obtained from every participant. The study was explained in English or a local language such as Twi, and participants were shown illustrations to facilitate understanding. In some cases, due to cultural norms, patients had to consult relatives before consent could be given. After obtaining verbal consent, participants were asked to sign or thumbprint to document their approval.

The confidentiality of study participants was always ensured. Patients were given study IDs, and questionnaires and biological samples were labelled with these IDs. Only the research personnel had access to the study data. Research personnel kept hard copies of questionnaires under lock and key at the respective institutions. Electronic data entry tools, including Confirmit, designed at the Karolinska Institute, and Medidata Rave, from the Mayo Clinic, were only accessible through password enabled accounts, only accessible by those involved in the studies. Participants were free to withdraw without consequence to their routine medical care. Participants who were called back to complete interviewing received compensation for their transport and time.

In studies II and IV, no new patients were actively recruited, however, review of register-based data and medical records were retrospectively reviewed, which also needed careful ethical consideration. Although we did not need to obtain informed consent due to the nature of the data [71], ethical approval was sought and granted before data collection began. Furthermore, it was still very necessary to ensure confidentiality, limit access to patient information, and store data securely and responsibly. We ensured that data obtained and analysed were de-identified and anonymised so that it could not be traced back to an identifiable individual. Where we asked laboratory or medical staff for clarification of hospital-based procedures, no employee was coerced to provide information or neglect clinical duties to facilitate data collection. Besides obtaining ethical approval, permission from heads of institutions involved in the study was also obtained.

5 MAIN FINDINGS

5.1 Clinical profile of ambulatory end-stage liver disease patients and utility of alpha fetoprotein and APRI score (Study I)

The sociodemographic characteristics and laboratory characteristics of HCC, cirrhosis and chronic HBV cases are shown in **Table 2**. There was a higher male preponderance among ESLD compared with chronic HBV controls, with a male to female ratio of 3.2:1 for HCC and 2.3:1 for cirrhosis groups. The median age at diagnosis was younger for chronic HBV (35 years IQR 28 – 44) compared with ESLD cases. There was no significant difference in the median age at diagnosis of 44 years (IQR 36 – 54) for HCC cases, and 46 years (IQR 37 – 46) for cirrhosis cases (p=0.4), however, patients with HCV-associated HCC had an older age at diagnosis (53 years IQR 47 – 65) compared with patients who had HBV-associated HCC (43 years IQR 36 – 48) (p-value 0.03).

Table 2. Sociodemographic and laboratory information of study participants

	HCC n/N* (%)	**Cirrhosis n/N* (%)**	**Chronic HBV n/N* (%)**
Sociodemographic information			
Sex			
Men	106/139 (76.3)	150/214 (70.1)	125/218 (57.3)
Women	33/139 (23.7)	64/214 (29.9)	92/218 (42.2)
Age at diagnosis, median (IQR)			
Overall	44 (36-54)	46 (37-46)	35 (28-44)
HBV	43 (36-48)	42 (34-50)	-
HCV	53 (47-65)	49 (40.5-59.5)	-
ALD	42 (39-50)	51 (44-60)	-
Monthly household income in Ghana cedis (GHS) with USD ($) equivalent			
<500 ($90)	44/100 (44.0)	91/151 (60.3)	88/165 (53.3)
500-999 ($91-182)	29/100 (29.0)	24/151 (15.9)	39/165 (23.6)
1,000-2,499 ($182-454)	24/100 (24.0)	27/151 (17.9)	32/165 (19.4)
>2500 (>$454)	3/100 (3.0)	9/151 (6.0)	6/165 (3.6)
Laboratory information			
Platelet, mean (SD)	224.9 (150)	141.3 (104)	221.1 (59)
Prothrombin Time INR, mean (SD)	1.3 (0.7)	1.8 (1.3)	-
Albumin (g/L), mean (SD)	35.1 (11.9)	30.8 (9.9)	42.0 (7.0)
APRI score, median (IQR)	1.5 (0.7 – 2.9)	1.4 (0.7 – 3.0)	0.3 (0.3-0.5)
AFP (ng/mL), median (IQR)	528.1 (31.45 – 3149)	4.6 (2.6 – 8.7)	-
HBV DNA (IU/mL), median (IQR)	21615 (8580-122500)	1903.5 (20-76399)	3503 (489-15300)

n/N* The total number of patients was 141 HCC, 216 liver cirrhosis and 218 chronic HBV, however, not all questions were answered by all participants. Missing laboratory data: Platelet count (HCC 36, cirrhosis 50, and chronic HBV 117); INR (HCC 59 and cirrhosis 103); Albumin (HCC 33, cirrhosis 47, and chronic HBV 99); AFP (HCC 80 and cirrhosis 147); HBV DNA (HCC 86, cirrhosis 89, and HBV 132).

1This table is reproduced from Nartey et al. BMC Gastroenterology 2020 [72]

The median APRI score was 0.3 (IQR 0.3-0.5) in chronic HBV cases without cirrhosis, 1.5 (IQR 0.7 – 2.9) in HCC and 1.4 (0.7 – 3.0) in cirrhosis cases **(Table 2).** We found that less than half of study participants with liver cirrhosis or HCC on a background of cirrhosis had an APRI score above the WHO recommended cut-off of 2. At this cut-off, the sensitivity and specificity of APRI score in the diagnosis of cirrhosis among HBV positive patients were 45.4% and 95%, respectively (**Table 3**). A cut-off of >1 yielded sensitivity of 75.9% and specificity of 89%. In the diagnosis of HCC, the sensitivity of AFP at the diagnostic cut-off of 400ng/ml was 52.5%, and the specificity was 98.6% for any-stage HCC. At the same diagnostic cut-off, the sensitivity and specificity were 87.5% and 98.6%, respectively for BCLC stage 0 or stage A disease**.** It was noted that less than half of all ESLD patients could afford AFP testing in their diagnostic workup. After adjusting for age, the odds of having an AFP test performed was higher among higher-earning than lower-earning study participants.

Table 3. Sensitivity and specificity of APRI score in the diagnosis of cirrhosis and AFP in the diagnosis of HCC

APRI cut-off	Sensitivity (%)		Specificity (%)	
0.67	84%		86%	
1	75.9		89	
2	45.4		95	
	Any stage HCC		**Early-stage HCC (BCLC 0 and A) ***	
AFP (ng/ml) cut-off	Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)
7.0	86.9	68.1	100	68.1
29.5	75.4	91.3	87.5	91.3
116.2	57.4	97.1	87.5	97.1
400	52.5	98.6	87.5	98.6

*8 patients with BCLC stage 0 or stage A

2 This table is adapted from Nartey et al. BMC Gastroenterology 2020 [65]

Table 4 shows the common presenting symptoms, staging and risk factors associated with liver cirrhosis and HCC among study participants. Anorexia and abdominal pain or discomfort were more frequently present in HCC patients than those with cirrhosis ($p<0.001$). Weight loss was reported by most study participants in both disease groups (89.8% for HCC and 84.3% for cirrhosis). A greater proportion of ESLD patients presented with Child-Pugh class B or C compared with class A, whilst among HCC cases, only 1.2% and 11.1% were classified as BCLC stage 0 and stage A, respectively. Most HCC patients presented at an advanced stage of disease (BCLC stage C or D), with extrahepatic involvement, which only qualified for best supportive therapy.

