GHANA COLLEGE OF PHYSICIANS AND SURGEONS

FACULTY OF INTERNAL MEDICINE

(SUBSPECIALTY - GASTROENTEROLOGY)

NONALCOHOLIC FATTY LIVER DISEASE IN TYPE 2 DIABETES MELLITUS PATIENTS ATTENDING THE DIABETES CLINIC AT THE GREATER ACCRA REGIONAL HOSPITAL

A RESEARCH DISSERTATION SUBMITTED TO THE FACULTY OF INTERNAL MEDICINE IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE CONFERMENT OF A FELLOW OF THE GHANA COLLEGE OF PHYSICIANS (FGCP)

BY

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DECLARATION

I, **Dr Sally Afua Bampoh**, declare that this study and its findings are of my own original research. Where applicable, I have cited, duly acknowledged, and referenced work done by others. I also declare that this dissertation has not been submitted elsewhere for another degree either in whole or in part.

S Bampoh ..... 26/08/2021 .....

Signature Date

CERTIFICATION

We certify that this work was carried out by Dr Sally Afua Bampoh (F2015050874) at the Greater Accra Regional Hospital, Accra under our supervision in fulfilment of her Fellowship programme to be admitted as a Fellow into the Faculty of Internal Medicine. We also supervised the writing of the dissertation.

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DEDICATION

This work is dedicated to all the type 2 diabetes mellitus patients attending the Greater Accra Regional Hospital, especially those who wholeheartedly accepted to partake in this study and to the Late Prof. Jacob Plange-Rhule for encouraging me throughout this fellowship programme.

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ABSTRACT

Background: Nonalcoholic fatty liver disease is currently the leading cause of chronic liver disease and the second leading indication for liver transplantation in developed countries. It may be complicated by cirrhosis and hepatocellular carcinoma. The pathogenesis of primary nonalcoholic fatty liver disease involves insulin resistance, which also occurs in type 2 diabetes mellitus. The prevalence of nonalcoholic fatty liver disease is increasing worldwide. However, published data on this condition in Ghana is scarce.

General Objective: To determine the prevalence of, and factors associated with nonalcoholic fatty liver disease in type 2 diabetes mellitus patients attending the diabetes clinic at the Greater Accra Regional Hospital in Accra, Ghana.

Methodology: This study was a cross-sectional, hospital-based study conducted at the Greater Accra Regional Hospital from May to October 2018. Two hundred and thirty-five type 2 diabetes mellitus patients with insignificant alcohol history and negative screens for hepatitis B and C infection were recruited using systematic random sampling, after giving informed consent. Socio-demographic and clinical data were obtained using a semi-structured questionnaire. Anthropometric measurements, general and abdominal examinations were performed for each participant. Laboratory investigations included full blood count, fasting blood sugar, glycated haemoglobin, lipids and liver biochemistry. Abdominal ultrasonography was performed to assess for liver size and echogenicity and to measure the abdominal subcutaneous fat thickness. Non-invasive scoring systems were used to predict the presence of nonalcoholic steatohepatitis and fibrosis. Captured data was imputed and analysed using SPSS 23.0.

Results: The prevalence of nonalcoholic fatty liver disease among this cohort was 38.7% (91/235). Grade 3 disease was the most prevalent (46.1%). Prevalence of nonalcoholic steatohepatitis and fibrosis using non-invasive methods was estimated to be 23.1% and 26.4% respectively, in the population diagnosed with nonalcoholic fatty liver disease. Factors associated with presence of nonalcoholic fatty liver disease included age (p-value = 0.008), shorter duration of diabetes (p-value = 0.004), body mass index (p-value <0.0001), waist circumference (p-value = 0.006), waist/hip ratio (p-value = 0.001), abdominal subcutaneous fat thickness (p-value <0.0001), hepatomegaly (p-value <0.0001), glycated haemoglobin (p-value = 0.003) and hypertriglyceridaemia (p-value = 0.021). Increasing age (OR = 0.949; 95% CI: 0.918 - 0.980),

body mass index (OR = 1.108; 95% CI: 1.048- 1.171), hepatomegaly (OR = 4.230; 95% CI: 1.957 - 9.144) and elevated gamma-glutamyltransferase (OR = 3.591; 95% CI: 1.631 - 7.906) were found to be independent predictors of nonalcoholic fatty liver disease among this cohort.

Conclusions: Roughly one-third of type 2 diabetes mellitus patients seen in clinics may have nonalcoholic fatty liver disease. Predictors of nonalcoholic fatty liver disease in this study include established non-modifiable risk factors such as age, as well as modifiable risk factors such as increased body mass index.

Recommendations: Clinicians should utilize non-invasive markers to aid in the diagnosis of nonalcoholic fatty liver disease, especially in at-risk patients. Aggressive management of type 2 diabetes mellitus is needed to improve the outcome of the disease in Ghana. Further studies on the performance of non-invasive markers compared to liver biopsy are warranted in order to find alternate forms of diagnosis in low resource settings.

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LIST OF ABBREVIATIONS

AAR :	AST/ALT Ratio
AASLD:	American Association for the Study of Liver Diseases
ADA :	American Diabetes Association
ALT :	Alanine aminotransferase
APRI :	AST to platelet ratio
AST :	Aspartate aminotransferase
BMI :	Body mass index
ChREBP:	Carbohydrate response element-binding protein
CPT-1:	Carnitine acyltransferase I
CT scan:	Computerized tomography scan
CVS :	Cardiovascular system
DM :	Diabetes Mellitus
DNL :	De novo lipogenesis
EASL :	European Association for the Study of the Liver
FBC :	Full Blood Count
FBS :	Fasting Blood Sugar
FFA :	Free fatty acid
FIB-4 :	Fibrosis-4 score
GARH:	Greater Accra Regional Hospital
GGT :	Gamma-glutamyltransferase
GIT :	Gastrointestinal Tract
HbA1c:	Glycated Haemoglobin
HCC :	Hepatocellular carcinoma
IDF :	International Diabetes Federation
IGT :	Impaired fasting glucose
IL- :	Interleukin
INR :	International normalized ratio
IR :	Insulin resistance
IRS-1 :	Insulin receptor substrate-1
LFT :	Liver function test
MRI :	Magnetic resonance imaging
MRS :	Magnetic resonance spectroscopy
NAFL :	Nonalcoholic fatty liver
NAFLD:	Nonalcoholic fatty liver disease
NASH :	Nonalcoholic steatohepatitis
NFS :	NAFLD fibrosis score
NLR :	Neutrophil-lymphocyte ratio

NPV :	Negative predictive value	**TPN :**	Total parenteral nutrition
PPV :	Positive predictive value	**TNFα :**	Tumour necrosis factor alpha
SREBP-1c:	Sterol regulatory element binding protein	**USA :**	United States of America
		USG :	Ultrasonography
T2DM:	Type 2 diabetes mellitus	**WHO :**	World Health Organisation
TGF-β:	Transforming growth factor-β		

CHAPTER 1

INTRODUCTION

1.1 BACKGROUND

Nonalcoholic fatty liver disease (NAFLD) is a chronic liver condition with a clinical and pathological spectrum that is characterized by excessive lipid deposition in the liver parenchyma in the absence of significant alcohol consumption.[1] This spectrum ranges from simple steatosis to steatohepatitis and fibrosis. It was first described by Ludwig and colleagues in the 1980s among 20 patients without significant alcohol history but had liver disease with histopathologic features similar to that of alcoholic hepatitis.[2] Nonalcoholic fatty liver disease in that study was more common in women than in men. Also, majority of those with NAFLD had features of moderate obesity which was a body mass index (BMI) within the range of 30.0kg/m^2 to <35.0kg/m^2. This study was subsequently followed by other studies mainly in developed countries to reveal the epidemiology, pathophysiology, natural history, and possible treatment modalities.

Currently, NAFLD is the commonest cause of chronic liver disease worldwide ahead of alcoholic liver disease and viral hepatitis.[3,4] This was observed in a study involving 5,783 participants from different ethnic groups.[4] The incidence or prevalence of NAFLD depends on the study population and also on the method of diagnosis. Histology is the gold standard for its diagnosis and has the highest diagnostic accuracy. Although data on the incidence of NAFLD are limited, five studies from Asia and Israel recorded the current rate as a range from 28.01 per 1,000 person-years to 52.34 per 1,000 person-years.[5,6] The current worldwide prevalence of NAFLD ranges between 24% and 30%. In a 2016 study, Younossi et al[6] through a meta-analysis and systematic review of prevalence studies done between 1989 and 2015, estimated the global prevalence of NAFLD as 25.24%. This study recorded the highest prevalence in the Middle East and South America and the lowest prevalence in Africa, that is, 31.79%, 30.45%, and 13.48% respectively. Forty-two out of the 57 studies analysed in this meta-analysis diagnosed hepatic steatosis with imaging.

Nonalcoholic fatty liver disease can be classified using its aetiologic factors or histopathologic features. Based on features observed on histology, it can be classified into nonalcoholic fatty liver (NAFL) and nonalcoholic steatohepatitis (NASH).[7] This classification is supported by both the American Association for the Study of Liver Diseases (AASLD) and the European Association

for the Study of the Liver (EASL) guidelines.[8,9] These guidelines define NAFL as NAFLD with histological features of simple intrahepatic steatosis and NASH as NAFLD with histological features of steatohepatitis with or without fibrosis. The true prevalence of NASH in the general population is difficult to assess because of the invasive nature of liver biopsies. Younossi et al[6] recorded the overall prevalence of NASH among NAFLD patients diagnosed by histology as 59.10%. However, the prevalence differed among those with clinical indication for biopsy and those without, that is, 60.64% to 69.25% and 6.67% to 29.85% respectively.[6]

Based on aetiology, NAFLD can either be primary or secondary. Primary NAFLD is defined as the presence of excess amounts of lipids in the hepatocytes with no obvious aetiology. Secondary NAFLD is the presence of excess amounts of lipids in the liver from underlying agents like toxins, medications, and some medical conditions but with no significant history of alcohol use.

Primary NAFLD, which is of interest in this study, is now known to be associated with insulin resistance (IR) and also with components of the metabolic syndrome like hyperlipidaemia, obesity, type 2 diabetes mellitus (T2DM), or impaired glucose tolerance (IGT).[10–12] A recent study by Younossi et al[6] supported this evidence when they reported that, NAFLD was closely associated with obesity, T2DM, dyslipidaemia, and hypertension. Because of this association, NAFLD was initially considered to be part of the metabolic syndrome but it is now known to occur even in non-T2DM or non-obese patients.[13,14] Nonalcoholic fatty liver disease can either precede or follow the onset of any of the components of the metabolic syndrome especially T2DM.[15]

The current increase in the prevalence of NAFLD is strongly linked with the increase in obesity and T2DM which have now become epidemics worldwide and a major burden for public health.[11] Prevalence of NAFLD is higher in T2DM than in the general population. Byrne and colleagues in a 2015 report estimated this prevalence as 70% which was about twice the prevalence in the general population.[16] However, Younossi et al in a 2019 meta-analysis of studies done between 1989 and 2018 recorded this current prevalence as 59.48%.[17] The prevalence of NASH is also higher in T2DM patients than in the general population with Golabi et al[18] reporting it as 65.26%. Nonalcoholic steatohepatitis in this study was diagnosed by histology. Both NAFL and NASH can eventually progress to liver cirrhosis and hepatocellular carcinoma (HCC) which have high morbidity and mortality rates.[19] This risk of progression is higher in NASH than in NAFL. It is reported that, over a 15-year period, NAFL had a 0.7% risk of progression to cirrhosis with a liver-

related mortality rate of 0.9% but NASH had a 10.8% risk of progression to cirrhosis and a higher rate of liver-related mortality, that is, 7.3%.[20] The risk of progression from NASH to cirrhosis and HCC has made NASH the second leading indication for liver transplantation in the United States of America (USA)[21] and is expected to be the leading cause by the year 2020.[11] Type 2 DM serves as a risk factor for progressive liver disease and liver-related mortality in NAFLD. This was confirmed by Younossi et al[22] who recorded an 18.2% liver-related mortality rate in NAFLD patients with T2DM compared with 2.3% in those without T2DM.

Research works done by Armstrong et al[23] and Vanni et al[24] showed that NAFLD had extrahepatic manifestations involving mainly the cardiovascular system (CVS) and the kidneys but can also cause malignancies especially in the gastrointestinal tract (GIT). Targher and colleagues reported that primary NAFLD serves as an independent determinant of cardiovascular diseases especially in T2DM and is also a risk factor for mortality in such patients.[25] This has emphasized the need for a multidisciplinary approach in the management of NAFLD.

1.2 STATEMENT OF PROBLEM

Primary NAFLD is known to be increasing worldwide with a higher prevalence in people with lifestyle-related diseases. Worldwide, NAFLD has a prevalence of about 30% in the general adult population but this prevalence is higher in obese people on high-fat diets and inactive lifestyles.[3] In Ghana, the prevalence of obesity has gradually increased from 5.5% to 25.4% in the last decade.[26,27] Nonalcoholic fatty liver disease is therefore expected to increase gradually among this group of people.

Globally, DM is one of the most common lifestyle-related diseases with the majority of cases being T2DM, that is, about 90% of all DM cases.[28,29] Type 2 DM is another metabolic condition that increases the risk of developing NAFLD. The high prevalence of NAFLD in T2DM than in the general populace is a result of the pathogenesis of primary NAFLD being closely linked to IR. Insulin resistance also explains the pathogenesis of T2DM. Cross-sectional and prospective cohort studies in Europe and North America have revealed that the prevalence of NAFLD in asymptomatic adults in the general populace is between 19% and 30% but ranges between 21% and 78% in those with T2DM.[30–32] In West Africa, only three cross-sectional studies on NAFLD in T2DM are readily available. These studies were carried out in Lagos, Nigeria between 2009 and

2018 by Onyekwere et al[33], Olusanya et al[34] and Afolabi et al[35] and recorded the prevalence of NAFLD in T2DM as 9.5%, 16.7% and 68.8% respectively. All studies from Nigeria used ultrasonography (USG) to diagnose NAFLD and brought to fore the increasing prevalence of NAFLD in the last decade.

In Ghana, information on NAFLD in the general populace is limited. The only study readily available is by Ansumana Bockarie and recorded the prevalence of NAFLD diagnosed by USG among patients attending the medical clinic at the Cape Coast Teaching Hospital between January and October 2016 as 24.8%.[36] Data on NAFLD among the at-risk groups in Ghana including T2DM is however lacking. The prevalence of T2DM in Africa, including Ghana, is known to be rising.[37,38] The World Health Organization (WHO) estimated the prevalence of DM among adults older than 18 years in Africa, which includes Ghana, as 7.1% in the year 2014.[39] The International Diabetes Federation (IDF) in 2015 stated that about two-thirds of DM in Africa remain undiagnosed.[37] The prevalence of T2DM is expected to double by the year 2040 as a result of the ageing population and urbanization of the population.[37] Hence, NAFLD is also expected to increase with a resultant increase in its complications if not prevented or effectively managed.

This study seeks to determine the prevalence of NAFLD in T2DM patients and also reveal the factors associated with NAFLD in these patients. The results of this study will help guide patient management and improve outcomes in affected patients.

1.3 JUSTIFICATION OF STUDY

Patients with end-stage liver disease are seen in most health facilities in Ghana but data on its incidence is scarce. These patients usually present at an advanced stage resulting in high morbidity and mortality. In 2006, Wiredu et al[40] documented liver cancer as the first and third leading cause of cancer-related mortality at the Korle Bu Teaching Hospital (KBTH) in males and females respectively. Concerning cirrhosis and HCC, only few studies exist on patient characteristics and clinical profile but chronic viral hepatitis, especially chronic hepatitis B infection, and alcoholic liver disease are well documented as leading causes.[41–43] Some patients however have negative screens for chronic viral hepatitis B and C and also have no history of significant alcohol use. Nonalcoholic fatty liver disease may play a role in the cause of cirrhosis and HCC in such patients

as it is now known to be the leading cause of chronic liver disease in developed countries and gradually increasing in developing countries.

The prevalence of metabolic diseases such as obesity and T2DM has risen in Ghana over the last 20 years.[27,38] This may be attributed to changes in diet and other lifestyle habits. Nonalcoholic fatty liver disease and T2DM have common underlying pathogenesis and an increase in one may occur with an increase in the other. Nonalcoholic fatty liver disease may cause hepatic and extrahepatic complications.[19,24] Therefore, the coexistence of NAFLD and T2DM increases the risk of cardiovascular, cerebrovascular, and peripheral artery diseases. The morbidity and mortality associated with complications of both NAFLD and T2DM have a direct impact on the socio-economic status of the individual and the country.

This study aims at determining the prevalence of NAFLD among T2DM patients and also identifying the prevailing factors associated with NAFLD in T2DM. This will be essential in the risk stratification of patients in relation to optimal management of their T2DM and other diseases and therefore, improving outcomes in affected patients.