Risk factors associated with ESLD were determined based on clinical history, laboratory findings and imaging results, with no liver biopsies performed for any study participants. HBV infection was associated with 69.5% of HCC and 47.2% of cirrhosis cases, whilst HCV was present in 6.4% and 3.7% of HCC and cirrhosis cases, respectively. There was a higher proportion of alcohol-related cirrhosis than alcohol-related HCC (29.2% vs 10.6%: p-value <

0.001). The aetiology of ESLD was undetermined in 15.6% of HCC and 18.5% of cirrhosis patients.

Table 4. Symptoms, classification, and risk factors of ESLD patients at presentation

	HCC n/N* (%)	**Cirrhosis n/N* (%)**	**p-value**
Abdominal pain or discomfort	110/129 (85.2)	122/204 (59.8)	<0.001
Jaundice	38/128 (29.7)	59/204 (28.9)	0.88
Weight loss	115/128 (89.8)	172/204 (84.3)	0.15
Anorexia	67/128 (52.3)	64/205 (31.2)	<0.001
Fever	61/126 (48.4)	85/203 (41.9)	0.25
Ascites (clinically determined)			
None	60/133 (45.1)	57/203 (28.1)	0.001
Mild	24/133 (18.0)	60/203 (29.6)	0.02
Moderate-Severe	49/133 (36.8)	86/203 (42.4)	0.3
Hepatic encephalopathy			
none	118/129 (91.5)	194/202 (96.0)	0.08
Grade 1-2	11/129 (8.5)	8/202 (4.0)	0.08
Child-Pugh Class			
A	12/32 (37.5)	17/83 (20.5)	0.01
B	15/32 (46.8)	35/83 (42.2)	0.9
C	5/32 (15.6%)	31/83 (37.3)	0.01
Risk factors			
HBV	98 (69.5)	102 (47.2)	<0.001
HCV	9 (6.4)	8 (3.7)	0.3
Alcohol	15 (10.6)	63 (29.2)	<0.001
Autoimmune	1 (0.7)	4 (1.9)	0.3
NAFLD	0 (0)	3 (1.4)	0.1
Unknown**	22 (15.6)	40 (18.5)	0.4

* The total number of patients was 141 HCC, 216 liver cirrhosis and 218 chronic HBV however, not all questions were answered by all participants **Unknown after testing for viral hepatitis and without history suggestive of other causes

3 This table is reproduced from Nartey et al. BMC Gastroenterology 2020 [65]

5.2 Hepatitis B in-hospital testing and burden of disease in Ghana (Study II)

Based on findings from study I, in which HBV infection was the leading risk factor associated with ESLD, we evaluated in-hospital testing and burden of disease of HBV in Ghana. We found a high testing volume in teaching hospitals compared with faith-based, regional and district level hospitals. For example, in 2020, of 48,972 HBsAg RDT tests performed in 18 centres, 26,861 (54.85%) were performed in teaching hospitals alone. Additionally, only 2 out of 22 facilities could perform HBV DNA testing. HBV serological profile testing was predominantly RDT based, with 12 out of 22 sites unable to perform ELISA-based testing.

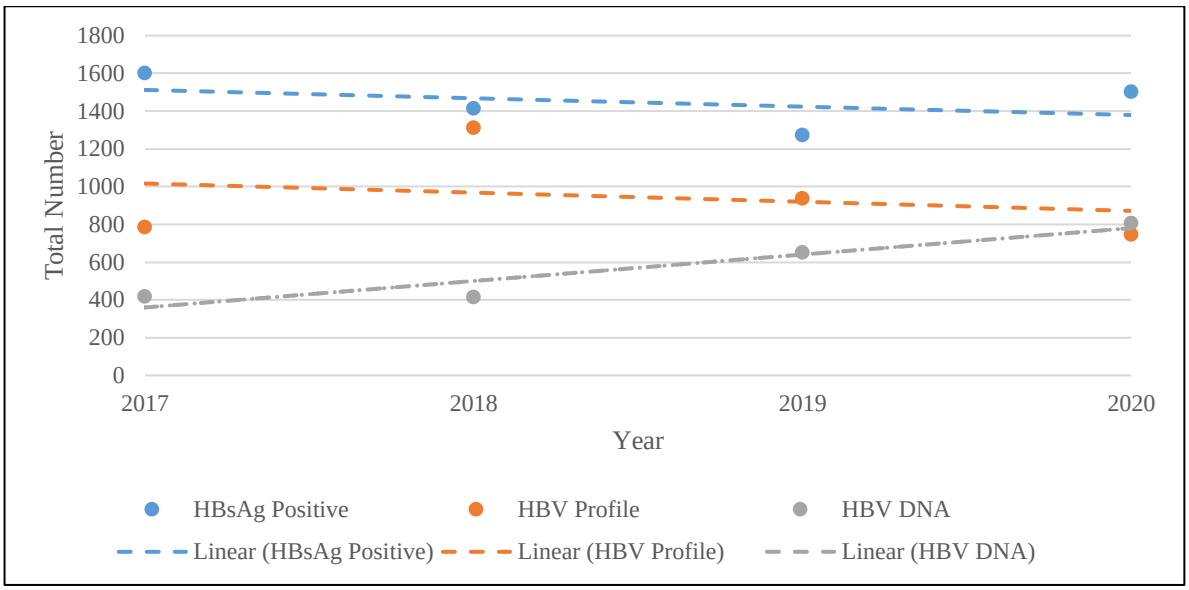

Figure 7. Crude number of HBsAg, HBV serological profile* and HBV DNA tests and their linear trends in two teaching hospitals, 2017-2020 *HBV Serological profile includes Hepatitis B e antigen, Hepatitis B core antibody, Hepatitis B e antibody, Hepatitis B surface antibody. Abbreviations: HBsAg=hepatitis B surface antigen; HBV DNA=hepatitis B virus deoxyribonucleic acid

We reviewed testing volumes of HBV-related investigations at the two largest teaching hospitals between 2017 – 2020. We found a mismatch between crude numbers of HBsAg positive cases compared to the crude number of HBV profiles and the HBV DNA tests performed **(Figure 7).** Although there was an increase in the crude number of HBV DNA tests performed between 2017 to 2020, the value fell short of the total number of people testing HBsAg positive by almost 50%.

In-hospital RDT based testing in laboratory-based registers for 139,966 inpatients and outpatients seen between 2016 – 2020 showed regional differences in HBsAg seroprevalence **(Figure 8)**. Regions with the highest seroprevalence were the Savannah (22.7%), Northern (21.6%), Upper West (18.0%), and Upper East (15.6%) regions. The crude in-hospital HBsAg seroprevalence estimate was 8.48% (95% CI 8.25 – 8.57%), and the pooled estimate was 11.40% (95% CI 10.44 – 12.35). An estimated 6.28% (95% CI 4.73 – 7.84) of patients with chronic HBV infection were e antigen positive. The HBsAg seroprevalence among children under 5 years of age was 1.87% (95% CI 1.07-3.27), and the highest seroprevalence was seen in the 40-49 years age-group. Age was a predictor of a positive HBsAg RDT test (OR 1.005 95% CI 1.002 – 1.007), and females had lower odds of testing positive (OR 0.81 95% CI 0.74 – 0.88).