1.4 SCOPE OF STUDY

The scope of this study is limited to recruiting 235 T2DM patients attending the DM clinic at the Greater Accra Regional Hospital (GARH) in Accra, Ghana. Patients aged 18 years or more will be eligible for recruitment and this will be done over a period of 5 months. Data will be extracted using a semi-structured questionnaire and each participant will have blood samples taken and also undergo an abdominal USG.

1.5 RESEARCH QUESTION

The research questions to be answered are;

1. What is the prevalence of NAFLD in T2DM patients attending the DM clinic at the GARH in Accra, Ghana?

2. What is the influence of sociodemographic, clinical, anthropometric, and biochemical factors on the development of NAFLD among T2DM patients attending the DM clinic at the GARH in Accra, Ghana?

3. Using serum alanine aminotransferase (ALT) level and neutrophil-lymphocyte ratio (NLR), what proportion of the T2DM patients with NAFLD attending the DM clinic at the GARH have NASH?

4. What is the prevalence of fibrosis among the T2DM patients with NAFLD attending the DM clinic at the GARH in Accra, Ghana?

1.6 AIM AND OBJECTIVES

1.6.1 Aim of Study

To determine the prevalence of and factors associated with primary NAFLD in T2DM patients attending the DM clinic at the GARH.

1.6.2 Specific Objectives

1. To determine the prevalence of primary NAFLD in T2DM patients using features on USG.

2. To assess the prevailing factors associated with NAFLD in T2DM patients diagnosed by USG.

3. To determine the proportion of T2DM patients with NAFLD presenting with NASH diagnosed by the level of serum ALT and NLR.

4. To determine the proportion of T2DM patients with NAFLD presenting with advanced liver fibrosis using non-invasive scoring systems.

1.7 RESEARCH HYPOTHESIS

The null hypothesis (H_0) this research seeks to answer is;

1. The prevalence of NAFLD-associated risk factors in T2DM patients attending the DM clinic at the GARH does not show any significant difference in those with and without NAFLD.

The alternative hypothesis (H_A) this research seeks to answer is;

1. The prevalence of NAFLD-associated risk factors in T2DM patients attending the DM clinic at the GARH shows a significant difference in those with and without NAFLD.

CHAPTER 2

LITERATURE REVIEW

2.1 INTRODUCTION

Fatty liver is a spectrum of disorders defined by the abnormal accumulation of lipids in the liver. The amount of lipids found in a normal adult human liver is approximately 5% of its mass. Therefore, fatty liver is present when the amount of lipids in the hepatocytes exceeds 5%.[44] Lipids in the liver are mainly triglycerides.

Fatty liver can be classified into macrovesicular and microvesicular based on the size and number of triglyceride droplets observed in each hepatocyte during histology.[45] The type of fatty liver present may serve as a clue to the underlying cause. In macrovesicular steatosis (figure 1), the cytoplasm of the hepatocyte is filled with 1 or 2 fat globules which are large enough to displace the nucleus to the periphery of the cell. This type of steatosis may be seen in NAFLD.[46] It is also seen in alcoholic liver disease, chronic hepatitis C virus infection, Wilson's disease and in drug-induced steatosis from methotrexate and corticosteroids.[8,47] In microvesicular steatosis, however, the cytoplasm of the hepatocytes is filled with numerous small vesicles of fat but without displacement of the nucleus as illustrated in figure 2. This type can be seen in pregnancy, that is, acute fatty liver of pregnancy and HELLP syndrome, and also in Reye's syndrome and drug-induced steatosis from valproate and antiretrovirals.[8]

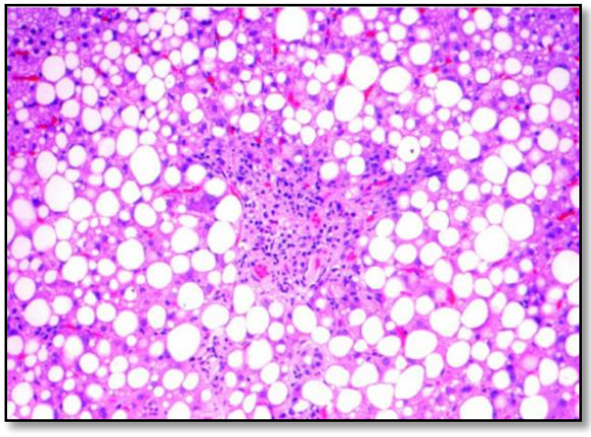


Figure 1: Macrovesicular Steatosis

Source: Clinical Pharmacology: Advances and Applications48; cited on 29th March 2020.

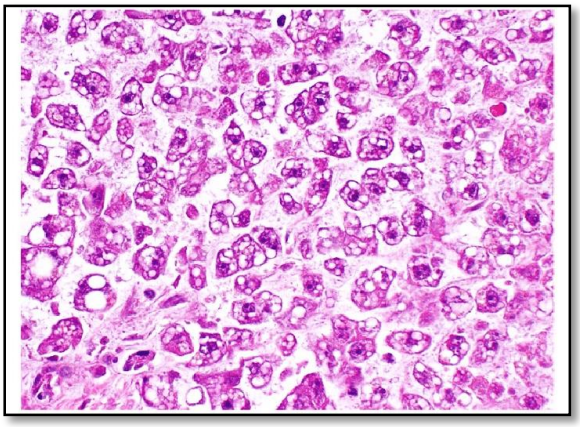


Figure 2: Microvesicular Steatosis
Source: Hepatology Communications[49]; cited on 29th March 2020.

Fatty liver may also be classified as alcoholic fatty liver disease and NAFLD depending on the presence or absence of significant alcohol history. Alcoholic fatty liver disease is the presence of hepatic steatosis on histology in a person with a history of significant alcohol use. The chronic use of alcohol in significant quantities result in decreased lipolysis with the accumulation of triglycerides in the hepatocytes.[50] This usually presents as simple macrovesicular steatosis although microvesicular or mixed steatosis may occur.[47,51] Steatosis can progress to steatohepatitis and finally result in cirrhosis and HCC.[51] The amount of alcohol consumed and the duration of alcohol use is directly related to the development of these hepatic complications.[52]

Although there are inconsistencies in the definition of significant alcohol intake, Bellentani et al[53] in a cross-sectional, observational study among 257 participants in Northern Italy documented that, when daily alcohol consumption exceeded 20g in women and 30g in men, hepatic steatosis can occur. These limits are endorsed by both the EASL and AASLD guidelines which now considers a daily consumption of more than 30g and 20g of alcohol in men and women as significant.[8,54] Ten grams of alcohol is equivalent to a unit of alcohol.[55] Therefore, a daily alcohol intake of 30g and 20g is equivalent to 3 units and 2 units. By inference, weekly alcohol intake in quantities greater than 21 units and 14 units in men and women is considered significant.

According to the NHS in the United Kingdom,[56] the units of alcohol in a drink can be calculated using this formula;

Units of alcohol = (Quantity of alcohol (mL) x Alcohol by volume (%)) ÷ 1000

Worldwide, the alcohol content in beer, wine and spirit ranges from 2% - 5%, 10% - 20% and 38% - 40% respectively. Therefore, 250mL of beer contains 0.5 - 1 unit of alcohol whiles 200mL of wine contains 2 - 4 units of alcohol. A 30mL shot of whiskey contains 1.14 - 1.25 units of alcohol. In Ghana, the alcohol content in the locally produced beer is 5%. Therefore, a 330mL and a 625mL bottle of beer contain 1.65 and 3.12 units of alcohol. The locally brewed spirit, akpeteshie, has an alcohol content of 40% - 50%.[57,58] Hence, 30mL of akpeteshie contains about 1.2 - 1.5 units of alcohol.

2.2 NONALCOHOLIC FATTY LIVER DISEASE

Nonalcoholic fatty liver disease is the presence of steatosis in the hepatocytes without a significant alcohol history, that is when the amount of triglyceride amount is >5% of liver mass.[5] The NAFLD spectrum of steatosis to steatohepatitis and fibrosis can ultimately result in cirrhosis.[1,54] Steatosis describes the presence of fat in the liver without inflammation whilst steatohepatitis is the presence of fatty liver with inflammation of the parenchyma with or without necrosis.[59] This forms the basis of the histological classification of NAFLD into NAFL and NASH which is endorsed by both EASL and AASLD.[8,9] The term, NASH, was first coined by Ludwig et al[2] in 1980 at the Mayo Clinic after they observed that, the biopsy specimen from 20 obese patients with no history of significant alcohol intake demonstrated a histologic resemblance to alcoholic hepatitis. A later study by Pais and colleagues reported that progression from NAFL to NASH could occur in some patients.[60] This was after they had followed up 6 patients with an initial histologic diagnosis of simple steatosis for 5 years and a repeat of the biopsy at completion showed a progression from steatosis to steatohepatitis in 5 out of the 6 patients.

The classification of NAFLD by its aetiology into either primary or secondary is broadly used.[54] Primary NAFLD forms the majority of NAFLD cases and may either occur alone or in combination with components of the metabolic syndrome via its pathogenesis which involves IR.[54] The metabolic syndrome includes central obesity, dyslipidaemia, systemic hypertension, T2DM or impaired glucose tolerance and microalbuminuria.[11] This type of NAFLD was initially considered as the hepatic component of the metabolic syndrome.[14] The assumption was supported by Cortez-Pinto et al[61] who after studying 30 patients with NAFLD, reported the presence of

obesity, hypertension and T2DM in 80%, 50% and 33% of these patients respectively. Dyslipidaemia was also recorded in 80% of the patients. However, some later studies led to the rejection of this concept as they recorded the presence of NAFLD in persons with no features of the metabolic syndrome. One such study was by Margariti et al at an outpatient clinic in Greece between 2002 and 2010 and involved 162 participants.[62] These researchers found NAFLD in about 12% of persons with normal BMI and with no features of metabolic syndrome. Diagnosis of NAFLD in this study was by either raised ALT, raised GGT or both plus evidence of steatosis on USG or histology. Although NAFLD is no longer regarded as a component of metabolic syndrome, it is still of public health interest since it has strong associations with non-communicable diseases and may result in poor outcomes if not properly managed.

Secondary NAFLD which rarely occurs in adults is not known to be linked to IR or metabolic syndrome.[54] The use of the causative factor and resultant pathology (e.g.) drug-induced steatosis, is currently preferred to the term 'secondary NAFLD'.[63] The possible underlying agents for this type of NAFLD include toxins, steatogenic medications and an array of clinical conditions.[64] Clinical conditions like chronic hepatitis C infection, Wilson's disease, coeliac disease, acute fatty liver of pregnancy and disorders of lipid metabolism like abetalipoproteinemia, hypobetalipoproteinaemia, lipodystrophy and glycogen storage diseases may cause hepatic steatosis.[65] Additionally, toxins and nutritional factors like starvation, total parenteral nutrition (TPN) and severe surgical weight loss also cause this type of NAFLD. Total parenteral nutrition causes depletion of carnitine and choline. Carnitine works by facilitating the transfer of free fatty acid (FFA) from the cytoplasm of hepatocytes to the mitochondria for oxidation whiles choline promotes the secretion of lipoproteins from the liver. The depletion of these 2 facilitates the deposition of FFA in the hepatocytes, resulting in steatosis.[65] The severity of the hepatic disease is directly related to the duration of TPN.

Steatogenic medications may also cause NAFLD. The commonly used ones in our environment include corticosteroids, methotrexate, amiodarone, tamoxifen and some antiretroviral medications. Amiodarone causes a mild elevation of transaminases in about 25% of all patients but only 1% - 3% of patients will develop significant hepatic injury. This is similar to the histologic appearance of NASH but exhibits a characteristic lesion known as phospholipidosis.[65] Tamoxifen, a drug commonly used in the treatment of breast cancer, causes steatosis especially in those with risk

factors for NAFLD. Bruno et al,[66] studied 5,408 women in a prospective randomized controlled study and showed a 2-fold increase in the risk of developing NAFLD in patients on tamoxifen whose BMI was above normal. Progress from steatosis to steatohepatitis and fibrosis may occur with tamoxifen but progression to cirrhosis is uncommon in these patients. Another chemotherapy agent, methotrexate, at high doses also cause steatohepatitis with necroinflammation which can progress to cirrhosis.[65] The occurrence of cirrhosis is commonly seen in patients with a history of alcohol abuse, obesity and DM but may also occur in the absence of these risk factors. Another group of patients who may develop NAFLD are those on highly active antiretroviral therapy (HAART) especially protease inhibitors and nucleoside/nucleotide reverse transcriptase inhibitors.[67] These patients may develop a microvesicular type of steatosis. Lastly, corticosteroids can cause hepatic steatosis via activation of enzymes needed for de novo lipogenesis (DNL) and also by inhibition of enzymes of the lipid peroxidation and mitochondrial beta-oxidation pathways.[65]

2.3 PATHOPHYSIOLOGY OF NONALCOHOLIC FATTY LIVER DISEASE

Fat metabolism occurs in adipose tissue, skeletal muscle and the liver. This results in the conversion of FFA to triglycerides which is stored in the liver or used in the synthesis of very-low-density lipoprotein (VLDL). In the normal liver, FFA is derived from either intestinal absorption of dietary fat, lipolysis in adipose tissue or is synthesized in the liver via DNL using Acetyl-CoA.[59] De novo lipogenesis is tightly regulated by insulin and is the primary source of hepatic FFA in the fed state as insulin levels are normally elevated.[68] The sources of FFA in the liver and the various complex biochemical processes of DNL via the Kreb's cycle and glycolysis pathways are shown in figures 3 and 4.

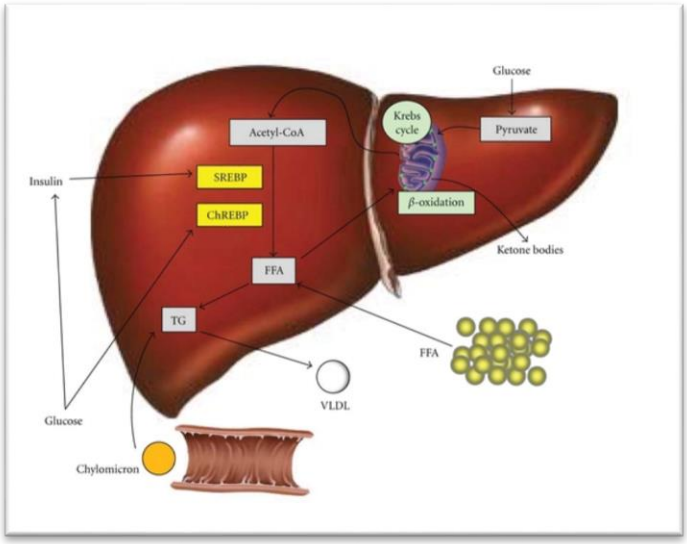


Figure 3: Sources of FFA in the liver and their mediators

Source: Journal of Hepatology[69]; cited on 21st January 2020

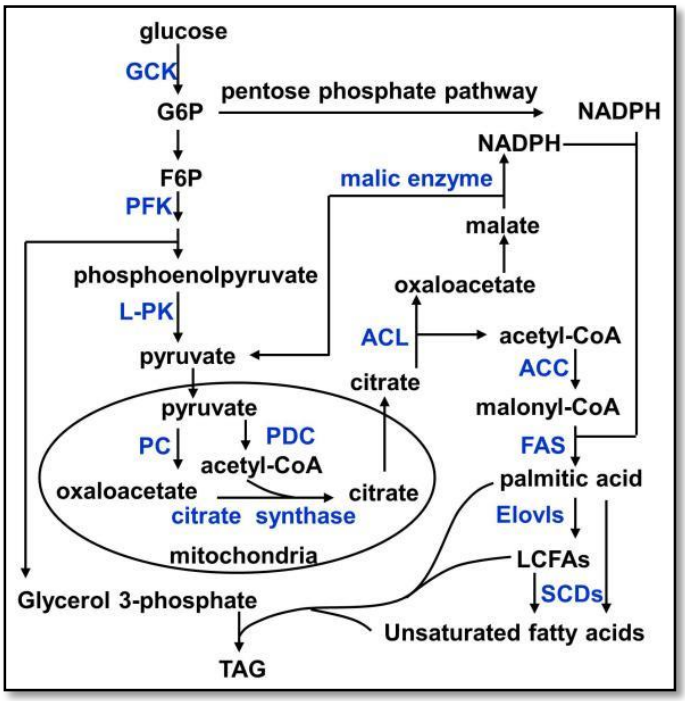


Figure 4: De novo lipogenesis via the glycolysis pathway and Kreb's cycle

Source: Comprehensive Physiology: Energy metabolism in the liver[70]; cited 5th February 2020.

Lipogenesis is regulated by 2 main mediators, sterol regulatory element-binding protein (SREBP-1c) and carbohydrate response element-binding protein (ChREBP). The SREBP-1c is activated by insulin and acts by activating the genes that promote the production of malonyl-CoA[71] whiles ChREBP is activated by hyperglycaemia and acts by activating the enzymes of the glycolytic pathway and those that promote lipogenesis.[72]

Lipolysis occurs via β-oxidation with FFA and glycerol as end products. Aldehydes are produced as by-products and these include 4-hydroxynonenal and malondialdehyde.[73] Lipolysis is increased during fasting and decreased in the fed state.[70] Carnitine acyltransferase I (CPT-1) is the main regulator of lipolysis with its action being the rate-limiting step. It is inhibited by malonyl-CoA and thereby decreasing the rate of lipolysis.[74]

For maintenance of equilibrium, the rate of lipogenesis must equal the rate of lipolysis as illustrated in figure 5.

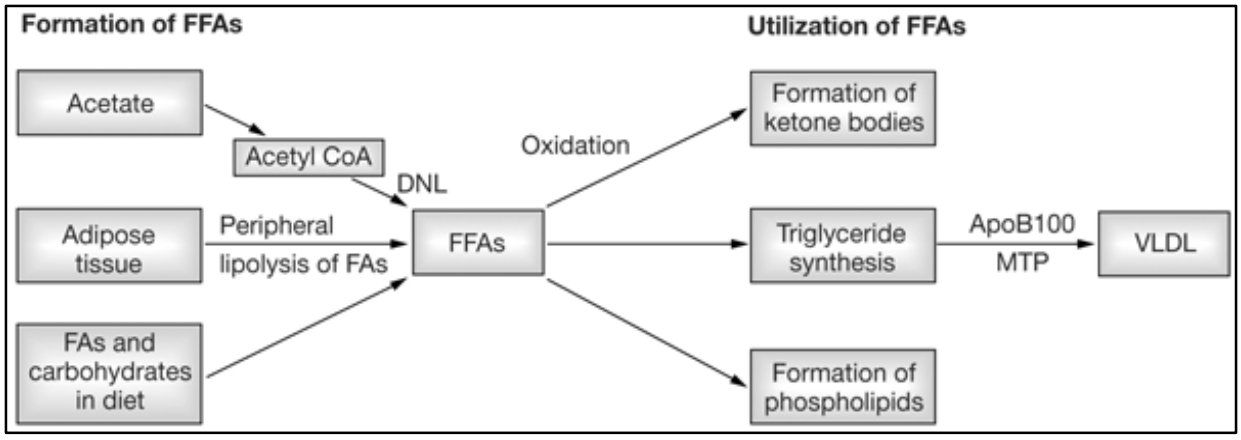


Figure 5: Summary of fat metabolism in the liver

Source: Nature Clinical Practice Gastroenterology & Hepatology[75]; cited on 28th July 2019.

The pathophysiology of NAFLD is poorly understood. It was initially explained by the "two-hit" hypothesis (figure 6) conceptualized at Pessayre's lab by Benson et al.[76] This theory was based on a first and second hit principle and was able to link the presence of hepatic steatosis to the oxidation of lipids which may progress to steatohepatitis and fibrosis. The first hit in this theory involves the development of hepatic steatosis from IR, obesity, lack of physical activity and intake of foods

with high-fat content. This primes the hepatocytes for a second hit which is initiated by environmental or genetic factors. These factors promote excessive production of free radicals which act as a second insult to the liver and initiates lipid peroxidation. This increases the production of cytokines and endotoxins like tumour necrosis factor-α (TNFα), interleukin 8 (IL-8), intercellular adhesion molecule 1 (ICAM-1) and E-selectin. Also, the by-products of peroxidation may cause necroinflammation and fibrosis. Peroxidation explains the histological features of NASH and has been demonstrated in studies involving animal and human models.[77,78] Additionally, free radicals are produced by an increase in oxidative stress from excessive FFA deposition in the hepatocytes and also from increased expression of cytochrome P450 2E1 (CYP2E1) as seen in some patients with NASH. Persistence of this cascade causes a progression from NASH to fibrosis and cirrhosis.

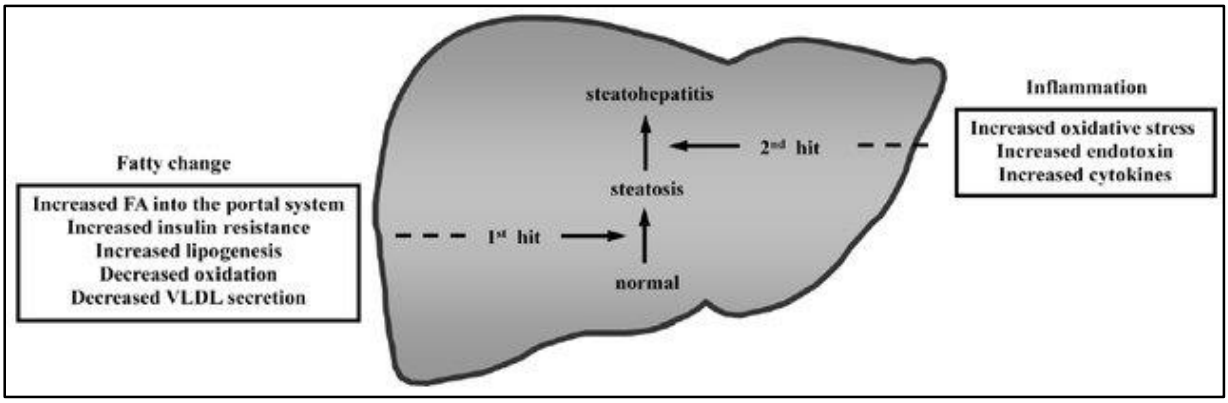


Figure 6: The "two-hit" hypothesis in the pathophysiology of NAFLD

Source: International Journal of Molecular Sciences[77]; cited on 21st July 2019

The "two-hit" theory was however discarded as it was considered by experts as simplistic and unable to explain why the majority of patients with hepatic steatosis did not progress to NASH. This led to the birth of the "multiple hit" theory, which is multifactorial and currently used to explain the pathophysiology of NAFLD.[79] This hypothesis involves genetic, environmental and dietary factors, which may cause hepatic steatosis from IR, hyperinsulinemia or changes in the gut microbiome. Subsequently, other harmful factors act on the already vulnerable liver causing the progression of steatosis to NASH.

Genes involved in NAFLD include the PNPLA3 gene, also known as adiponutrin. According to Romeo and colleagues who performed genome studies among participants in the Dallas Heart Study, PNPLA3 was associated with both hepatic steatosis and inflammation diagnosed by magnetic resonance spectroscopy (MRS).[80] This association was with the variant, PNPLA3-148M, which has specific sites for SREBP-1c and ChREBP.[81] PNPLA3-148M in this study was commonly seen in Hispanics than in other ethnic groups. Another gene, transmembrane 6 superfamily member 2 (TM6SF2) has been linked to NAFLD as it interferes with VLDL secretion and contributes to the remodelling of hepatic triglycerides.[82] Apart from genes, dietary and environmental factors like increased intake of saturated fat with a lack of physical activity, intestinal bacterial overgrowth, obesity, and IR contribute to NAFLD. In addition to this role, dietary factors, which are also risk factors for the development of obesity, may also facilitate the progression of NAFLD to fibrosis.[83]

In the multiple-hit theory, there is an interaction between IR, adipose inflammation, adipokines and other factors in the presence of genetic factors. The initial hit involves the dysregulation of the insulin-mediated processes of fat metabolism through the down-regulation of insulin receptor substrate-1 (IRS-1), thereby, triggering an imbalance between lipogenesis and lipolysis with a resultant increase in adipose lipolysis.[84] This leads to excessive production and release of FFA into the portal blood.[59] Besides the down-regulation of IRS-1, IR decreases glucose utilization and increases hepatic gluconeogenesis resulting in hyperglycaemia.[85] For normoglycaemia to be maintained, excess insulin is produced and the resultant hyperinsulinemia increases the rate of DNL and lipolysis in adipose tissue, causing an influx of FFA into the liver. The IR and the resultant steatosis serve as the first hit in the "multiple hit" theory. This, together with hyperinsulinemia is now considered to be at the centre of NAFLD progression via various mechanisms.[59]

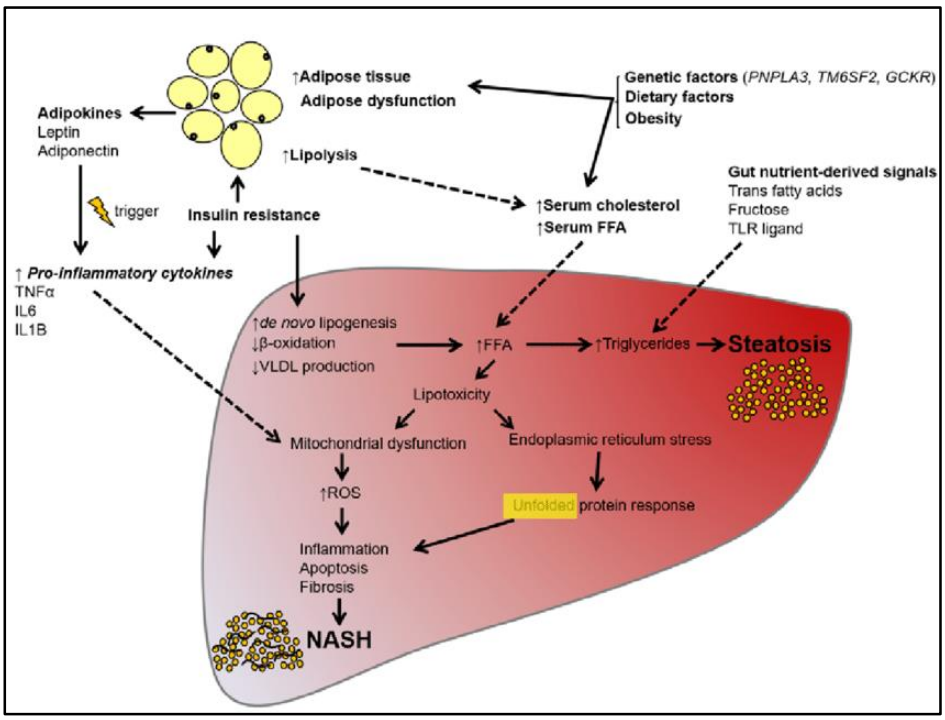


Figure 7: The "multiple hit" theory in the pathophysiology of NAFLD
Source: International Journal of Molecular Sciences[86]; cited on 7th of February, 2020

With the development of hepatic steatosis, the liver becomes vulnerable and susceptible to other harmful agents. These agents may arise from the lipotoxicity caused by excessive FFA deposition or by the by-products of fat metabolism.[79] These act as multiple hits to the already vulnerable liver, causing simple steatosis to progress to NASH and fibrosis.[87] Consequently, the negative effects of the multiple hits on the endoplasmic reticulum and mitochondria gives rise to oxidative stress with a release of reactive oxygen species (Figure 7). The liver remains in a chronic inflammatory state which then acts as a source of persistent injury to the hepatocytes, contributing to the progression from steatosis to steatohepatitis, fibrosis and eventually cirrhosis. Apoptosis and activation of transforming growth factor-beta (TGF-β) also influence the progression to NASH.[88] Other factors implicated in the pathophysiology of NAFLD, particularly NASH, include tumour necrosis factor α (TNF-α), leptin and adiponectin.[59,89] Both elevated TNF-α as well as low adiponectin level promote steatohepatitis. Elevated TNF-α works by impairing the action of insulin whiles decreased adiponectin levels causes the loss of the antilipogenic effect. The role of adiponectin in NAFLD is consistent with that in obesity, T2DM and in persons with IR.

In addition to the above pathophysiology, alteration in the gut flora increases the permeability of the small intestines, promoting unrestricted absorption of FFAs into the portal circulation with subsequent deposition in the liver.[90] Furthermore, alteration in the gut microbiome causes an increase in the activation molecules of the inflammatory pathways resulting in the secretion of inflammatory cytokines such as IL-6 and TNF-α.

Currently, the effectiveness of the "multiple-hit" theory is being debated. This is because, despite the difference in the role of IR, both the "two-hit" and "multiple-hit" theories consider NASH as a progression from NAFL but only limited documented evidence of this progression exists.[91] Various research works have suggested the possibility of NAFL and NASH being independent but related entities which means NASH may occur without prior evidence of NAFL. This has led to the suggestion of the conceptualization of a new theory for the pathogenesis of NASH and the development of non-invasive diagnostic methods to aid further research work. This suggested theory may influence the treatment of NAFL and NASH.

Although NAFLD is usually considered a consequence of metabolic syndrome, it may precede the onset of T2DM and other components of the metabolic syndrome.[92–94] Musso and colleagues were the first to publish a meta-analysis and reported a doubling of the incidence of T2DM among NAFLD patients.[93] Subsequently, a longitudinal study by Zhang et al[92] at Shandong Provincial Hospital between 2005 and 2011 also concluded that NAFLD may precede the onset of T2DM and the metabolic syndrome, but this risk decreased when NAFLD was successfully managed.[92] Another meta-analysis of prospective studies by Ballestri and colleagues,[94] analysed 117,020 participants with NAFLD who were followed up for a period of 3 to 15 years with a median of 5 years. They reported a 1.97 relative risk of these participants developing T2DM. Also, the relative risk of developing metabolic syndrome was 1.80 among 81,411 participants during a median follow-up period of 4.5 years.

2.4 EPIDEMIOLOGY OF NONALCOHOLIC FATTY LIVER DISEASE

In recent years, research into primary NAFLD has increased due to the increasing incidence of NAFLD-related cirrhosis and HCC but data on NAFLD incidence are limited. Nonalcoholic fatty liver disease is currently considered a major health burden in developed countries and gradually

increasing in developing countries due to urbanization and change in lifestyle habits. The rising NAFLD prevalence is associated with the current increase in obesity and T2DM worldwide.[8,11]

2.4.1 Global Nonalcoholic Fatty Liver Disease

There is a dearth of data on the incidence of NAFLD compared to data on prevalence. Early studies reported the global incidence as ranging between 19.6 to 26.8 cases per 1,000 person-years.[64] A prospective study done in Japan over a 5-year period from 1997 to 2002 among participants with metabolic syndrome reported its incidence as 31 cases per 1000 person-years.[10] This study employed the elevation of aminotransferases as an indicator of NAFLD and showed an association between elevated aminotransferases and risk factors for NAFLD including male sex, high BMI and decreased HDL. Another follow up study, the Dionysos study, done in Northern Italy between 1991 and 2002, recorded NAFLD incidence after 8.5 years of follow-up as 18.5 cases per 1,000 population.[95] Nonalcoholic fatty liver disease in this study was diagnosed with USG. Later, a retrospective study from England in 2007 showed a much lower incidence rate of 29 cases per 100,000 population among the general populace in West Suffolk with a third of these patients presenting with NASH.[96] Out of these confirmed NAFLD cases in the England study, 5.5 cases per 100,000 population had cirrhosis whilst 23.5 cases per 100,000 population showed no evidence of cirrhosis. Nonalcoholic fatty liver disease in this study was diagnosed by USG and biopsy. Data on the current global incidence of NAFLD are not readily available. However, Younossi et al[6] in their 2016 meta-analysis of studies done between 1989 and 2015 reported the incidence in Israel and Asia as 52.34 and 28.01 per 1,000 person-years. Studies in this analysis were by non-invasive methods. Data on NAFLD incidence among T2DM patients are not readily available.

The worldwide prevalence of NAFLD in the general population over the past 20 years is between 2.8% and 46%.[64] The variation is dependent on the study population and the diagnostic method used. Using USG, the 3rd National Health and Nutrition Examination Survey estimated the prevalence of NAFLD in the USA between 1988 and 1994 as 19%.[32] Williams and colleagues later used USG and histology to record the prevalence of NAFLD in the USA as 46%.[97] Their study was conducted between 2007 and 2010 in 400 participants between the ages of 18 to 70 years. The higher prevalence recorded could be attributed to the use of histology, which is the gold standard for the diagnosis of NAFLD. Current data by Younossi et al[6] in 2016 documented the global prevalence of NAFLD as 25.24%. About 75% of the studies used in this meta-analysis

diagnosed steatosis with imaging. Prevalence in this study varied between continents (Figure 8). The low prevalence in Africa was attributed to the scarcity of data from the region. Prevalence was lower when laboratory findings were used, that is, between 9.26% and 13.00%.