There was a significant increase in testing numbers among parturient women between 2017 – 2020 **(Figure 9)**. The proportion of women giving birth in hospitals who undertook HBsAg RDT testing increased from 87.2% in 2017 to 94.3% in 2020 ($p<0.001$). Among 83,920 women managed at labour wards between 2016 - 2020, the HBsAg seroprevalence was 6.14% (95% CI 5.97-6.31). The pooled estimate was 6.36% (95% CI 5.70-7.02). Seroprevalence was highest in the 20-29 years (6.18%) and 30-39 years (7.01%) age groups.

There was no available data on the proportion of HBsAg positive parturient women linked to HBV-related services during and after delivery. Furthermore, there was no data on the proportion of HBV exposed babies who received HBIG or birth dose HBV vaccination from registers.

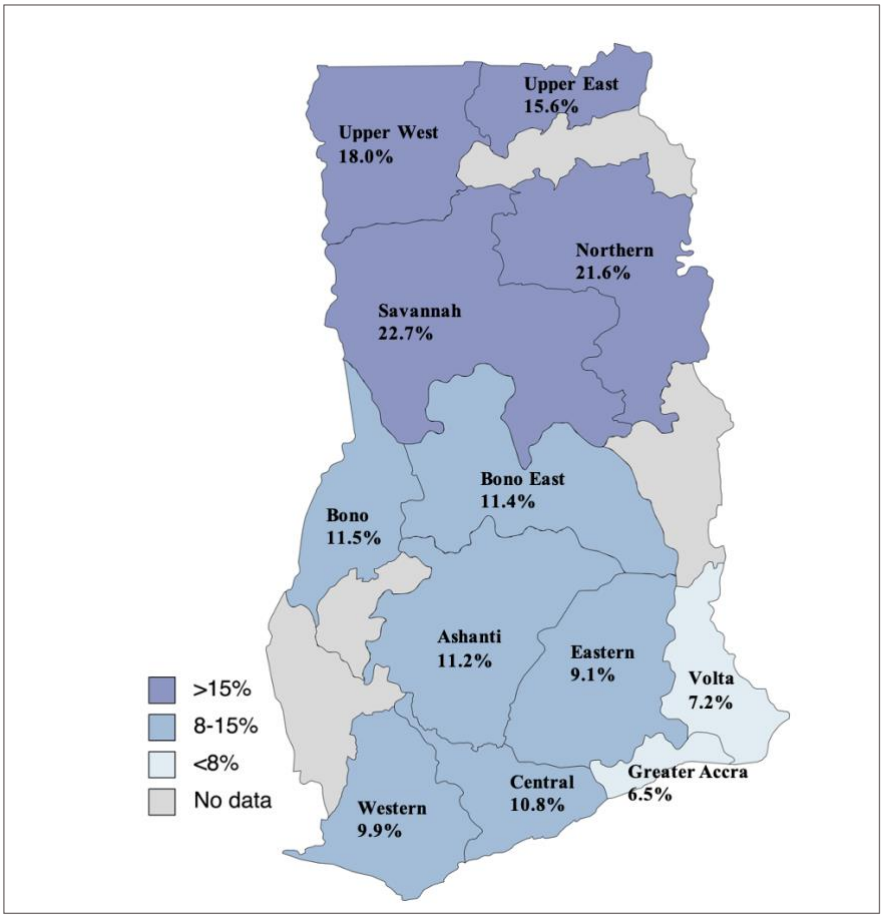

Figure 8. HBsAg seroprevalence based on laboratory-based RDT tests by region, 2016–2020. Map source: https://www.mapchart.net/africa-detailed.html

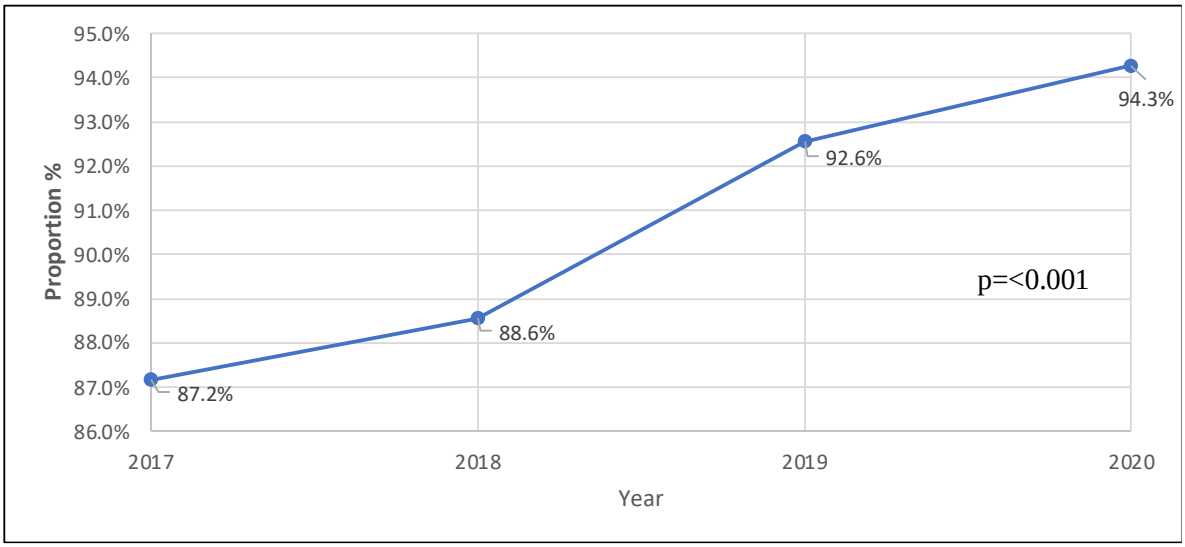

Figure 9. Proportion of pregnant women with hepatitis B surface antigen (HBsAg) test by time of delivery, 2017-2020

5.3 Nutritional management of cirrhosis patients (Study III)

Study I found a high proportion of ESLD patients reporting substantial weight loss at the time of diagnosis and a higher proportion of cirrhosis patients presenting with moderate-severe ascites. Since weight loss and ascites may be related to macro- and micronutrient intake, we assessed the nutritional management of cirrhosis patients qualitatively in Study III.

We found both healthcare workers and cirrhosis patients reporting some nutritional advice and recommendations during hospital visits, however, a lack of formal nutritional assessment and long-term follow-up were of significant concern (**Table 5**). Participants reported that no screening tools for malnutrition or for nutritional assessment were used during nutritional consultations. Additionally, there appeared to be a lack of synchrony between physicians, nurses and dieticians in nutritional management. Recommendations were based on European guidelines. The need for local guidelines was highlighted as crucial to improving the management of these patients.