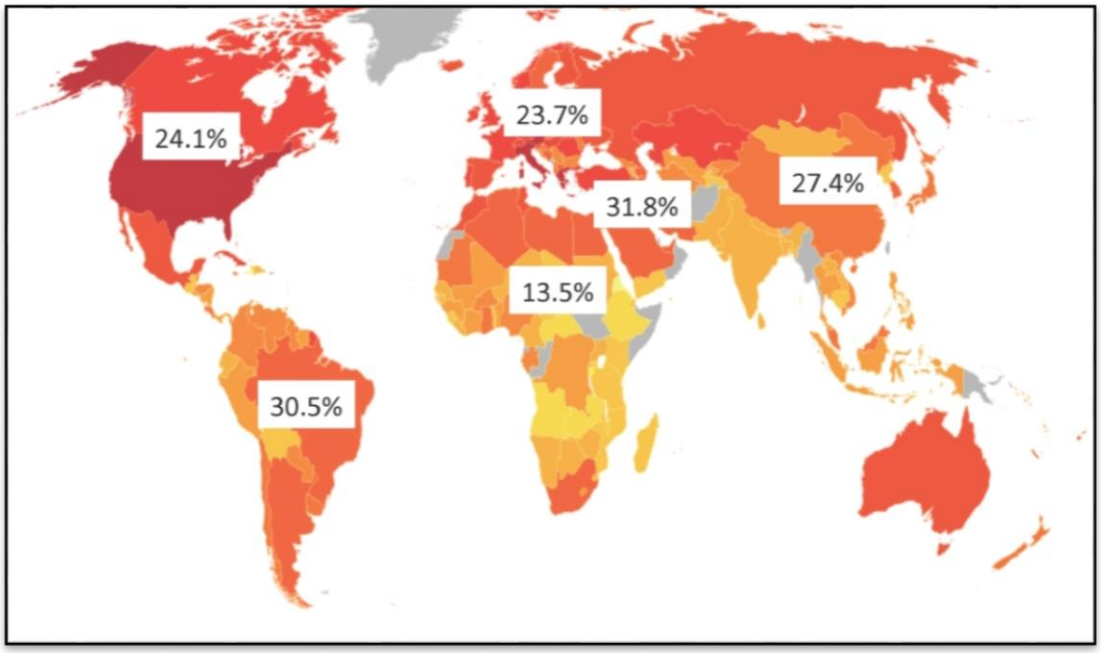


Figure 8: Current global prevalence of NAFLD by region

Source: Hepatology[98]; cited on 21st January 2020.

Nonalcoholic fatty liver disease is higher in persons with metabolic syndrome and in those who do not engage in regular physical activity.[32,97] Various studies done over the years have also reported a higher prevalence of NAFLD in T2DM compared to that in the general population. Earlier cross-sectional and prospective cohort studies in Europe and North America revealed the prevalence of NAFLD in asymptomatic adults in the general populace as 19% to 30% but between 30% and 50% in those with T2DM.[30,32] In the Edinburgh Type 2 Diabetes Study (ET2DM) reported in 2011, Williamson and colleagues used USG and revealed the NAFLD prevalence in the T2DM population as 42.6%.[99] Later, a study by Portillo-Sanchez and colleagues[100] used proton magnetic resonance spectroscopy (H-MRS) to assess the prevalence of NAFLD in T2DM patients with normal aminotransferases and recorded prevalence among obese and non-obese patients as 50% and 36% respectively. Recent studies including one by Nengguang and colleagues in 2016 at the Shanghai First People's Hospital in China recorded the prevalence of USG-diagnosed NAFLD

in T2DM patients as 56.5%.[101] Younossi et al[17] in a recent systematic review of studies done between 1989 and 2018 documented the global prevalence of NAFLD in T2DM as 55.48%. The lowest prevalence was recorded in Africa, 36.29%. The AASLD in its 2018 guidelines also suggested that NAFLD was present in about a third to two-thirds of T2DM patients but failed to emphasise the method of diagnosis.[5]

The prevalence of NASH and fibrosis is thought to be lower than NAFL. Variations in prevalence may result from the diagnostic method used. Non-invasive methods may underdiagnose NASH and fibrosis. In 2010, Bellentani reported the prevalence of NASH as 2% to 3% in the general population of Western countries.[30] As seen in NAFLD, the prevalence of NASH has also increased in the last decade. Data gathered by the United Network for Organ Sharing and Organ Procurement and Transplantation Network (UNOS/OPTN) registry in the USA reported that, between 2004 and 2013, the number of new patients registered for liver plantation increased by 170% and this made NASH-related cirrhosis the second leading cause of liver transplant in 2013.[21] Younossi et al in 2016 also estimated the prevalence of NASH by USG in the general population diagnosed without biopsy as 1.5% to 6.45%.[102] Using histology, NASH prevalence was higher with a rate of 59.10% but ranged from 60.64% to 69.25% and 6.67% to 29.85% in patients with and without clinical indications for liver biopsy.[6] Data on incidence is still lacking.

Concerning T2DM, the prevalence of NASH and fibrosis followed the same pattern as seen in NAFLD, that is, higher in T2DM patients than in the general population. In 2012, fibrosis in T2DM patients diagnosed using the NAFLD fibrosis score was reported by Williamson and colleagues as 16.4%.[103] Steatosis in this study was diagnosed with USG and laboratory investigations. In 2018, Golabi and colleagues performed a meta-analysis on studies from January 1989 and May 2017 and reported the overall prevalence of biopsy-proven NASH among T2DM as 65.26%.[18] Prevalence in this study was highest in the Western Pacific regions. There was no data from Africa. Advanced fibrosis in this study, that is F3 and F4, was 15.05%. Another study in 2019 by Younossi et al used histology and reported the prevalence of fibrosis among T2DM patients with NAFLD as 17.0%.[17] Kumar and colleagues however used a FibroScan in 2018 and documented the prevalence of fibrosis in T2DM patients with NAFLD as 34%.[104]

Age and gender are known risk factors of NAFLD. A study by Kneeman et al[65] reported that the highest prevalence of NAFLD was seen between the ages of 40 to 59 years but noted an increasing

prevalence in childhood NAFLD which could be due to the current epidemic in childhood obesity. The prevalence of NAFLD increased with increasing age. This finding was supported by Hu et al.[105] They reported a similar age-specific prevalence in a Chinese population. They also documented a gradual increase in prevalence with increasing age with a peak prevalence between age 50 and 65 years (Figure 9). Another study among Japanese adult patients also recorded a similar finding and went further to report a similar pattern in NASH.[106]

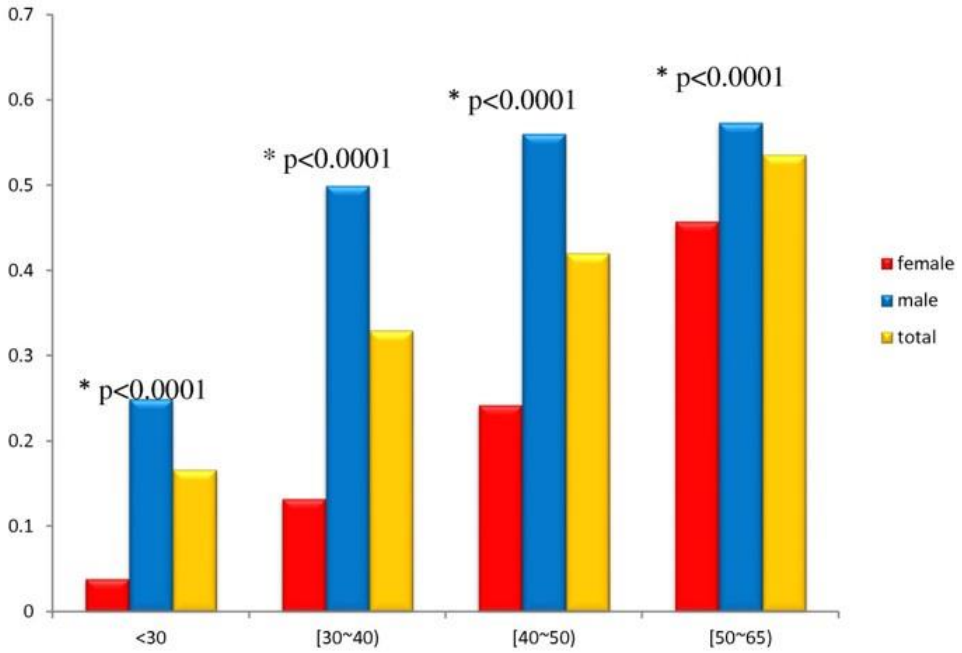


Figure 9: Trend of NAFLD prevalence by age and gender

Source: BMC Gastroenterology[105]; cited on 15th May 2020.

Concerning gender, earlier studies suggested NAFLD occurred more often in females than in males.[107] However, recent studies from America and Europe have shown prevalence to be higher in males than in females.[32,99] Bellentani documented a male to female ratio of 2:1.[30] The lower prevalence in women was thought to be evident in the pre-menopausal years and was assumed to be as a result of the protective nature of female hormones.[64] The difference in fat distribution in the 2 genders with men having more visceral fat than women, may also account for this difference. Another report by Hashimoto et al noted a similar gender preference in both NAFL and NASH prevalence. Nonalcoholic fatty liver disease was 2- to 3-fold higher in men below the age of 60

years but higher in women after this age.[106] From Figure 9, Hu et al in their study, however, reported that the higher NAFLD prevalence in men existed in all age groups.[105]

The prevalence of NAFLD is also influenced by race. This was demonstrated in a study by Angulo et al.[108] In their study, the prevalence of NAFLD among Hispanics, Caucasians and Blacks was 45%, 33% and 24% respectively. Another study by Williams et al in 2011 however recorded a higher prevalence in Caucasians than in Hispanics and African Americans, that is, 62.5%, 22% and 11% respectively.[97] The recent meta-analysis by Younossi et al however supported the findings of Angulo et al, recording a higher prevalence in South America than in Europe or North America.[6] Familial clustering has been observed in NAFLD with about 27% of cases showing evidence of this.[109] This may be associated with the progression to NASH.

2.4.2 Nonalcoholic Fatty Liver Disease in Africa

In Africa, data on NAFLD are scanty. The earliest research was by Nail et al[110] at the Ibn Sina Hospital in Sudan between January 2002 and December 2004 and involved 1,800 participants. Fatty liver disease diagnosed by USG was present in 100 of these participants. Forty-four of the 100 participants, that is, 2.4% of the overall study population, had primary NAFLD. Kruger et al[111] in 2010, followed this with a study in South Africa. This study described the clinical characteristics of NAFLD among a population who were mostly Caucasians with few Africans. The prevalence of NAFLD in their study was 47.6% and correlated well with studies from American and Europe. Nonalcoholic fatty liver disease in this study was confirmed by histology and showed a strong association with IR. Later, a study from Egypt used USG to record the prevalence of NAFLD in obese patients as 65.3% and 62.7% in children and adults.[112] This prevalence was higher compared with other studies from the continent and demonstrated an association between NAFLD and age, BMI, and features of the metabolic syndrome. Almobarak et al[113] also used USG to diagnose NAFLD in the general population at the Ibn Sina Hospital in Sudan and found the prevalence to be 20%. This prevalence was higher than a similar study done about 7 years earlier in the same hospital. This supports the worldwide evidence of the increasing prevalence of NAFLD. The study also showed a relationship between NAFLD and increasing BMI and age. The mean age in the study was 53 years and showed no disparity in the two genders.

Amongst T2DM patients, a dissertation by Kimani done at the Kenyatta National Hospital, Kenya in 2013 recorded the prevalence of primary NAFLD in T2DM patients as 34.4%.[114] He was also able to show that, NAFLD had a close relationship with obesity and high triglyceride levels. Currently, the mean prevalence of NAFLD among T2DM patients in Africa according to Younossi et al is 36.29%.[17]

In West Africa, information on NAFLD is from 3 studies in Nigeria and 1 unpublished study from Ghana.[33–36] The reported prevalence of NAFLD using USG in the general population in Nigeria and Ghana was 4.5% and 24.8% respectively.[33,36] The studies from Nigeria recorded an upsurge in the prevalence of NAFLD among T2DM patients from 9.5% in 2011 to 68.8% in 2018.[33,35] This showed that the prevalence of NAFLD in both the general population and in T2DM was rising in Nigeria just as is seen in the rest of the world.

2.5 DIAGNOSIS OF NONALCOHOLIC FATTY LIVER DISEASE

Most patients with NAFLD are asymptomatic. In such patients, hepatic steatosis may be detected incidentally during imaging or suspected when liver enzymes are elevated on routine investigations. Symptomatic patients may present with right upper quadrant pain, malaise and fatigue or with symptoms of advanced disease. A detailed medical and alcohol history is needed to make a diagnosis of NAFLD as its histologic features are similar to that of alcoholic liver disease and other causes of secondary NAFLD.[115]

In the evaluation of NAFLD, both non-invasive and invasive methods can be used. After confirmation, staging based on the degree of inflammation and fibrosis must be done to assess the severity of the disease.[116]

2.5.1 Invasive Methods

Liver histology is the gold standard for NAFLD diagnosis. It has the advantage of distinguishing between NAFL and NASH and also excludes other causes of steatosis. Samples are obtained by liver biopsy, either through the trans-jugular route or the percutaneous route with or without USG guidance.

Histologically, NAFLD is usually characterized by macrovesicular steatosis. The percentage of hepatocytes containing FFA can be used to grade steatosis as follows[19];

S1 or Mild: >5% to <10% of hepatocytes contain FFA.

S2 or Moderate: 10% to 30% of hepatocytes contain FFA.

S3 or Severe: >30% of hepatocytes contain FFA.

Characteristic features of NASH shown in Figure 10 are lobular inflammation, ballooning of hepatocytes, mitochondrial abnormalities and fibrosis.[115,117] These may be absent in advanced disease.

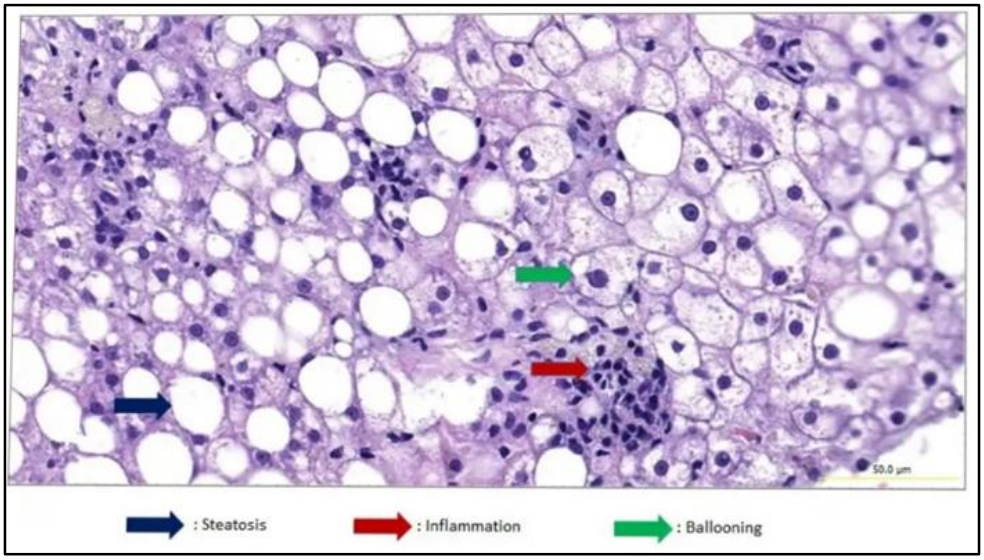


Figure 10: Histologic features of NASH

Source: http://www.kbimed.com/NewsDis-abd0058bb6de48f3870aa89fa6434755.htm[118]; cited 28th July 2019.

Lymphocytes, neutrophils and other mononuclear cells are the predominant cells in lobular inflammation.[115] Fibrosis usually occurs around the terminal hepatic veins in zone 3 but may involve the portal tracts.

According to Matteoni and colleagues[119] who analysed 132 histology-confirmed NAFLD specimen presented at the pathology department of the Cleveland hospital between 1979 and 1987, the NAFLD spectrum has different features and can be classified into 4 subtypes;

Type 1: Simple hepatic steatosis without inflammation.

Type 2: Hepatic steatosis with inflammation in the lobular region.

Type 3: Hepatic steatohepatitis with ballooning of hepatocytes only.

Type 4: Hepatic steatohepatitis with ballooning of hepatocytes, Mallory's hyaline, and sinusoidal fibrosis.

In this study, patients with types 3 and 4 had increased mortality from hepatic complications. Features of types 3 and 4 are equivalent to that of modern-day NASH.[120]

Currently, liver biopsy has limited use because it is invasive, costly and has a risk of complications.[121] This risk is minimal in USG-guided percutaneous biopsy. Histology is also observer-dependent. Reports have shown variations in results depending on the pathologist.[122] Furthermore, sampling errors have been reported depending on the site accessed.[123] Samples may be reported as normal when NASH lesions are focal.[115] The limited number of hepatologists and interventional radiologists to perform biopsies has also restricted the routine use of histology. These limitations and the current high prevalence of NAFLD has made histology impracticable as the gold standard in diagnosis.[124] This led to the development of non-invasive methods for diagnosis.