Table 5. Summarised themes and sub-themes of perceptions and experiences of patients and healthcare workers on nutritional management of liver cirrhosis patients in Ghana

Theme	**Sub-theme**
Nutrition as part of care during hospital visit	Nutritional status assessment
	Referral to a dietitian
Nutritional recommendations	Nutritional advice
	Source of nutritional recommendations
	Expectations about diet advice
Dietary changes and long-term practice involvement	Adherence to nutritional advice
	Expectations about improving dietary care

5.4 Hospital mortality from end-stage liver disease (Study IV)

We evaluated the proportion of liver-related deaths associated with HCC and liver cirrhosis in Ghana and determined the proportional prevalence of risk factors associated with these two conditions by reviewing 1224 MCCD certificates from 11 referral hospitals in Ghana signed between January 2018 and December 2021. We found that among liver-related deaths, 48.8% (95% CI: 46.0 – 51.8), were associated with HBV infection,10.0% with alcohol (95% CI: 8.3 – 11.7) and 7.0% (95% CI: 5.6 – 8.5) with HCV infection (**Figure 10).** There were more HBV-related HCC deaths (69.8%) than HBV-related liver cirrhosis deaths (25.0%) and more alcohol-related cirrhosis (33.3%) than alcohol-related HCC (4.1%) deaths ($p<0.001$). Roughly a third of all-liver related deaths had no associated cause identified in MCCD registers.

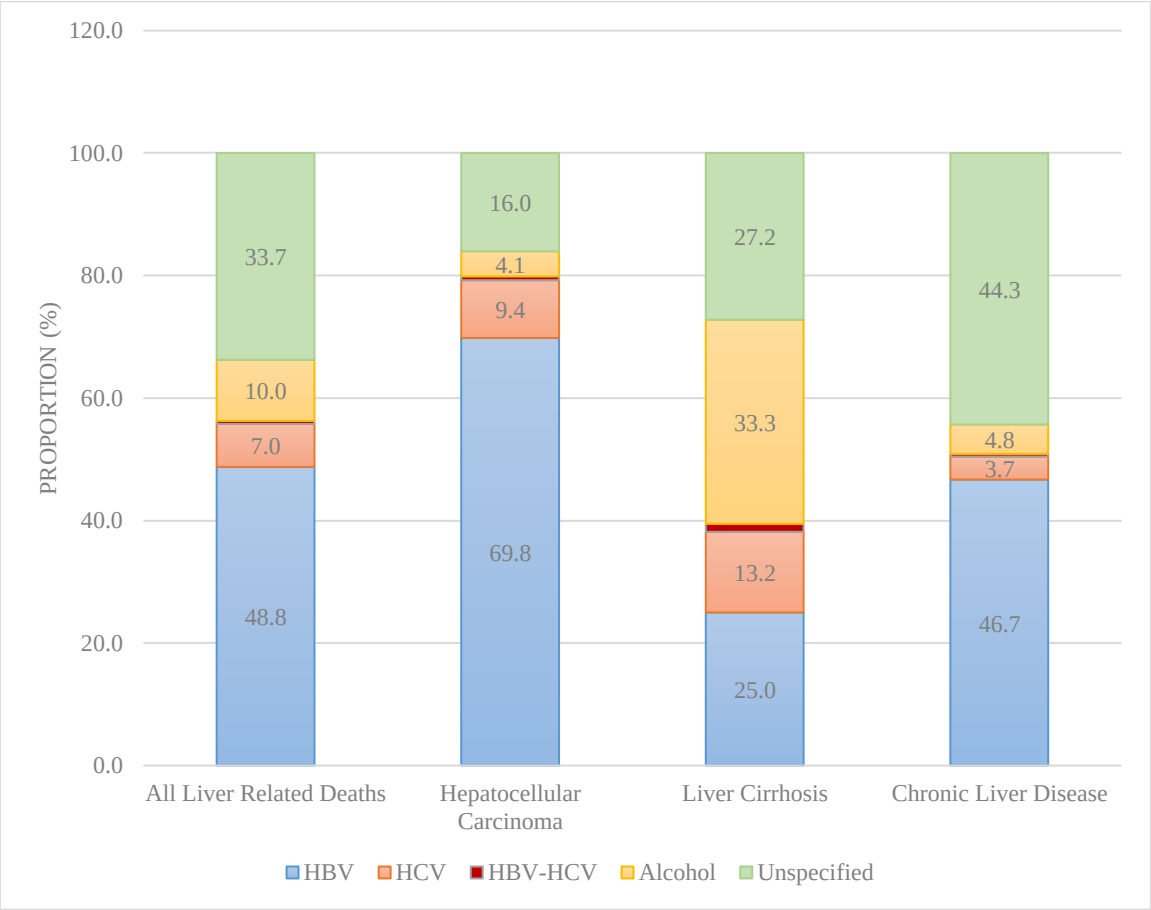

Figure 10. Proportion of risk factors associated with death from hepatocellular carcinoma, liver cirrhosis, and chronic liver disease in 11 referral hospitals in Ghana.

Of 8995 reported deaths between 2018 - 2020 in the Ghana District Health Information Management System-2 (DHIMS2) database from 7 referral centres, 578 deaths (6.4%, 95% CI: 5.9 – 6.9) from all causes in all ages were because of liver-related diseases. In adults aged 18 years and above, 8.8% (95% CI: 8.1 – 9.5) of deaths were liver-related. Specifically, 1.9% (95% CI: 1.5 – 2.2) were due to HCC, 2.1% (95% CI: 1.8 – 2.5) were due to liver cirrhosis, and 4.8% (95% CI: 4.3 – 5.3) were due to unspecified chronic liver disease.

We further assessed the predictors of in-hospital mortality in 172 liver cirrhosis and HCC patients admitted to a tertiary referral centre in a retrospective cohort study. On multivariable analysis, predictors of in-hospital mortality among cirrhosis patients were elevated WBC (OR=1.14, 95% CI: 1.00 – 1.30) and MELD-Na score (adjusted OR=1.24, 95% CI: 1.01 – 1.54), whilst female sex (adjusted OR=3.74 95% CI 1.09 – 12.81) and the presence of hepatic encephalopathy grade 1 (adjusted OR=5.66 95% CI 1.10 – 29.2) were predictors of mortality in HCC patients (**Table 6)**. Survival analysis using the Kaplan-Meir method revealed no difference in in-hospital mortality between HCC and cirrhosis patients (log-rank test p=0.21). We found that patients with higher Child-Pugh and MELD-Na scores had poorer in-hospital survival ($p<0.05$) **(Figures 11 & 12).**

Table 6. Multivariable logistic regression analysis of predictors of inpatient mortality from liver cirrhosis and HCC

Cirrhosis			
	OR	95% CI	P-value
WBC ($X10^9$/L)	1.14	1.00 - 1.30	0.04
INR	0.39	0.11 - 1.36	0.14
Total Bilirubin (mg/dl)	0.99	0.98 - 1.00	0.21
MELD-Na	1.24	1.01 - 1.54	0.04
Hepatic encephalopathy			
Grade 1	2.27	0.59 – 88.31	0.66
Grade 2	9.48	0.52 – 173.43	0.13
Grade 3	1.59	0.17 – 14.87	0.69
Child-Pugh Score			
B	0.28	0.03 – 2.44	0.25
C			
HCC			
Gender (Female)	3.74	1.09 – 12.81	0.04
Hepatic Encephalopathy			
Grade 1	5.66	1.10 – 29.2	0.04
Grade 2	0.71	0.04 – 13.49	0.82

Multivariable model adjusted for age (continuous) gender (male, female), WBC (continuous) MELD-Na (continuous).
Abbreviations: INR, international normalised ratio; WBC, white blood cells; MELD-Na, Model for End-Stage Liver Disease-Sodium

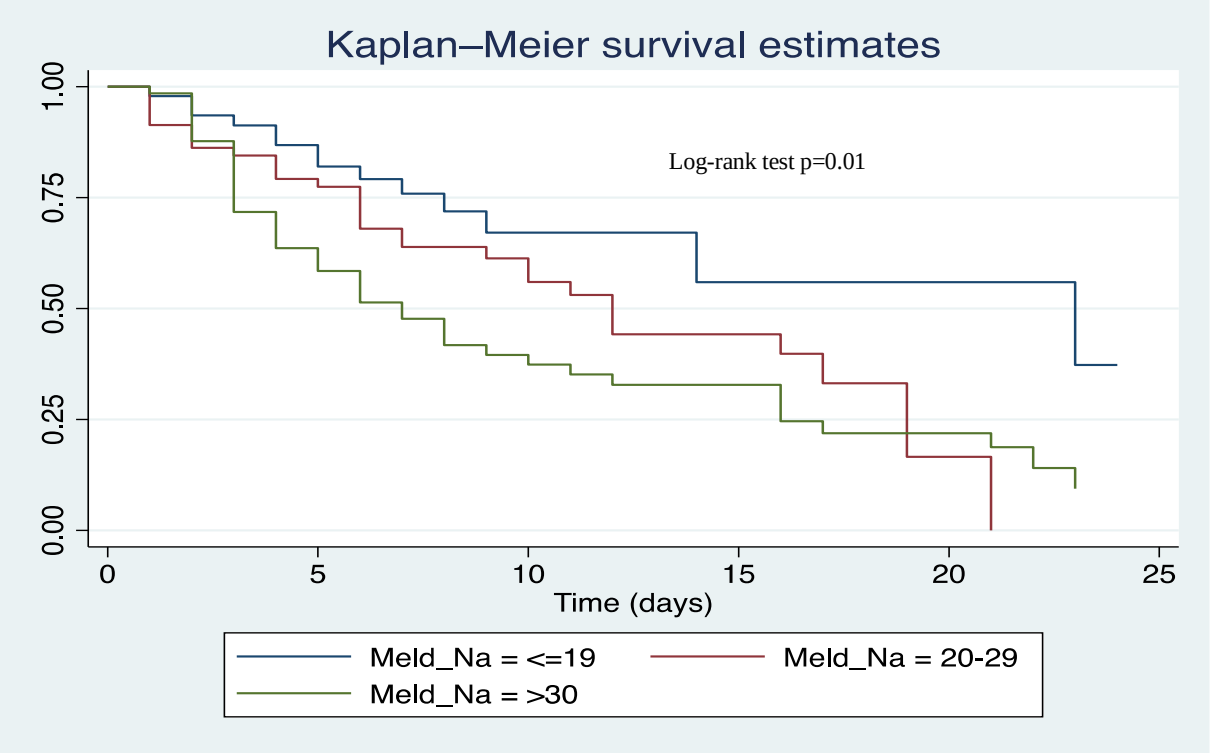

Figure 11. Kaplan-Meier survival curves comparing inpatient survival by MELD-Na score.

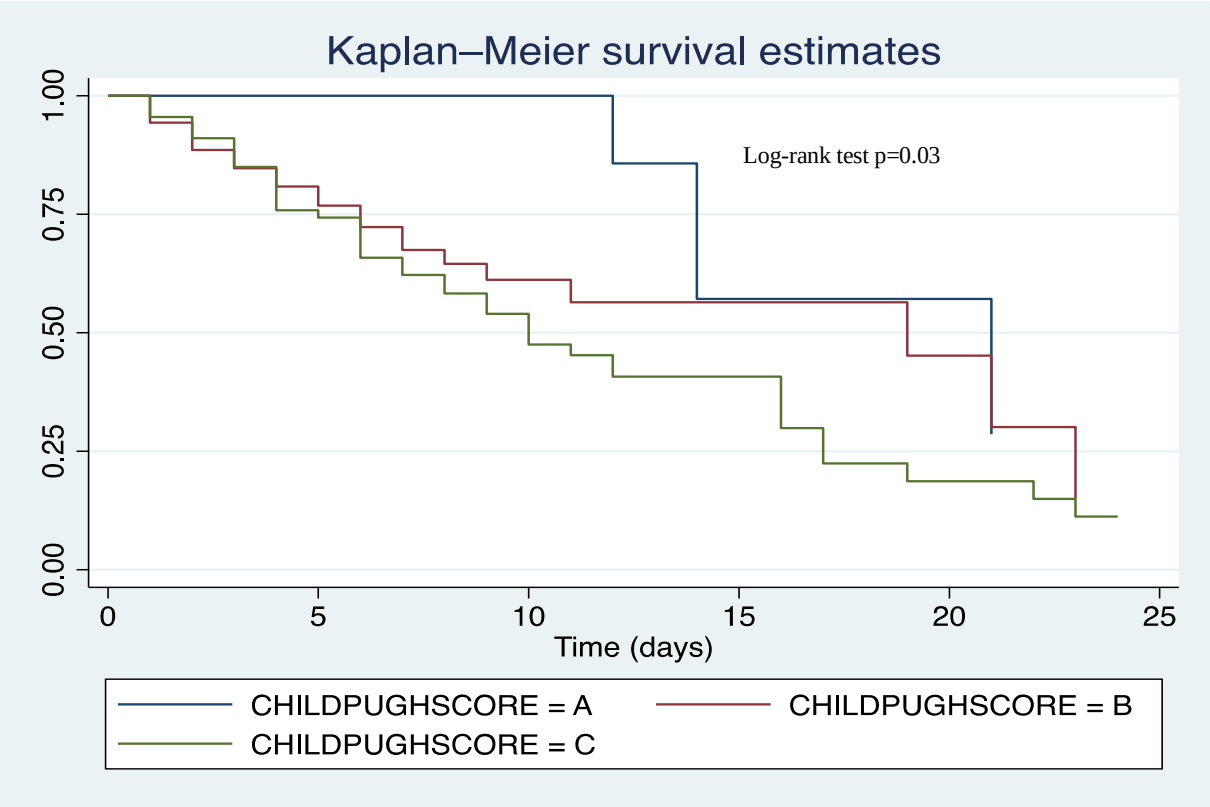

Figure 12. Kaplan-Meier survival curves comparing inpatient survival by Child-Pugh score

6 DISCUSSION

This thesis has described the clinical profile of liver cirrhosis and liver cancer patients commonly presenting to the outpatient departments at multiple referral centres in Ghana and the performance of non-invasive diagnostic markers for liver cirrhosis and liver cancer. Additionally, it has provided insight on the burden of Hepatitis B virus infection, the commonest risk factor for ESLD in Ghana, and has shed light on the nutritional management of and mortality associated with ESLD patients in the country.