2.5.2 Non-invasive Methods

These methods include laboratory investigations and imaging. Newer methods have the ability to diagnose and stage NAFLD, thereby, limiting the need for biopsies.[116] However, their sensitivities and specificities are not comparable to histology.[125] A combination of non-invasive methods and clinical examination can be used to assess for advanced disease.

2.5.2.1 Laboratory Investigations

Laboratory investigations may reveal active inflammation or decreased synthetic activity of the liver. These may suggest the presence of NASH, fibrosis or cirrhosis.

(i) <u>Liver Biochemistry</u>

The entire spectrum of NAFLD can occur without derangement in liver enzymes. Clinical features of obesity, T2DM or obstructive sleep apnoea with deranged liver enzymes should suggest the likelihood of NAFLD.[116] Although the majority of patients with NAFLD have normal enzymes, raised transferase levels is an indicator of NAFLD and the commonest mode of presentation.[126]

This is significantly found in patients with NAFLD and T2DM.[29,127] This elevation of transferase levels however is minimal and usually does not exceed four times the upper limit of normal. Neuschwander-Tetri studied 1500 samples of biopsy-proven NAFLD and reported that the rise in ALT and AST was typically seen in the early stages of NASH and fibrosis but normalized in advanced fibrosis and cirrhosis.[128] Agarawal and colleagues went further to explain that early disease was associated with a rise in ALT but normalized as the disease advanced whiles AST levels rise with disease progression.[29]

Elevated serum gamma-glutamyltransferase (GGT) may occur in NAFLD and is an indicator of advanced disease. In a study involving 50 patients with biopsy-proven NAFLD, serum GGT had a sensitivity and specificity of 83% and 69% for predicting advanced fibrosis.[129] Also, Nagaraj et al.[127] reported significant increases in GGT in patients with both NAFLD and T2DM. Elevated GGT is known to be associated with increased mortality.[116,126]

Synthetic dysfunction of the liver, usually seen in advanced disease, is evidenced by hypoalbuminemia, hyperbilirubinemia and elevated INR or prothrombin time (PT).[116,126,130]

(ii) Full Blood Count

The level of platelet is an indication of the synthetic function of the liver. Thrombocytopenia in the setting of chronic liver disease may be as a result of hepatic dysfunction or indicate the presence of portal hypertension.[131]

White blood cell differentials, especially neutrophils and lymphocytes, can be used to predict the presence of NASH as described by the neutrophil-lymphocyte ratio (NLR).

(iii)Serum Ferritin

This is an acute phase reactant protein and has been found to be a predictor of NASH.[132] Raised ferritin levels have been observed in about 50% of patients with NASH.[133] Its involvement in the pathophysiology of NASH is unclear.[134] Ferritin level however does not correlate with the level of iron in the liver.

(iv)Novel Markers

The novel markers for inflammation include TNF-α, serum adiponectin, IL-6, cytokeratin-18 fragments and homeostasis model assessment-insulin resistance (HOMA-IR).[126,135] These are however costly and not readily available.

2.5.2.2 Imaging

Various imaging techniques are available for assessing steatosis and this includes CT scan, MRI, USG and transient elastography. An ideal imaging technique should be able to quantify steatosis and stage the disease.

(i) Ultrasonography

Hepatic steatosis shows on USG as echogenic or bright liver. Echogenicity is assessed by comparing the liver parenchyma to the cortex of the kidney, spleen or the walls of the intrahepatic vessels. This may be diffused or focalized near the porta hepatis. According to Lee et al[136], hepatic steatosis can be graded on a four-point scale from 0 to 3 depending on the severity of the echogenicity with grade 0 being the absence of steatosis. In their study, echogenicity was established by comparing the liver parenchyma to the cortex of the kidney, diaphragm and also the walls of the intrahepatic vessels. Adibi et al[137] used this grading system and documented grade 1 NAFLD as the most common finding on USG in a cross-sectional study among 483 participants of the general population in Iran. This is presented in Figure 11 and shows the overall prevalence of NAFLD as 39.3% with 21.1% as grade 1. Normal in this study indicated the absence of NAFLD. In Nigeria, Afolabi et al[35] however reported grade 2 NAFLD as the most prevalent (32.5%), followed by grade 3 NAFLD (20.0%) and grade 1 NAFLD (16.56%).

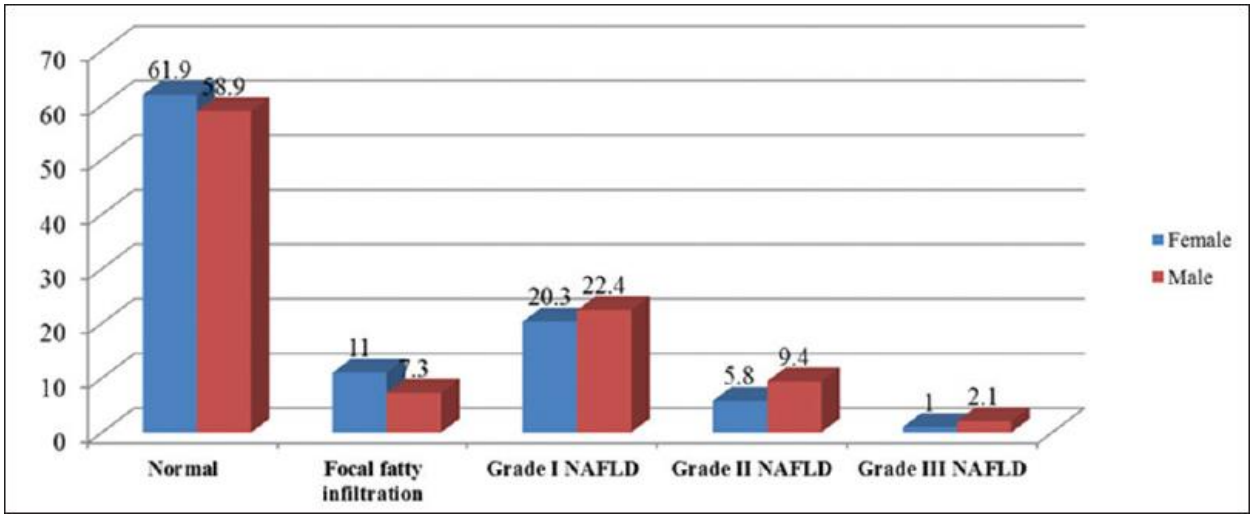


Figure 11: Prevalence of different grades of NAFLD diagnosed by USG

Source: Advanced Biomedical Research[137]; cited on 21st January 2020.

Ultrasonography is widely available, cheap and can be easily performed at the bedside.[138] It has comparable sensitivity to other imaging techniques when steatosis is greater than 30%, that is, 92.3%.[139] These advantages of USG makes it suitable as the imaging of choice in large screening programmes. Its use is however limited because it is operator dependent and this can lead to overestimation of NAFLD prevalence in inexperienced hands. The measurement of steatosis is only qualitative and shows a low specificity and sensitivity when steatosis is less than 30%.[136,140] Also, the sensitivity and specificity are decreased in morbid obesity.[141] Routinely, USG is unable to differentiate between NAFL and NASH. This can be overcome with the addition of the ultrasonic contrast agent, levovist.[142]

(ii) <u>Computed Tomography Scan</u>

Although its diagnostic yield is similar to that of the USG, CT scan has the advantage of quantifying steatosis by measuring the difference between hepatic and splenic attenuation.[116] It is accurate when hepatic steatosis is greater than 30%. At this grade of steatosis, it has a sensitivity and specificity of 73% to 100% and 95% to 100% respectively.[126] The non-contrast CT scan is preferred to the contrast type.

Computed tomography scans are generally expensive and not readily available. It also exposes patients to the hazards of radiation, especially those in the younger age bracket. Its use is unremarkable when steatosis is less than 30% and is also unable to differentiate between NAFL and NASH. There is a risk of misdiagnosis as other pathologies like siderosis affect hepatic attenuation.

(iii)Magnetic Resonance Imaging

In the detection of hepatic steatosis, MRI is superior to USG and CT scan.[143] It uses the difference in the chemical shifts between water and fat and can accurately diagnose even when steatosis is less than 30%. The use of MRS has enhanced the quantification of steatosis.[126] However, it is expensive with limited availability. It is also unable to stage NAFLD and hence, unable to differentiate between NAFL and NASH.

(iv)Transient Elastography

This is also known as the FibroScan and uses the principle of USG to assess liver stiffness, which is a measure of fibrosis.[144] Although initially validated for chronic hepatitis C infection, its use in NAFLD has now been validated by various studies. In a study among 97 NAFLD patients in Japan, Yoneda et al[145] showed a relationship between increased liver stiffness detected by FibroScan and histology-confirmed fibrosis. The diagnosis of cirrhosis, moderate and severe fibrosis in this study, showed an AUROC of 0.99, 0.88 and 0.91. Another study involving 246 patients recorded similar results with an AUROC for fibrosis stages F2, F3 and F4 as 0.84, 0.93 and 0.95 respectively.[146]

The use of the FibroScan is time-saving and can easily be done in an OPD or bedside setting. However, it has limited use in about 5% of people. For instance, in obese patients, there is a likelihood of not recording a reading because of excess abdominal fat. This has improved with the addition of the XL probe.[144,147]

Magnetic resonance imaging can be used for transient elastography instead of USG, that is, magnetic resonance elastography (MRE). This has enhanced the ability of the FibroScan in detecting all grades of fibrosis, thereby, improving its sensitivity and specificity to 98% and 99% respectively.[148] Although diagnostic efficiency is improved, the MRE is more expensive and not widely available.

(v) Abdominal subcutaneous fat thickness

Abdominal fat can be evaluated by either measuring the visceral fat or subcutaneous fat thickness using imaging techniques. Using MRI, Ducluzeau did not find an association between steatosis and abdominal subcutaneous fat.[149] Parente also used MRI in a prospective study involving 62 patients with T2DM and reported that the abdominal visceral fat measured correlated well with the degree of steatosis diagnosed by histology and was also able to predict NASH and fibrosis.[150] In this study, abdominal subcutaneous fat did not correlate with NAFLD but was able to predict NASH. Although these earlier studies did not find a correlation between NAFLD and abdominal subcutaneous fat, Hegazy and colleagues in 2019 used USG and documented a correlation between these 2 parameters.[151] Subcutaneous fat thickness recorded in the umbilical region and left lobe of the liver is a significant predictor of hepatic steatosis. Lee et al also documented an association between these 2 parameters in obese children.[152]

2.6 SCORING SYSTEMS

Various scoring systems use clinical and laboratory parameters in the prediction of steatosis, steatohepatitis, fibrosis and cirrhosis in patients with NAFLD. This increases the sensitivity and specificity of diagnosis. The scoring systems include the NAFLD fibrosis score, APRI score, fatty liver index, BARD score, AST/ALT ratio (AAR) and fibrosis-4 (FIB-4) index.[124,153,154] The AASLD guidelines documented that, the NAFLD fibrosis and FIB-4 scores were better predictors of advanced fibrosis than the other scores.[5] The scores used in this study are described below.

2.6.1 AST / ALT Ratio

Both AST and ALT are parameters of the liver biochemistry test and can be run easily in most clinics. The AAR is used to predict the presence of significant fibrosis and cirrhosis. It was developed by McPherson and colleagues who compared the ability of some non-invasive test to detect advanced fibrosis in patients with histology-proven NAFLD.[155] In the prediction of advanced fibrosis, AAR showed a sensitivity and specificity of 74% and 78% respectively with an AUROC of 0.83. It was however not able to differentiate NAFL from NASH. Later, a study by the Nonalcoholic Steatohepatitis Clinical Research Network (NASH CRN) in the USA, confirmed AAR was better at predicting cirrhosis than NASH with an AUROC of 0.81 and 0.59

respectively.[128] The AAR in NASH patients was low but increased gradually with progression from fibrosis to cirrhosis with values >1 correlating with cirrhosis on histology.

The prediction accuracy of AAR was enhanced with the addition of clinical and laboratory parameters like BMI and comorbidities. This improved the AUROC to 0.79 and 0.91 for NASH and cirrhosis respectively. This new finding gave rise to other scoring systems. Currently, AAR is either used singly or as a parameter in other scoring systems including the BARD and NAFLD fibrosis scores.

2.6.2 NAFLD Fibrosis Score

This score was designed mainly to assess the presence of significant fibrosis in NAFLD patients. Variables of the score are AAR, serum albumin level, platelet count, hyperglycaemia, BMI and age. It was developed at the Mayo Clinic by Angulo and colleagues during a study among 733 patients with histology-confirmed NAFLD.[156] They discovered that the 6 variables of the score were all independent predictors of fibrosis in NAFLD. Applying all variables in a single model increased the accuracy of prediction and hence, reducing the need for liver biopsy in the diagnosis of fibrosis. In the study by Angulo et al[156], participants were assigned to either an estimation or a validation group. With cut off values of -1.455 and 0.676, the estimation group had the negative predictive value (NPV) and positive predictive value (PPV) as 93% and 90% respectively whiles the validation group had values of 88% and 82% respectively. In their study comparing various scoring systems, McPherson et al recorded the NPV and PPV of this score as 92% and 72% respectively.[155] Later studies also proved its accuracy of excluding significant fibrosis in different populations including Japanese and Chinese.[154,157–159]

The formula for NAFLD fibrosis score is;

$$(-1.675 + 0.037 - \text{age (years)} + 0.094 - \text{BMI (kg/m}^2) + 1.13) \times (\text{IFG / DM} + 0.99) \times (\text{AAR} - 0.013) \times (\text{platelet count } (\times 10^9/\text{l}) - 0.66) \times (\text{albumin (g/dl)})$$

In this formula, a value of 0 or 1 is given when IFG or DM is absent or present respectively. The current cut-offs for this score are <-1.455 which signifies the absence of significant fibrosis and > 0.676 which correlates with significant fibrosis.[5]

2.6.3 APRI Score

The APRI score is used for assessing fibrosis and cirrhosis. This score is easy to calculate as the parameters used are easy to obtain and also inexpensive. The formula for the score is;

APRI score = [(AST value ÷ Upper limit of normal AST range) x 100] ÷ Platelet (10^9/L)

It was originally used and validated by Wai et al among chronic hepatitis C patients.[160] They documented that, the score accurately predicted significant fibrosis and cirrhosis by 51% and 81% respectively in patients who had never received treatment. Subsequently, the APRI score has been used among NAFLD patients. Cales et al[159] assessed for fibrosis and cirrhosis among 235 patients with NAFLD and reported an AUROC of 0.866 and 0.842 for significant fibrosis and cirrhosis respectively. Other studies done to evaluate the APRI score including that by McPherson et al among 135 NAFLD patients, however, reported a lesser AUROC and hence, was less accurate in predicting fibrosis.[155] In NAFLD patients, an APRI score of ≥1.5 was found to be associated with significant fibrosis whiles values of <0.5 was a negative predictor of fibrosis.

2.6.4 BARD Score

The BARD score was designed to predict the absence of advanced disease. It incorporates 2 clinical variables and the AAR. The variables and points assigned are shown in Table 1.

Table 1: Variables and points of the BARD score

VARIABLE	**POINTS ASSIGNED**		
	0	**1**	**2**
BMI	<28	≥28	-
Presence of DM / IFG	No	Yes	-
AAR	<0.8	-	≥0.8

After summation of the individual points, a score from 0 to 4 may be obtained. A higher score is a predictor of advanced fibrosis. A score of 2 to 4 correlates with F3 and F4.[103] A score of 0 or 1 is a negative predictor of significant fibrosis. In the study by Ruffillo et al[161] among 138 people

with NAFLD diagnosed by histology, the NPV and PPV for BARD was 81% and 45% respectively. The sensitivity and specificity were reported as 51% and 77% respectively. However, McPherson and colleagues reported a higher NPV of 95% but a lower PPV of 25%. Sensitivity was 89% whiles specificity was 44%.[155]

2.6.5 Fibrosis-4 Index

This score uses 4 parameters, that is, age, platelet count and ALT and AST levels, to predict the presence of fibrosis. Among NAFLD patients, it showed a sensitivity and specificity of 85% and 65% respectively with an NPV of 95% in the prediction of significant fibrosis.[126] Earlier studies reported that FIB-4 had an advantage over NAFLD fibrosis score, AAR, APRI score and BARD score in predicting the presence of advanced fibrosis.[126] However, Imajo et al in a study comparing the ability of the various scoring systems and MRE with histology in predicting advanced fibrosis reported that, FIB-4 score's ability to predict significant fibrosis was comparable to that of NAFLD fibrosis score and MRE.[162]

The formula of the score is;

$$\text{Age (years)} \times \text{AST (U/L)} / [\text{Platelet } (10^9/\text{L}) \times \text{ALT}^{1/2} \text{ (U/L)}]$$

In NAFLD, a FIB-4 value of 1.30 or less rules out the presence of fibrosis whiles a value of 2.67 or more predicts the presence of significant fibrosis.[163]

2.6.6 Neutrophil Lymphocyte Ratio

The NLR has proven to be effective in distinguishing between NAFL and NASH. In a study among 101 patients undergoing liver biopsy for suspected NAFLD, Alkhouri and colleagues were able to predict the presence of NASH or fibrosis using the elevation of NLR.[164] In this study, patients with NASH had NLR of 1.9 to 3.3 with a mean of 2.5. It also showed an association between NLR and the NAFLD activity score. Also, the study showed that NLR increased with the degree of fibrosis with values of >2.9 correlating with F3 and F4. Tanoglu and colleagues also used NLR to predict cirrhosis.[165] These findings were supported by Abdel-Razik et al.[166]

2.7 COMPLICATIONS OF NONALCOHOLIC FATTY LIVER DISEASE

The complications of NAFLD are either hepatic or extra-hepatic and have high morbidity and mortality rates. Hepatic complications include cirrhosis and HCC. Both NAFL and NASH can progress to fibrosis but NASH has a greater risk of advancing.[167,168] A systematic review and meta-analysis by Singh et al[167] reported the fibrosis progression rate per year from stage 0 as 0.07 and 0.14 for NAFL and NASH respectively. They also documented that, patients who progressed from fibrosis stage 0 are either rapid or slow progressors with the majority being slow progressors. Only 20% were rapid progressors and these advanced from stage 0 through to F3 or F4. Slow progressors advanced from stage 0 to F1 or F2. Pais and colleagues had earlier reported that progression from NAFL to fibrosis occurred in patients who had steatosis with mild inflammation compared with those who had only steatosis at initial diagnosis.[168] Type 2 DM is a risk factor for the progression of NAFLD. This was evident in a study by Adams et al[169] which observed that 37% of its 103 participants showed an increase in fibrosis stage by histology within a 3-year period with DM and high BMI were independent predictors of progression to advanced fibrosis.

Both NAFL and NASH can lead to cirrhosis and HCC.[19] A study by Angulo et al[20] showed that the risk of progression to cirrhosis after 15 years of follow up, was 0.7% and 10.8% for NAFL and NASH respectively. The risk of HCC is also increased in NAFLD especially in those with NASH and in cirrhotic livers.[170] It may also occur in non-cirrhotic livers with T2DM and obesity as independent predictors.[171,172] As seen in fibrosis, HCC risk is also increased in T2DM. In a nationwide cohort study done in Denmark where records of patients diagnosed with DM were assessed, there was a 3-fold risk of HCC in T2DM.[172] This risk increased to 4-fold in the presence of chronic hepatitis, cirrhosis or alcohol use, suggesting a synergistic effect. Diagnosis of DM in this study preceded the diagnosis of HCC by many years.

Extra-hepatic associations of NAFLD include cardiovascular diseases, chronic kidney diseases and colorectal cancer.[23,173] Cardiovascular manifestations are the most common extrahepatic association and include coronary heart diseases, peripheral vascular diseases and cerebrovascular diseases. These manifestations are usually a result of its association with metabolic syndrome. According to Arslan et al[174] who conducted a prospective longitudinal study in 100 patients in Turkey, the risk of developing cardiovascular disease was increased by 9.0% among patients with NAFLD compared with 3.8% in those without NAFLD. This risk was also higher in patients with

metabolic syndrome. Concerning T2DM, Targher et al[25] revealed a 1.87-fold increase in the risk of cardiovascular events when NAFLD co-existed with T2DM. Nonalcoholic fatty liver disease also increases the risk of other T2DM complications including retinopathy and chronic kidney disease and also increases the daily insulin requirement.[175]

2.8 MANAGEMENT OF NONALCOHOLIC FATTY LIVER DISEASE

Currently, no pharmacologic therapy has proven effective in the treatment of NAFLD but research in this field is still ongoing. The mainstay of treatment is lifestyle modification but surgical and pharmacological methods can be used in patients who have advanced disease.[5,176] Effective management of NAFLD needs a multi-disciplinary approach involving hepatologists, endocrinologists and dieticians and must include aggressive management of all risk factors.[5]

2.8.1 Lifestyle Modification

Lifestyle modification is beneficial when NAFLD is detected early as management of advanced chronic liver disease is usually unsuccessful. It is also beneficial in the management of metabolic syndrome that may co-exist with NAFLD. Lifestyle modification involves a combination of physical exercise and dietary modification.[5,177] This must be specific for each patient and must be done in a period of 6 to 12 months with the aim of a reduction in total body weight. Resistance exercises have shown more benefits than aerobic exercises in the management of obesity and NAFLD. Hallsworth and colleagues reported an improvement in hepatic steatosis of 11 patients with NAFLD who were previously physically inactive but assigned to resistance training for a period of 8 weeks.[178] They concluded that even in the absence of significant weight loss, resistance exercises increased muscle bulk leading to a decrease in IR and increased adiponectin levels. These exercises also improved blood sugar levels.

Many studies have confirmed that lifestyle modifications may normalize aminotransferase levels and improve steatosis.[179,180] Most of these studies used imaging techniques or other markers for the diagnosis and monitoring of steatosis as liver biopsy is invasive. Lomanaco and colleagues, however, used liver biopsy to monitor patients on diet therapy and exercise and observed histological improvement in steatosis with a 3% - 5% loss in body weight.[12] Also, necroinflammation in NASH patients improved with a 7% - 10% loss in total body weight.

Recently, Barb et al[176] reported a 40% to 80% improvement in hepatic steatosis when body weight reduced by 8% to 10%.

2.8.2 Treatment of Nonalcoholic Steatohepatitis

Drug therapy in NAFLD management is not routinely done but may be used in the setting of steatohepatitis. Despite the unavailability of curative medications, the use of thiazolidinediones (TZD) and vitamin E is now considered the most effective in the management of NASH.[12]

The TZD, pioglitazone, acts by improving the sensitivity of insulin on adipocytes, hepatocytes and skeletal muscles, thereby, limiting lipolysis and reducing FFA levels. It also raises the plasma adiponectin levels. The effectiveness of pioglitazone was first reported by Belfort et al[181] in a controlled trial done over 6 months. In this trial, participants with T2DM or IFG and histology-proven NASH were randomly placed in one of 2 groups. Both groups were then placed on a low-calorie diet and one group assigned to pioglitazone whilst the other group was on placebo. At the end of the trial, there was a significant reduction in aminotransferase levels with improvement in blood glucose levels among patients on the pioglitazone arm. Also, there was an improvement in microscopic inflammation and the NAFLD activity score, by 73% in the pioglitazone group and 24% in the placebo group. Improvement in the degree of fibrosis was however insignificant. Aithal and colleagues[182] supported this finding in a study that recorded significant improvement in steatosis and inflammation with normalization of aminotransferases in participants on pioglitazone. In their study, all participants had histology-proven NASH without T2DM and were placed on a low caloric diet plus moderate exercises like walking and swimming. They were then placed on either the pioglitazone or placebo arm and monitored for 12 months.

In 2010, the PIVENS trial conducted by Sanyal et al[183] over a 2-year period in non-diabetic patients had 3 arms, that is, the vitamin E arm, the pioglitazone arm and the placebo arm. Participants on vitamin E and pioglitazone therapies showed improvements in aminotransferases, hepatic steatosis and inflammation compared with those on placebo but these improvements were more remarkable in the vitamin E arm. Fibrosis was however unchanged in all groups and participants on the pioglitazone arm reported significant weight gain. Vitamin E acts by inhibiting the intrinsic pathways for apoptosis and also protects the cells from oxidative radicals, acting as an antioxidant.[184] Recently, concerns about the safety of vitamin E have arisen because of an increase

in the overall mortality among patients who use vitamin E supplements.[185] Vitamin E supplementation has also been associated with an increased risk of haemorrhagic stroke and prostate cancer.

Pioglitazone is currently used in the treatment of T2DM but not recommended in NASH. This is because its long term benefits in the treatment of NASH is still unknown as all studies done lasted less than 24 months.[186] It can however be used as the drug of choice in NASH patients with T2DM. Vitamin E is recommended by AASLD in the treatment of NASH in patients without T2DM.[184] Vitamin E is cheap and effective at a dose of 400 units twice a day. However, because of its side effects, it is recommended that confirmation of NASH by histology be made before initiation of therapy.[185]

Research is still ongoing to produce medications for the definitive management of NASH and includes peroxisome proliferator-activated receptor (PPAR) and farnesoid X receptor (FXR) agonists.[187] Most of these studies are in phase 2 or 3 trials. Elafibranor, a PPAR currently in phase 3 trials, produced significant resolution of steatohepatitis in patients with severe disease in the GOLDEN phase 2b trial.[188] Other PPAR agonist currently being developed include lanifibranor, saroglitazar and seladelpar. Obeticholic acid (OCA), an FXR agonist, which plays a crucial role in the metabolism of bile acids, is currently in phase 3 trial. In the FLINT study, OCA caused a significant histologic resolution of NASH and fibrosis.[189] Other agents in trials include cenicriviroc and selonsertib.

Liver transplant may be considered in patients with cirrhosis.[5]

2.8.3 Treatment of Type 2 Diabetes Mellitus in Nonalcoholic Fatty Liver Disease

Research to unravel common treatment agents for NAFLD and T2DM has gained interest because of their common pathophysiology. These research works have involved mainly oral hypoglycaemic agents. Initial works involved metformin as it has the ability to decrease IR mainly in the liver.[190] Loomba et al[191] used metformin to treat participants with histology-proven NASH and recorded significant improvements in the levels of aminotransferase in patients with NASH along with improvements in steatosis and inflammation on repeat histology. Later studies however did not support these findings.[192,193] Although metformin remains the first-line treatment for T2DM, it is however not recommended in NASH.

The oral hypoglycaemic agent that has proved effective in both T2DM and NASH is pioglitazone which is discussed above. Studies using drugs like sitagliptin and the glucagon-like peptide-1 analogue, liraglutide, in the LEAN trial did not show remarkable benefits.[194] Investigations are still ongoing in this area.

In patients with both T2DM and NAFLD, lifestyle modification and blood glucose control improve overall outcome.

2.8.4 Treatment of Obesity in Nonalcoholic Fatty Liver Disease

The most effective treatment modality for obesity is lifestyle modification as described above. Although pharmacological agents like sibutramine and orlistat have been available for the past 20 years and previously used in the management of NAFLD, they did not show any remarkable advantage over the traditional diet and exercise regime.[195]

Bariatric surgery has been used for weight reduction and has shown a reduction in hepatic steatosis and steatohepatitis as well as improve the control of hypertension, T2DM and hyperlipidemia.[196] A foregut bariatric surgery is however recommended only in patients who have obesity co-existing NAFLD or NASH.[5]

2.8.5 Treatment of Dyslipidaemia in Nonalcoholic Fatty Liver Disease

Patients with either NAFLD or dyslipidaemia are at an increased risk of cardiovascular diseases.[197] In NAFLD, the commonest feature of the dyslipidaemia seen is low HDL with high triglycerides and LDL levels.

Fibrates are effective in lowering triglyceride levels whiles elevating HDL levels. The use of fenofibrate in about 16 patients with NAFLD in a study by Fernandez-Miranda et al recorded improvements in the level of aminotransferases and hepatocyte ballooning in some patients.[198] However, there was no resolution of steatosis. Other studies using other types of fibrates showed similar results.[199,200] A review article by Jelske and colleagues concluded that, although the use of fibrates may result in the normalization of aminotransferase levels, its ability to reduce hepatic steatosis was unremarkable.[201]

Statins are currently considered safe for treating dyslipidaemia in the setting of NAFLD.[202,203] However, this should be initiated at low doses and gradually increased to therapeutic doses whiles

monitoring the level of the aminotransferases. It should be withheld when liver injury, evidenced by increasing aminotransferase levels occurs. A combination of a statin and fenofibrate is the current treatment of choice for patients with elevated triglycerides and low HDL.[204]

2.9 PREVENTION OF NONALCOHOLIC FATTY LIVER DISEASE

To prevent the occurrence of NAFLD and its complications, risk factors like obesity, T2DM and hypertension must be effectively managed.[82] Effective preventive measure is similar to management of the condition. This involves modifying both dietary habits and physical activity. The role of resistance exercise is beneficial as demonstrated by Hallsworth et al.[178] Dietary habits that offer benefits include increased intake of vegetables, fruits, nuts and whole grains as observed in the Mediterranean diets.[205] Reduction in the consumption of meat also aids in the prevention of NAFLD.

2.10 PROGNOSIS OF NONALCOHOLIC FATTY LIVER DISEASE

Prognosis is dependent on the severity of the disease and the presence or absence of complications.[171] Nonalcoholic fatty liver has a favourable prognosis compared with NASH. Bhala et al reported a ten-fold increase in mortality among NASH patients with mortality mostly being as a result of hepatic causes.[171]

A study among 68 participants with NASH cirrhosis reported the 5-year survival as 75.2%.[206] Mortality in this study was as a result of decompensation of cirrhosis or the development of HCC. In another study done between 1995 and 2004 by Zipprich et al, the 1-year mortality rate in participants with compensated and decompensated cirrhosis was 5.4% and 20.2% respectively.[207] Compensated cirrhosis had longer median survival than decompensated cirrhosis, that is, 78.7 months and 29.5 months respectively. Age was a predictor of mortality among participants with compensated cirrhosis.

In most patients, HCC is diagnosed at an advanced stage and hence, the prognosis is usually poor. However, for patients who present with early disease, treatment improves prognosis. Benhammou et al after a median follow-up of patients with HCC caused by NAFLD, HBV or HCV documented a longer survival rate in those with NAFLD-related HCC who received any form of treatment

compared with those with HCC from other causes.[208] Orthotopic liver transplantation offered the best reduction in mortality. The recurrence rate was however higher in this group.

CHAPTER 3

METHODOLOGY

3.1 MATERIALS AND METHODS

3.1.1 Study Design

The study was a hospital-based, cross-sectional study among adults attending the DM clinic at the GARH in Accra, Ghana. It was conducted between May and October 2018.

3.1.2 Study Site

The GARH, formerly known as the Ridge Regional Hospital, is situated in Accra, the capital city of Ghana. Accra is located in the Greater Accra Region which has a population of about four million people.[209] Inhabitants of this region are of a multi-cultural origin. The hospital was built in the year 1929 with a 191-bed capacity but expansion works completed in the year 2016 has increased this capacity to 420. It is a tertiary facility under the Ghana Health Service. It provides emergency, in-patient and outpatient services in Obstetrics and Gynaecology, Child Health, Internal Medicine, Surgery and Dentistry. Clients of the hospital are either self-referred or referred from other health facilities in the region and neighbouring regions, mainly in the southern part of the country. In 2016, DM was the second most common cause of outpatient attendance at the hospital with complications of the disease being ranked as the seventh leading cause of mortality.

The Department of Internal Medicine runs daily physician specialist clinics on weekdays at the outpatient's department and weekly subspecialty clinics. According to the hospital's 2016 report, the department recorded an average monthly outpatient attendance of about 927 patients with an annual turnout of 11,119 patients. The department runs a weekly DM clinic on Tuesday mornings for patients. The average monthly attendance at this clinic in 2016 was 224 patients with an annual attendance of 2,694 patients. The clinic is manned by two endocrinologists, medical officers and house officers rotating in the department. They are supported by the nursing staff, the public health team and the dietitians. At every clinic, patients are educated on DM care and good dietary habits. Their blood pressure and fasting blood sugar are recorded followed by a consultation with the doctors.