6.1 INTERPRETATIONS AND IMPLICATIONS

6.1.1 Profile of Ambulatory ESLD patients and diagnostic utility of APRI-score and AFP

Study I examined the demographic and clinical characteristics of ambulatory liver cirrhosis and liver cancer patients presenting to tertiary referral centres for management. The relatively younger age at diagnosis of 44 years for HCC and 46 years for liver cirrhosis compared with Western countries has been described in several studies [3, 27, 34, 73-75]. There is debate over whether HCC truly occurs at a younger age in sub-Saharan Africans or whether these observations are related to the younger age structure of this population. Evidence to support the young age of onset was demonstrated in a study examining the impact of country of birth on age at HCC diagnosis in the United States by Yang et al., using the Surveillance, Epidemiology, and End Results (SEER) registry. Yang and colleagues found that being born in West Africa had a strong association with early-onset HCC (AOR 16.3; 95% CI, 9.2-27.9 [$p<0.01$]). Similarly, country of birth in South or central (AOR, 11.0; 95% CI, 4.5-23.7 [$p<0.01$]) or East Africa (AOR, 3.5; 95% CI, 1.5-6.8 [$p<0.01$]) was also associated with an earlier age of HCC diagnosis [76]. Factors responsible for the relatively younger age at HCC onset in SSA may include differences in clinical sequelae associated with HBV genotype [77], environmental exposure, including consumption of aflatoxin-contaminated food and chewing khat [78, 79], geographic and dietary differences in the gut microbiome [80], or emerging risk factors such as *Helicobacter pylori* infection which has a high prevalence in the region [81, 82]. The relevance of this finding is that further studies to ascertain attributable factors associated with the younger age at presentation will help develop strategies to reduce ESLD development in these populations.

This study also demonstrated the relatively low sensitivity of the APRI cut-off of 2 in determining the presence of cirrhosis. It showed improved sensitivity to 75.9 with a specificity of 89% when an APRI cut-off of 1 is used and sensitivity and specificity of 84% and 86% when a cut-off of 0.67 is used. The APRI score is a non-invasive method to diagnose liver fibrosis and can help identify patients with HBV who require treatment [62, 83] since alternate methods to determine fibrosis such as liver biopsy and transient elastography are not readily available in many SSA countries [3]. There is concern that applying the WHO recommended APRI threshold of 2 may miss the opportunity to treat patients. Similar studies in SSA have demonstrated low sensitivity of APRI and other non-

invasive tests in diagnosing liver fibrosis and liver cirrhosis [84] [85, 86]. The implications of this are that the WHO recommended threshold may be unsuitable for diagnosing cirrhosis in patients from SSA and may hinder efforts to increase treatment numbers and the overall target of eliminating viral hepatitis. Due to the scarcity of diagnostic tests such as transient elastography in the region, a review of the WHO guidelines may be warranted. Also, in study I, there was good performance of AFP in the diagnosis of HCC, however, frequency of undertaking the test was significantly limited by cost and inability of the patients to afford it. This poses significant challenges in HCC surveillance, therefore developing cheaper alternative, or undertaking studies to identify cheaper biomarkers, may be helpful to improve surveillance in the region.

Finally, this study also highlighted the significant role of viral hepatitis as a risk factor for ESLD in Ghana. HBV was the commonest risk factor in HCC and cirrhosis development, whilst HCV was the third commonest. Although HBV is a vaccine-preventable infection and HCV is curable with direct-acting antiviral (DAA) medication [87, 88], mortality from these infections is increasing globally [89]. Ghana is highly endemic for HBV infection, with a prevalence of 8-12.3% [38, 90] and the prevalence of HCV infection is estimated at 3% [91]. Reducing the burden of viral hepatitis in Ghana is an important strategy in reducing the ESLD burden in the country. However, the country faces similar challenges as others in SSA with failure to adopt birth dose HBV vaccination [42, 92] and high costs of testing and treatment for HBV and HCV [93]. In roughly 15-19% of cases in this ESLD cohort, the associated risk factor was not determined. Limitations in clinical workup, including performing liver biopsy, imaging such as MRI and CT scan and further diagnostic tests, are related to the cost of these tests, as was found in our study, and their lack of widespread availability [2, 3]. Additional risk factors, which are thought to play a significant role but are not routinely measured in clinical workup, such as aflatoxin exposure, are also likely to contribute to the ESLD cases whose risk factors may be classified as unknown or undetermined. Contamination in staple foods such as maise and nuts is common, and aflatoxin levels are found to be higher than the legal limit, with very little regulation of this carcinogen by governments in several SSA countries [94]. Regulation by appropriate agencies is therefore imperative to reduce the cancer risk associated with this and other environmental carcinogens.

6.1.2 Hepatitis B virus in-hospital testing and burden of disease

Study II explored the in-hospital testing patterns and disease burden of HBV infection, the major risk factor for ESLD in Ghana. The findings demonstrated higher testing volumes in teaching hospitals than in lower-level centres, with limited testing capacity for HBV DNA and ELISA in district, regional or faith-based institutions. Decentralisation of testing from larger referral centres or teaching hospitals is necessary to increase testing access and facilitate the identification of HBV positive individuals [2]. Furthermore, it is important to develop point of care and qualitative tests to aid in faster diagnosis instead of centralised tests (as was found in our study), which are slower to conduct and more expensive [95]. The

mismatch between total number of HBsAg positive test results and the total number of viral serological profile and HBV DNA tests being conducted within the same institution highlighted the testing gap that exists in the diagnosis of chronic HBV infection and the limitation in determining which patients qualify for treatment due to insufficient workup.

Although the study found that an increasing proportion of pregnant women in Ghana seen in health facilities are being screened for HBV infection, the proportion of these women linked to HBV associated and PMTCT care services, including tenofovir prophylaxis and HBIG and birth dose vaccination in HBV-exposed babies could not be obtained. However, a pilot study in the Eastern region of Ghana found that only 6% and 1% of women received HBeAg and HBV DNA testing, respectively, following an HBsAg positive test [96]. Since mother-to-child transmission remains a significant source of chronic HBV infection in SSA [24], the implications of inadequate PMTCT services are enormous, therefore, adequate linkage care and management are crucial in Ghana to reduce the incidence of chronic HBV and its sequelae of liver cirrhosis and HCC. It must also be considered that approximately one-fifth of women in Ghana deliver at home [97] due to socio-cultural factors and barriers such as the cost of facility-based delivery and long-distance to health centres or hospitals [98]. It is uncertain what proportion of these women receive antenatal care prior to delivery, and highly probable that babies born to HBV positive women in this group will not receive HBV birth dose vaccination or HBIG within 12 hours of delivery, as is currently recommended [99]. The efforts to reduce PMTCT in Ghana must move beyond in-hospital care to community-based education on the prevention of HBV infection, PMTCT and the importance and advantages of delivery in a healthcare institution or by a trained healthcare worker.

This study further demonstrated regional differences in the HBsAg seroprevalence of individuals tested in hospitals. There was a higher seroprevalence in regions in Northern Ghana compared with those in the South. This may be associated with higher rates of home deliveries in Northern Ghana, a more rural and less educated populace, and higher rates of poverty in the region [97, 100]. Policies by the Ghanaian government and Ministry of Health will therefore need to address these disparities if the country is to achieve HBV elimination.

6.1.3 Nutritional management of cirrhosis patients

Weight loss is one of the commonest symptoms at first clinical presentation for patients with ESLD in West Africa [3]. Poor nutritional intake and development of malnutrition may be related to factors such as early satiety when the patient has ascites, anorexia, impaired absorption due to portal gastropathy and alcohol-related aetiology of liver disease [101]. Furthermore, malnutrition is a predictor of adverse outcomes in cirrhosis patients, including increased infections and hepatic encephalopathy [102]. In low resource settings where ESLD treatment is primarily supportive and not curative [2, 3, 60], appropriate nutritional advice and prevention and treatment of malnutrition are an important part of non-pharmacological therapy.