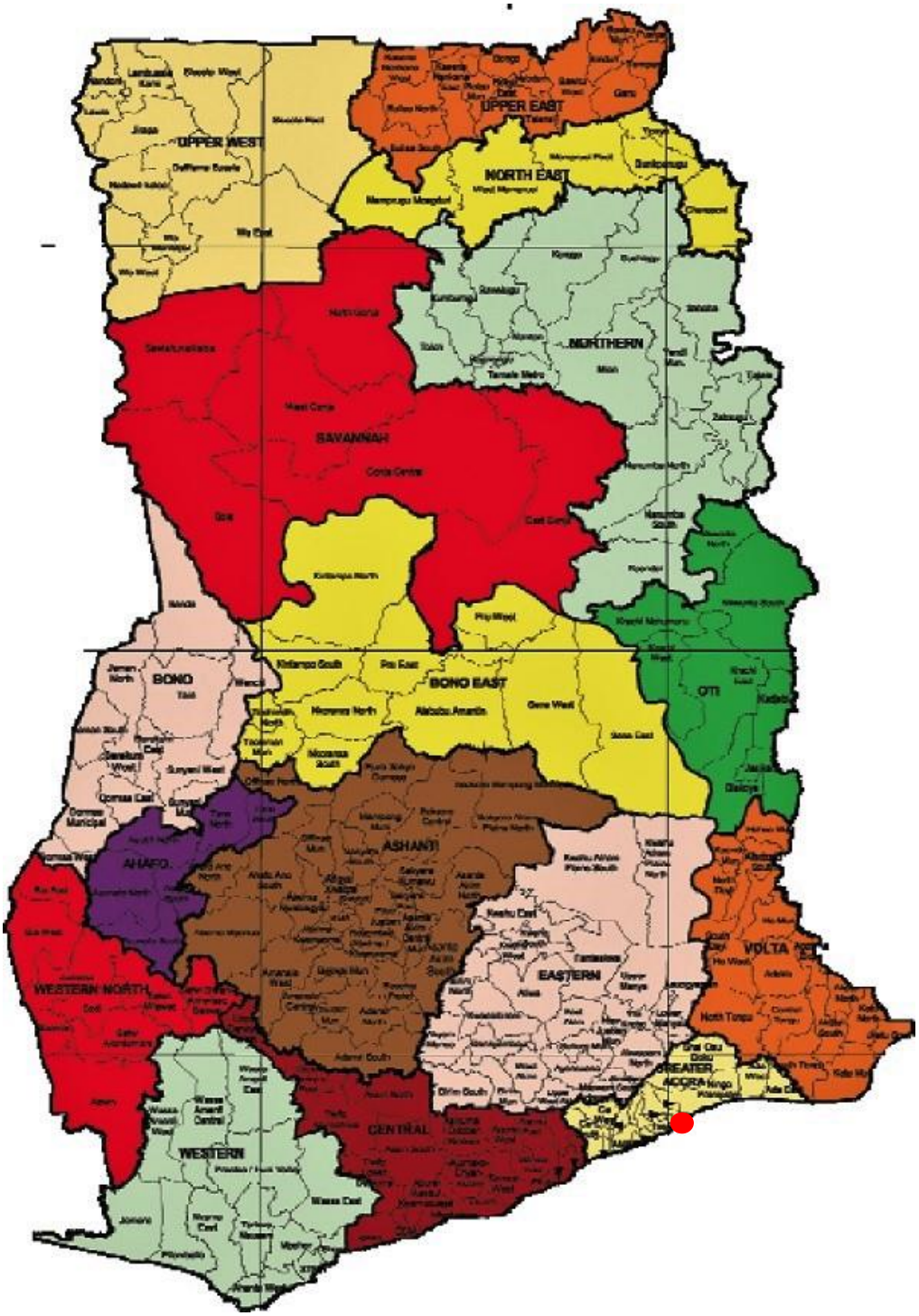


Figure 12: Map of Ghana showing Accra where GARH is located

Source: https://www.graphic.com.gh/features/features/evolution-of-the-regions-from-5-to-16.html cited on 1st April 2021.

Figure 13: Study site – The Greater Accra Regional Hospital
Source: https://www.livinspaces.net cited on 18th March 2021

3.1.3 Study Population

All patients with T2DM or IFG diagnosed at age 18 years or more and attending the weekly DM clinic were eligible for inclusion in the study. Patients with the above criteria and currently being managed with diet therapy or oral hypoglycaemic agents or previously managed successfully with oral hypoglycaemic agents but now on insulin therapy were also eligible for the study.

3.1.4 Selection Criteria

Selection of participants was done using the inclusion and exclusion criteria below.

3.1.4.1 Inclusion Criteria

All adult patients with T2DM or IFG who were diagnosed at age 18 years or above using the American Diabetes Association (ADA) guidelines and fulfilled the following criteria at the time of recruitment;

1. Being managed with diet therapy, oral hypoglycaemic agents or previously managed successfully with oral hypoglycaemic agents but now on insulin therapy.

2. Have a negative history of significant alcohol intake, that is, less than 30g per day in men and 20g per day in women. This is equivalent to less than 21 units and 14 units per week in men and women respectively.

3. Have a negative screen for viral hepatitis B and C using a rapid diagnostic kit, that is, tested negative for HBsAg and HCV Ab.

3.1.4.2 Exclusion criteria

1. Patients with a history of significant alcohol intake as described above.

2. Patients with serologic evidence of HBV or HCV at the time of recruitment, that is, positive tests for HBsAg and HCV Ab.

3. Patients with evidence of other causes of NAFLD (e.g.) drugs.

4. Patients with evidence of terminal diseases (e.g.) malignancies.

5. Patients with a diagnosis of gestational diabetes.

6. Pregnant women.

3.1.5 Sample Size Determination

The appropriate sample size for this study was determined by three factors:

(i) the estimated prevalence of NAFLD in T2DM in Sub-Saharan Africa
(ii) the desired level of confidence and
(iii)the acceptable margin of error

The sample size was determined using the equation below.

$$n = [z^2\ p\ (1\text{-}p)] \div d^2$$

n = sample size

z = confidence level at 95% (standard value of 1.96)

p = estimated prevalence of NAFLD in T2DM. This was 16.7% based on a study done in Nigeria.[34]

d = margin of error at 5% (standard value of 0.05)

Substituting these values,

$$n = [(1.96)^2 \times (16.7 \div 100) \times (1 - (16.7 \div 100))] \div (0.05)^2$$

$$n = [(1.96)^2 \times 0.167 \times (1 - 0.167)] \div (0.05)^2$$

$$n = [3.842 \times 0.167 \times 0.833] \div 0.0025$$

$$n = 0.534 \div 0.0025$$

$$n = 213.6$$

$$n = 214$$

To allow for contingencies (non-responses and incomplete data), the minimum sample size was increased by 10%. The minimum sample size for this study was therefore **235**.

3.1.6 Sampling Technique

The selection was by systematic random sampling with every fifth patient attending the weekly clinic being selected. The first participant was selected by simple random sampling.

This sample interval was determined using this equation;

$$\text{Sample interval (SI)} = \text{Population size (P)} \div \text{sample size (n)}$$

P = average monthly DM clinic attendance (224) x period of study (5 months)

n = 235

Substituting these values;

$$SI = [(224 \times 5) \div 235]$$

$$SI = 1120 \div 235$$

$$SI = 4.7$$

$$SI = 5$$

3.2 DATA COLLECTION

3.2.1 Data Collection: Questionnaire

On the morning of each DM clinic, an explanatory talk on the study was delivered to all attendants of the clinic. Both new patients and those reporting for follow up visits were included in the study. The research assistant selected folders of all eligible patients and arranged them in a pile. These folders were given numbers which were then entered in a ballot. The folder of the first participant was determined by an independent individual by simple random sampling using the numbers generated from balloting. Other potential participants were then selected by systematic random sampling with every fifth patient being selected.

During the time of checking the vital signs, selected patients who agreed to participate in the study were given further explanation on an individual basis and in a language of their choice. An interpreter was used where necessary. Hepatitis B and C virus screen was done for each patient using the rapid diagnostic test kits (Core Technology Company Limited, China). Patients who satisfied the inclusion criteria were recruited into the study and made to sign or thumbprint an informed consent form (Appendix III).

A total of 235 consenting patients were recruited using the above process. They were each given a unique code to hide their identity. Vital signs including blood pressure (BP), body weight in kilograms (kg) and height in meters (m) were recorded for each participant. Fasting blood sugar (FBS) levels were also checked with a glucometer. Participants were then referred to the researcher for data extraction.

A semi-structured questionnaire (Appendix IV) was used to extract data from participants. This was done by face to face interviews and also from participant's folders which contained their medical records. Data obtained included current and past medical histories, drug history and information on physical activity. The clinical data obtained with the aid of the semi-structured questionnaire were grouped into drug history, duration of DM, presence of current symptoms of

liver disease, non-specific examination features, stigmata of chronic liver disease on examination, features of IR, anthropometric measurements and liver characteristics.

3.2.1.1 Medical History

The medical history extracted included socio-demographic and clinical information. The socio-demographic information included age, sex, marital status, educational level, occupation, employment status, religion and ethnicity. These factors were grouped for easy analysis as seen in appendix IV. Their occupation was further grouped according to the International Standard Classification of Occupations.[210] The current residence of participants was also noted and classified as either urban or rural.

Information on the presence or absence of symptoms of liver disease was obtained. Duration of T2DM and compliance with anti-diabetic agents and diet therapy was also obtained. A personal and family history of metabolic diseases and also information on drugs used by patients in their management were extracted. Exercise history was also acquired.

3.2.1.2 Clinical Examination

A general and complete abdominal examination was performed on all participants by the researcher. The general examination included anthropometric measurements and examination for stigmata of chronic liver disease and features of IR.

Anthropometric measurements, weight (kg) and height (m), were taken with a Seca 756 mechanical column weighing scale, fitted with a Seca 222 mechanical telescopic measuring rod (Seca Deutschland, Germany). This was done without the participant wearing shoes and with minimal clothing. Two readings for each measurement was taken and the averages calculated and recorded.

The BMI (kg/m^2), which is a ratio of weight (kg) to the square of height (m) was calculated from the above parameters using the online app www.omnicalculator.com and classified according to the current WHO criteria.[211] The BMI classes and corresponding ranges are shown in Table 2.

Table 2: BMI classification according to WHO criteria

BMI Class	Weight range
Underweight	$\leq 19.9 kg/m^2$
Normal	$20.0 kg/m^2$ - $24.9 kg/m^2$
Overweight	$25.0 kg/m^2$ - $29.9 kg/m^2$
Class I	$30.0 kg/m^2$ - $34.9 kg/m^2$
Class II	$35.0 kg/m^2$ - $39.9 kg/m^2$
Class III	$\geq 40.0 kg/m^2$

Measurements for waist and hip circumference were also taken for the diagnosis of central obesity. Two different readings were recorded for each participants using a tape measure with minimal clothing barrier. The average of each of these measurements was calculated and recorded. Measurement was taken using the guidelines of the WHO STEPS protocol, that is, participant in the upright position with feet closed and hands by the sides.[212] The reference point for the waist circumference was midway between the lowest palpable rib and the iliac crest and measurement started and ended at the right mid-axillary line. The hip circumference was measured at the widest part of the buttocks with measurement starting and ending at the right mid-axillary line. The waist to hip ratio was then calculated using the app on www.omnicalculator.com. Central obesity was diagnosed when waist circumference was >80cm and >94cm for females and males respectively[213] and waist to hip ratio ≥0.90 and ≥0.85 for men and women respectively.[212]

Two resting BP readings were taken within a thirty-minute interval using an Omron M2 Intellisense digital BP monitor (Omron Corporation, Japan). This was usually taken on the right upper arm with the forearm resting on a flat table. The cuff of the sphygmomanometer was placed on the upper arm with the lower edge just above the elbow. The averages of the systolic and diastolic BP were calculated from these two readings and used for analysis. The highest value was used to classify BP according to the current ESH/ESC guidelines, that is, optimal, normal, high normal, grade I, grade II, grade III or isolated systolic hypertension.[214] The BP classes and their corresponding blood pressure range are shown in Table 3.

Table 3: Blood pressure classification according to 2013 ESH/ESC guidelines

BP Class	Systolic BP range (mmHg)	Diastolic BP range (mmHg)
Optimal	< 120	< 80
Normal	120 – 129	80 – 84
High normal	130 – 139	85 – 89
Grade I	140 – 159	90 – 99
Grade II	160 – 179	100 – 109
Grade III	≥ 180	≥ 110
Isolated systolic hypertension	≥ 140	< 90

A general examination was performed with the participant lying on a couch to assess for stigmata of chronic liver disease, specifically, pallor, jaundice, lymphadenopathy, spider naevi, clubbing, palmar erythema, and pedal oedema. Signs of IR were also assessed. These included acanthosis nigricans, xanthomata and arcus cornealis. General examination was classified either as asymptomatic or symptomatic based on the absence or presence of any of the above-mentioned signs.

An abdominal examination was also carried out to assess for advanced liver disease. The presence or absence of signs like abdominal distension, dilated veins, hepatomegaly, splenomegaly and ascites were documented. The normal liver size was a liver span of 12 to 15cm.[215] A span of more than 15cm was considered as enlarged (hepatomegaly). A shrunken liver had a span of less than 10cm. A palpable spleen of any size was noted as splenomegaly. Regarding ascites, the assessment was done mostly by shifting dullness and fluid thrill when there was a grossly distended abdomen. Abdominal examination was classified as normal when there was no abnormality and abnormal when any of the signs were present.

3.2.2 Data Collection Tools: Laboratory

Results of FBS and glycated haemoglobin (HbA1c) presented at each clinic visit were obtained from participants' medical records. The level of the HbA1c was used to determine DM control.

For this study, an HbA1c value of less than 7% was considered as controlled DM whiles a value of 7% or more was uncontrolled DM. This was based on the ADA 2017 guidelines.[216]

After this initial process, participants were scheduled for a second visit which coincided with their next clinic visit for sample taking and abdominal USG. They were made to undertake an 8 to 12 hour fast before drawing samples for fasting lipids and abdominal USG. For convenience, blood samples for all other tests were drawn at this particular time. Blood was drawn from the most accessible large calibre vein, which was usually from the cubital fossa of the left upper limb. Blood samples were drawn for FBC (to assess for haemoglobin level, white blood cell count with differentials and platelets count), INR, lipids and liver biochemistry including albumin. A total of 10mls of venous blood was drawn. About 4mls of this was placed in an EDTA tube for FBC analysis, 2mls in a sodium citrate tube for INR and 4mls in a gel separator tube for lipid and liver biochemistry analysis. All samples were correctly labelled with the assigned code of participant, gender, age, date and time of sample collection.

After collection, samples were gently inverted 8 times, placed in sealed bags and transported in an upright position in a chest containing ice packs to the Central Laboratory Department of the Korle Bu Teaching Hospital for analysis. The time between sample collection and processing was a maximum of 2 hours. Haematological samples and biochemical samples were analysed using the Hemix 5-60 auto-analyser (SFRI Medical Diagnostics, France) and Erba XL 200 (Erba Diagnostics Company, USA) respectively.

The synthetic activity of the liver was assessed in this study using the platelet and INR levels. Serum ALT level was used to assess for hepatic inflammation and hence, NASH. The lipid profile parameters comprising of total cholesterol, LDL, HDL, VLDL and triglycerides were used to determine the presence of dyslipidaemia. Derangements in laboratory findings were noted using the reference ranges specific to the laboratory. This is indicated on the questionnaire (appendix IV) and in appendix V.

3.2.3 Data Collection Tools: Ultrasonography

Each participant had an abdominal USG examination performed at the Imaging Department of the GARH. This was done by the researcher under the supervision of a radiologist. A Logiq E9

ultrasound machine (GE Healthcare, USA) was used. The patient was made to fast for a minimum of 8 to 12 hours before this procedure. This was usually done on the day of blood sample taking.

Figure 14: Set-up of USG room at the Imaging Department of the GARH

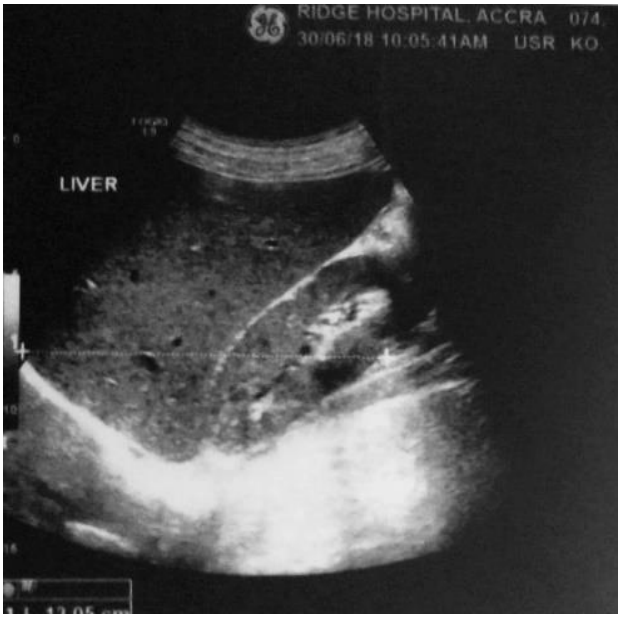
Figure 15: The curvilinear probe used for assessing liver characteristics during USG

With the participant in the supine position on a bed, the abdomen was exposed from the xiphisternum to the suprapubic area. Using a B-mode USG, liver size and characteristic features of steatosis were assessed with the aid of a low-frequency (2 – 5MHz) curvilinear probe (figure 15). The liver size was measured from the dome of the diaphragm to the lower edge of the liver along the mid-clavicular line, during the height of deep inspiration and recorded in centimetres (cm). Normal liver size was considered as a range of 10cm to 15cm.[217,218] The liver was considered as shrunken or enlarged (hepatomegaly) when its size was <10cm and >15cm respectively.