In this qualitative assessment, we described, for the first time, the experiences of patients and healthcare workers on the nutritional management of liver cirrhosis in Ghana. Although both patients and healthcare workers recognised the importance of dietary intake, there were challenges related to lack of human and logistical resources, including local guidelines, for adequate nutritional assessment and recommendations. EASL guidelines recommend screening for malnutrition, detailed nutritional assessment and global assessment tools in cirrhosis [68]. Currently, there are no studies assessing the nutritional status of cirrhosis patients in Ghana and these are warranted. Furthermore, local guidelines or consensus statements from the Ghana Association for the Study of Liver Diseases or the Ghana Academy of Nutrition and Dietetics are important to guide clinicians in assessing and managing ESLD patients. Local recommendations and guidelines are necessary because muscle mass, sarcopenia, rates of physical activity and diets vary among ethnicities and populations [103, 104]. Malnutrition should also be identified and managed because its presence may be associated with complications including ascites, hepatic encephalopathy and sepsis [105], which may lead to increased mortality in ESLD patients.

6.1.4 Mortality burden from End-stage liver disease

In study IV, where we reviewed records from Medical Certification of Cause of Death registers in Ghana, we found that 6.4% of reported deaths across all ages between 2018-2020 were due to liver-related causes and that in adults aged 18 years and above, liver diseases accounted for 8.8% of deaths. Specifically, HCC was associated with 1.5% of deaths in all ages, whilst cirrhosis was associated with 1.6%. To our knowledge, this study is the first in Ghana to report deaths from liver-related causes in an observational study. Previous estimates have relied on modelling data, which suggest that HCC accounts for 1.5% of total deaths [25, 106] and therefore appears to be consistent with our findings. However, it must be noted that close to 5% of deaths were associated with unspecified chronic liver disease. Since the definition of CLD encompasses a range of diagnoses, including viral hepatitis, liver cirrhosis and liver cancer [107], it is probable that many more deaths from HCC and cirrhosis occur in Ghana but are not reported as such.

This study also highlights the enormous burden of viral hepatitis-related HCC in Ghana, with almost 80% of reported HCC deaths (69.8% from HBV and 9.4% from HCV infection) due to these risk factors alone. Cirrhosis deaths were also commonly associated with HBV (25.0%), HCV (13.2%) and alcohol (33.3%). The high proportion of HBV and HCV-related deaths from ESLD are multifactorial but notably include the limited access to treatment for viral hepatitis in Ghana. A study in 2020 in Ghana found that the majority of patients admitted to and managed on the medical ward of a large teaching hospital for liver cirrhosis had not previously been prescribed any antiviral therapy for HBV or HCV [108]. Furthermore, surveillance systems for detecting fibrosis, cirrhosis or HCC are not commonplace in Ghana. A study of patients admitted to medical wards in the largest teaching hospital in Ghana demonstrated that none of those admitted for management of HCC was identified through surveillance [109]. The advanced stage of presentation of ESLD in Ghana

and SSA at large, which precludes the already limited curative services available, and the lack of transplant hepatology services, contribute to the mortality associated with these cases [3, 27, 60, 110].

In this study, predictors of poor in-hospital survival included elevated WBC, MELD-Na score, Child-Pugh score, and hepatic encephalopathy. Similar clinical factors have been reported in studies in Africa [111-114] and suggest poorer outcomes in patients with advanced liver disease. Treatable causes associated with hepatic decompensation, including management of sepsis and malnutrition, are important to prolong life and must be routinely evaluated in patients presenting for ESLD care.

6.2 METHODOLOGICAL CONSIDERATIONS

6.2.1 Study design

In study I, we performed a cross-sectional study that included a manual chart review to determine the demographic and clinical characteristics of ambulatory ESLD patients in Ghana. In the study, we described risk factors associated with ESLD development. Since this was a cross-sectional study, it was not possible to determine a temporal link between exposures such as viral hepatitis or alcohol use and the outcome of interest [115]. Other methodologies, such as a cohort study where we could follow research participants longitudinally or a case-control design which would be useful if the outcome of interest were rare, could be used. It takes 10-20 years for ESLD to develop, and this would be cost and resource-intensive in Ghana and, therefore, impractical. Furthermore, since we do not have large patient or population registers that chart sociodemographic and health-related data over time, it would not be possible to conduct a register-based study.

In study II we performed a hospital and laboratory-based register study to determine the in-hospital seroprevalence of HBsAg in Ghana. This study involved manually reviewing HBsAg test results for over 300,000 individuals across multiple health facilities at different levels of healthcare. This is the first of such a large observational study in Ghana, and the methodology was carefully considered. The country was zoned into 3 regions (upper, middle, and lower) to obtain a nationally representative picture of participants accessing healthcare. Since almost all records were paper based and data abstraction was done manually, human error could have occurred in some entries. Electronic registries may have reduced the possibility of these errors, however these are not readily available in Ghana.

In study III, a qualitative study was performed. Qualitative studies are helpful in exploring and better understanding the views and experiences of study participants [116]. One important question to address is the determination of sample size in qualitative studies. In this study, theoretical saturation was used to determine sample size, in which participants were consecutively interviewed until no new information was obtained [117]. This choice of study design was important to fully understand the barriers, challenges and practices associated

with the nutritional care of cirrhosis patients. In this study, data were collected through in-depth interviews. An alternative method could have been focused group discussions with patients and healthcare workers, however, focused group discussions are at times difficult to control. Additionally, not everyone in the group may participate [118]. Finally, due to the patient and healthcare worker dynamic, participants may not have felt comfortable expressing their opinions or perceptions in the presence of colleagues or carers because they might be worried about how these views may be perceived.

Study IV assessed factors associated with in-hospital mortality in a retrospective cohort design. The medical records of patients admitted to a tertiary referral centre in Ghana were reviewed from day of admission until death or discharge. An alternative method could have been to collect this data prospectively, and this may have limited challenges such as missing or incomplete data in medical records. Using a retrospective cohort design, although cheaper and faster, has the disadvantage of losing out on certain information, including confounders of interest, if these are not captured routinely in medical records [119]. An advantage of this study design was that the temporal sequence of risk factors and outcomes (death or discharge) could be assessed.

6.2.2 Confounding

Confounding is defined as a variable that is associated with both the exposure and outcome and is not an intermediate in the causal pathway that exists between the exposure and the outcome [120]. Controlling for confounding can begin at the study design stage, through randomisation, restriction or matching [121], or it can be during the analysis stage through, for example, stratification or the use of multivariate models. To control for confounding in studies I, II and IV, logistic regression was used to obtain adjusted odds ratios. Confounders adjusted for in study I included age, sex, and level of education in the assessment of factors associated with failure to undertake AFP and HBV DNA testing. In study IV, covariates included in the multivariable model to obtain an adjusted odds ratio included age, sex, type of risk factor (e.g., HBV and HCV status, alcohol use, herbal medication use, and laboratory parameters such as haemoglobin, platelet, and albumin level).

6.2.3 Information bias (misclassification)

In this thesis, cases of ESLD were diagnosed based on clinical history and examination and laboratory and abdominal ultrasound findings. Only a small proportion of patients undertook abdominal CT scans due to the cost of this investigation, and no patient undertook liver biopsy for histopathological confirmation of fibrosis, cirrhosis, or HCC. Misclassification of the outcome is, therefore, a possibility and may lead to information bias. This could have occurred in the diagnosis of very early stage (BCLC 0) or early-stage (BCLC A) HCC, where tumour sizes are less than 2cm or 3cm, respectively, but is unlikely to have occurred in the diagnosis of intermediate or advanced stage HCC. This is because the sensitivity of abdominal USG in the diagnosis of any-stage HCC is 84 -93% but is reduced to between 47 -

64% in early-stage disease [122, 123]. In these studies, misclassification of the outcome is likely to be non-differential, which may have biased ratios towards the null [124].

The possibility of interviewer bias in the data collection, mainly because of the qualitative methodology used in study III, was also carefully considered. Interviewer bias is a systematic error that results from the interviewer's subconscious influence in data collection [125]. The interviewer's ideas or expectations could affect how questions are phrased and follow-up questioning. It could also lead to social desirability bias, where the respondent may answer questions based on what they perceive the interviewer wants to hear. With these considerations, the choice of interview for the study was important. A master's degree student who had no affiliation with any of the study sites and no influence as a healthcare provider to patients or colleague to healthcare workers in the study was selected to minimise the possibility of interviewer bias since the PhD student was either a colleague or healthcare provider to respondents. Furthermore, the questionnaire was standardised, and a pilot study was undertaken prior to data collection.

6.2.4 Selection Bias

Selection bias occurs in relation to how study participants are selected [125]. In studies I and II, our source population was end-stage liver disease patients. Since ESLD patients with advanced disease are more likely to have hepatic encephalopathy, poorer performance status, and therefore the inability to consent or provide sufficient information for a study, some selection bias could have been introduced in that patients with less severe disease were included, whilst those with more advanced disease were not. Whilst this may have been possible, it is worth noting that in our analysis, most cases were moderate to severe stage cirrhosis or HCC, as measured by higher Child-Pugh and BCLC disease stages, so selection bias may not have been a significant source of bias in our studies.

6.2.5 Missing data

Missing data was noted in studies I, II and IV. In studies I and IV, not all patients could afford to undertake the required tests, therefore, some laboratory investigations required to calculate clinical staging were missing. In study II, age and sex data were missing for some individuals tested for HBsAg. Missing data were determined as missing completely at random because the missing data was unrelated to the outcome of interest. We omitted entries with missing data in the analyses and therefore performed available case analysis.

6.2.6 Generalizability

Generalizability is explained as the extent to which the findings of a study can be applied in 'real world' or other settings [126]. In study I, we found that there is a relatively younger age at diagnosis of ESLD, particularly HCC, among the Ghanaian patients, with a higher male to female ratio. Furthermore, we found the low sensitivity of the APRI score at the threshold of 2. In study II, we found a high burden of in-hospital HBV seropositivity and limited follow-up testing to determine which patients fit treatment criteria. These findings can be generalised

to countries with a high burden of HBV-associated ESLD, with similar healthcare systems and infrastructure, such as other West African countries like Nigeria, Sierra Leone, and The Gambia. Furthermore, the outlook of liver-related mortality, including the prevalence of associated risk factors and predictors of in-hospital mortality, may be generalisable to low resource settings and settings where patients present with advanced ESLD where curative treatment is not a frequent option.

7 CONCLUSIONS

- There is a young age at onset and a higher male to female ratio among ambulatory end-stage liver disease patients in Ghana, and patients often present with an advanced stage of disease. (Study I)

- The performance of the APRI score in the detection of cirrhosis at the current threshold of 2 may miss a significant proportion of patients who require treatment, and a revision of the cut-off to a lower threshold may present the opportunity to identify and treat more patients at-risk of ESLD. (Study I)

- There is a high HBsAg positive seroprevalence rate in patients presenting to health facilities in Ghana, with limited access to follow-up testing such as HBV ELISA and DNA testing outside of large teaching hospitals. (Study II)

- Nutritional management of cirrhosis in Ghana requires local assessment tools and guidelines to improve care. (Study III)

- Liver related mortality is predominantly associated with viral hepatitis, including HBV and HCV infection in Ghana. In-hospital mortality is associated with causes of elevated white count (such as sepsis), hepatic encephalopathy, and advanced disease, including increased MELD-Na and Child-Pugh scores. (Study IV)

8 FUTURE PERSPECTIVES

In recent decades, the incidence of end-stage liver disease, primarily hepatocellular carcinoma, has been increasing [127]. In the world's poorest regions, such as countries in sub-Saharan Africa, the most significant risk factors for ESLD remain hepatitis B and hepatitis C virus infection [20, 128]. The WHO has set out elimination targets for viral hepatitis by 2030. If achieved, this is likely to reduce the morbidity and mortality associated with liver cirrhosis and liver cancer in areas with a high disease burden. Elimination efforts require strategies such as increased testing and treatment and preventing mother-to-child transmission. Furthermore, reducing the mortality from ESLD requires early detection through effective surveillance systems and treatment through increased access and availability of antiviral medication such as tenofovir for HBV and pan-genotypic directly acting antiviral medication for HCV.

The studies in this thesis highlight the situation regarding ESLD risk factors, diagnosis, management, and mortality in an LMIC with similar health infrastructure to many sub-Saharan African countries with a high burden of ESLD

In study I, there was a significant association between HBV and HCV to ESLD in Ghanaian patients, and the utility of the APRI score and AFP were also demonstrated. Efforts to reduce the incidence of viral hepatitis in Ghana include the institution of birth dose vaccination of HBV, which is yet to be implemented in the country, and adequate linkage to care for HBV and HCV infected persons. Additionally, free or subsidised treatment for viral hepatitis is necessary to manage patients to reduce the incidence of ESLD. In study I, there was also a significant proportion of cases in whom associated risk factors could not be determined., and this should be an avenue of further research in Ghana. Specifically, emerging risk factors such as Helicobacter pylori infection and the alterations in the gut microbiome should be investigated, and the contribution of known risk factors such as aflatoxin exposure and NAFLD should be further studied.

Study II, where the in-hospital HBsAg seroprevalence was determined across the country, has important policy implications for HBV elimination in Ghana. Further studies are needed to determine the reasons for the higher seropositivity rate in Northern Ghana. Additionally, access to HBV DNA testing should be expanded, and the cost of testing reduced so that all patients who test positive for HBV can be adequately assessed for treatment. There is also a need to study the proportion of HBV exposed babies who currently receive the birth dose vaccination and HBIG in Ghana.

Studies III and IV highlight how management approaches can be implemented to improve morbidity and mortality associated with ESLD. Further studies on the prevalence of malnutrition could help determine the burden among ESLD patients and identify those who are most at risk. Improved nutritional care, and management of sepsis and hepatic encephalopathy, can potentially reduce morbidity and improve survival. Ultimately, cirrhosis and HCC surveillance are required in those at-risk to identify cases at the early stages of

disease, where curative therapies can be instituted. Curative therapies, including liver transplantation, should be made available in Ghana. A prospective follow-up study examining treatment modalities and survival is warranted to obtain an accurate picture of management and outcomes of these patients in sub-Saharan Africa. Additionally, validation of new models estimating survival, such as the MESIAH score [129], should be conducted in the African setting.

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