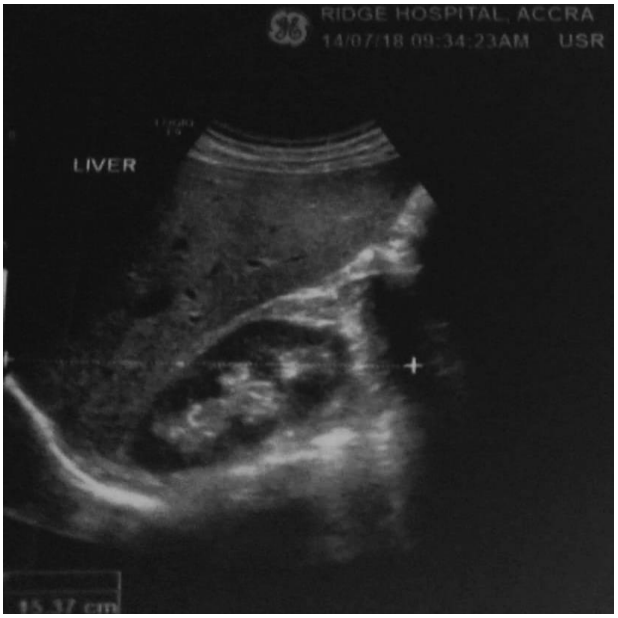
The echogenicity of the liver was assessed by comparing it with the cortex of the kidney and also by observing the sharpness of the liver edges, the diaphragm and hepatic vessels. Characterization was done as per the criteria documented by Lee et al.[136] This is shown in table 4 and figure 16.

Table 4: Grading of hepatic steatosis

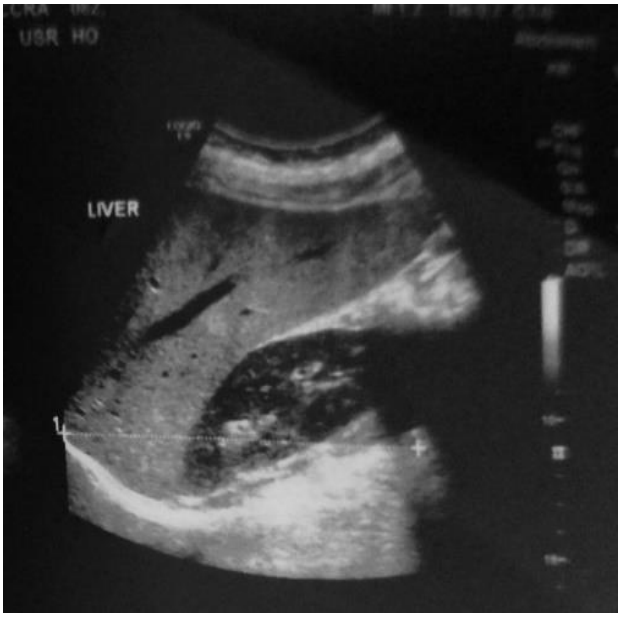
Grade	0	1	2	3
Impaired hepato-renal contrast	No	Minimal	Moderate	Marked
Impaired diaphragm visualization	No	No	Slight	Marked
Blurred hepatic vessels	No	No	Slight	Marked
Visible edges	Yes	Yes	No	No



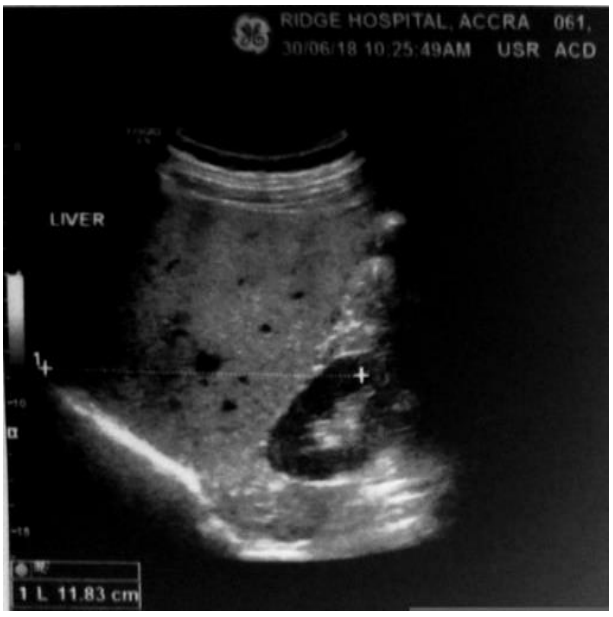
(a) Grade 0 NAFLD



(b) Grade 1 NAFLD



(c) Grade 2 NAFLD



(d) Grade 3 NAFLD

Figure 16: Sonographic images of the grades of NAFLD

In the assessment for advanced disease, the curvilinear probe was used to search for the presence, number and size of any focal lesions in the liver as this could indicate the presence of HCC. Portal hypertension which is a complication of cirrhosis was assessed by the presence of ascites and by measuring the size of the spleen. Splenomegaly, that is, spleen size of more than 12cm from one pole to the other,[218] and ascites are known indicators of portal hypertension.

Figure 17: Ultrasonography process

The abdominal subcutaneous fat thickness was measured for each participant whiles in the supine position and during normal respiration. A high-frequency linear probe (figure 18) with a frequency of 9 - 11MHz was used with minimal pressure on the abdominal wall. This measurement was taken about 1cm caudal to the umbilicus.[152] The distance between the skin layer and the muscle layer was considered as the abdominal subcutaneous fat thickness. The subcutaneous thickness measured was then categorized into one of the following groups, ≤20mm, >20mm to 25mm, >25mm to 30mm and >30mm.

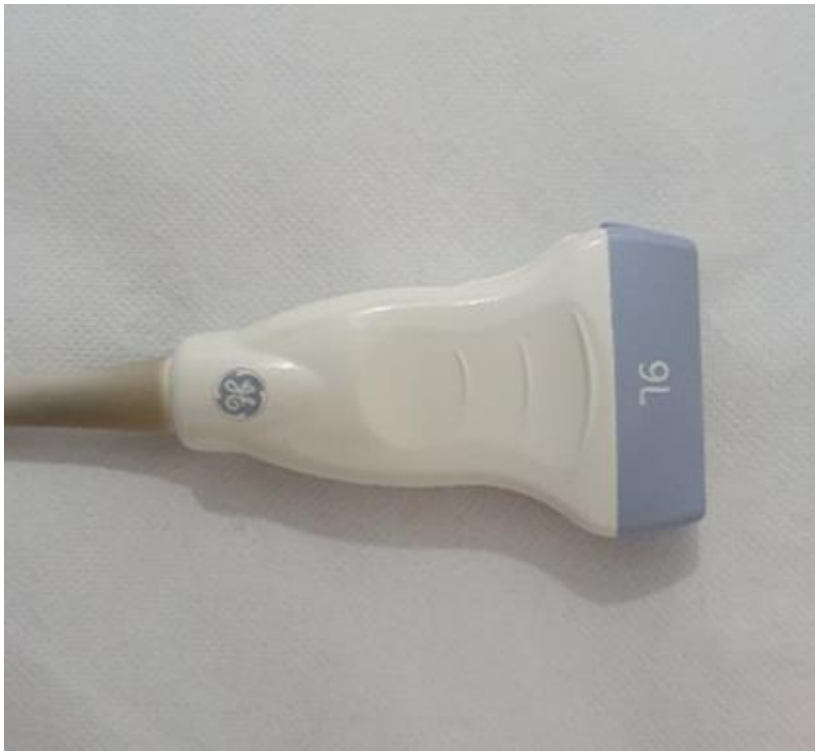


Figure 18: Linear probe used to measure abdominal subcutaneous fat thickness

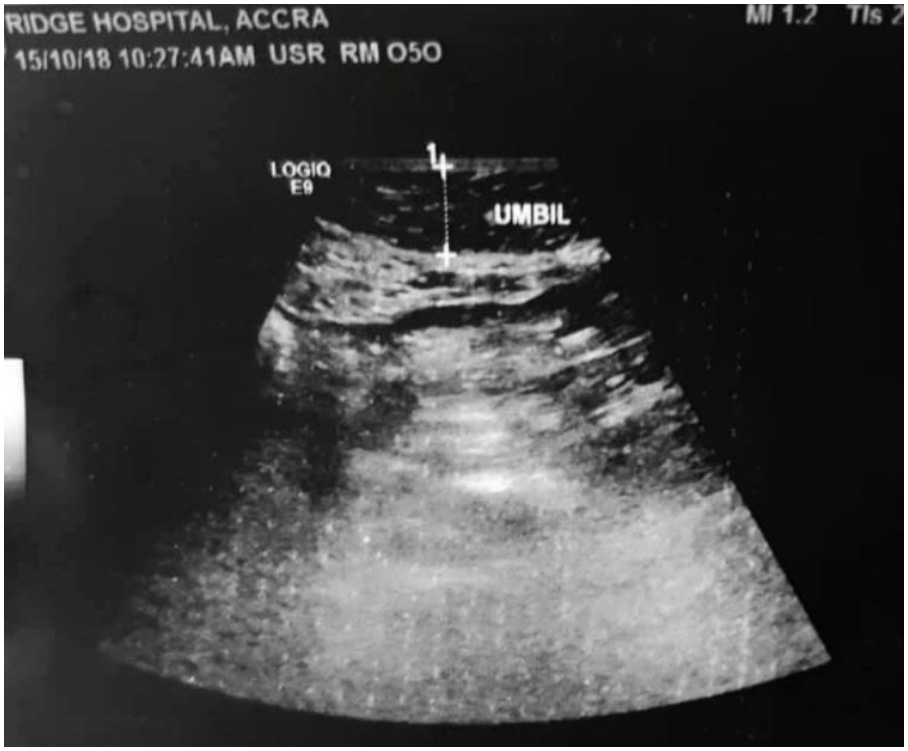


Figure 19: Measurement of abdominal subcutaneous fat thickness using the linear probe

3.2.4 Data Collection Tools: Calculated scores

Results from the laboratory and anthropometric measurements were used to calculate the NLR, AAR, APRI score, BARD score, FIB-4 score and NAFLD fibrosis score using online calculators on the website, http://www.gihep.com. The reference ranges of these scores can be seen in appendix V. The NLR was used to predict the presence of NASH whiles the presence or absence of advanced fibrosis was predicted with the aid of the AAR, APRI score, BARD score, FIB-4 score and NAFLD fibrosis score.

3.3 STUDY DEFINITIONS

Primary NAFLD was diagnosed by;

1. Evidence of hepatic steatosis on USG.[136]

AND

2. Absence of any other cause of liver disease, that is, no history of significant alcohol use[1] and no evidence of chronic viral hepatitis, medical conditions or drugs that can cause secondary NAFLD.

Nonalcoholic steatohepatitis was diagnosed by;

1. Primary NAFLD diagnosed by the criteria stated above.

PLUS

2. A negative or indeterminate prediction for fibrosis using non-invasive scores.

PLUS

3. A neutrophil to lymphocyte ratio of 2 or more.[164]

AND / OR

4. Elevated ALT[54], that is, above the upper limit of the laboratory reference range of 69U/L.

Advanced fibrosis was diagnosed by;

1. Diagnosis of primary NAFLD by the above criteria.

PLUS

2. A NAFLD fibrosis score of > 0.676. This score has specifically validated for NAFLD. For this study, it was assumed to be a good predictor of significant fibrosis in the absence of histology and elastography.

Type 2 DM patients were diagnosed at the DM clinic of the GARH using the ADA criteria below[216];

1. FBS level of 7mmol/L or higher; fasting was defined as no food intake for at least 8 hours.

OR

2. Random blood sugar (RBS) of 11.1mmol/L or higher in a patient with classic symptoms of hyperglycaemia, that is, polyuria, polydipsia, polyphagia, weight loss or hyperglycaemic crisis.

OR

3. HbA1c level of 6.5% or higher.

Impaired fasting glucose was diagnosed at the DM clinic of the GARH using the ADA criteria below;

1. FBS level between 5.6mmol/L and 6.9mmol/L

OR

2. HbA1c level of 5.7% to 6.4%.

Dyslipidaemia was considered as;

1. HDL was below the lower limit of 1.55mmol/L

PLUS

2. Total cholesterol, LDL, triglycerides and VLDL above the upper limit of, 6.20mmol/L, 3.90mmol/L and 2.25mmol/L respectively.

Hepatic dysfunction was diagnosed with;

1. Platelet level below 150 x 10^9/L

AND / OR

2. An INR level greater than 1.2.

Glycaemic control was diagnosed as[216];

1. HBA1c level of < 7%

Hepatomegaly was diagnosed as;

1. Liver size of >15cm on USG.[217,218] Ultrasonography was used because it was more accurate when compared to physical examination. Physical examination is known to underestimate liver size.[219]

3.4 DATA MANAGEMENT AND ANALYSIS

3.4.1 Data Management

Each research participant was assigned a code to conceal their identity and ensure confidentiality. Recorded data in the questionnaire were checked for completeness. This was then extracted and

entered into an IBM SPSS statistics spreadsheet. The cleaned data were analysed by a technician who was blinded.

Completed questionnaires were stored in a secured cabinet and used for only academic purposes. These questionnaires are not made available to anyone outside the study and will be kept for a period of two years before destruction by burning.

3.4.2 Data Analysis

The data captured from the questionnaire were entered onto an SPSS 23.0 (SPSS Inc., Chicago, USA) spreadsheet for analysis. Descriptive statistics were performed for all continuous variables and presented as mean, standard deviation and range. The nominal variables were expressed as graphs or charts where appropriate.

Proportions were used to determine the prevalence of NAFLD among T2DM patients sampled. The chi-square test was used to test the association between categorical variables and NAFLD. Student t-test and analysis of variance (ANOVA) were used to test the association between continuous variables and NAFLD where appropriate. The Mann-Whitney test was used to determine the association between the grades of NAFLD and selected clinical and biochemical variables.

The proportion of participants with NASH and advanced fibrosis among NAFLD participants was also calculated. Receiver operating characteristic (ROC) curves were used to assess the predictive strengths of ALT and NLR in distinguishing between patients who had and those who did not have NASH based on the cut-off points documented. The predictive strengths were determined from the models by calculating the area under the ROC curve (AUC). The same approach was used to assess the predictive strengths of the various fibrosis scores and liver size (by examination and USG) among participants with confirmed NAFLD. Additionally, all scoring systems used in the study were subjected to independence testing to determine whether there was a significant difference in their scores among patients with and without NAFLD. A test of agreement between the NAFLD fibrosis score and all other scores used to predict fibrosis in this study.

Multivariate regression analysis was done using statistically significant variables to determine the predictors of NAFLD. The variables were waist to hip ratio, BMI, HbA1c, GGT, triglycerides, total cholesterol, liver size determined by USG and subcutaneous thickness by USG. These

variables were entered into the multivariable regression analysis model and were presented as odds ratio, 95% confidence interval (CI) and p values. Also, the model was adjusted for age, sex and duration of DM. A forward stepwise regression of the model was also performed with the final model concluded after 4 simulations.

All statistical analysis was carried out at a 95% significance level with p values < 0.05 considered as statistically significant.

3.5 ETHICAL AND LEGAL CONSIDERATIONS

The proposal for this study received approval from the Ghana College of Physicians and Surgeons. Permission was obtained from the GARH to enable the researcher to conduct the study in the facility. This letter (Appendix II) was dated 6th February 2018. An ethical approval with the number, GHS-ERC004/03/18 (Appendix I), was also obtained from the Ghana Health Service Ethics Review Committee (GHS-ERC) on the 8th of May, 2018.

As part of consenting patients, an explanatory talk on the study was given to all attendants before the start of each clinic. Interested patients were then given further details in the language of their choice. They were allowed to involve an impartial witness of their choice during this process. Patients were assured that enrolment was voluntary and their refusal to participate in the study would not affect their care in any way. They were also informed about their right to remain in this study or withdraw at any stage without any repercussions or the need to give explanations. Those who agreed to be part of the study were made to sign an informed consent (Appendix III) before enrolment.

To ensure confidentiality and anonymity, participants were assigned codes instead of using their names. Contact numbers recorded during recruitment for easy scheduling of the second meeting was discarded and not included in the final data set after data management and analysis were concluded. Also, each participant was interviewed separately and in a private consulting room to ensure their privacy and confidentiality.

This study presented only a minimal risk to participants. The process of sample taking was associated with mild discomfort at the site. This was transient and did not pose any danger to participants. Participants did not incur any additional cost by participating in the study neither did

they receive any remuneration. Participants' second visit was scheduled to coincide with their routine clinic visit to reduce the loss of productive time.

As a direct benefit to participants, all those diagnosed with NAFLD were enrolled at the GI clinic of the hospital for monitoring and further management.

CHAPTER 4

RESULTS

4.1 RECRUITMENT FLOW CHART

During the period in which the study was conducted, a total of 387 patients with T2DM attending the DM clinic at the GARH were screened. Out of this number, only 289 met the inclusion criteria. Of the 289 patients who satisfied the inclusion criteria, 54 declined to be enrolled or lost interest after initially giving their consent because of various reasons. These reasons included a lack of interest in the study, fear of discovering a new medical condition and time constraints.

After this screening process, a total of 235 patients gave their informed consent and were therefore enrolled in the study. The recruitment flow is exhibited in Figure 20.

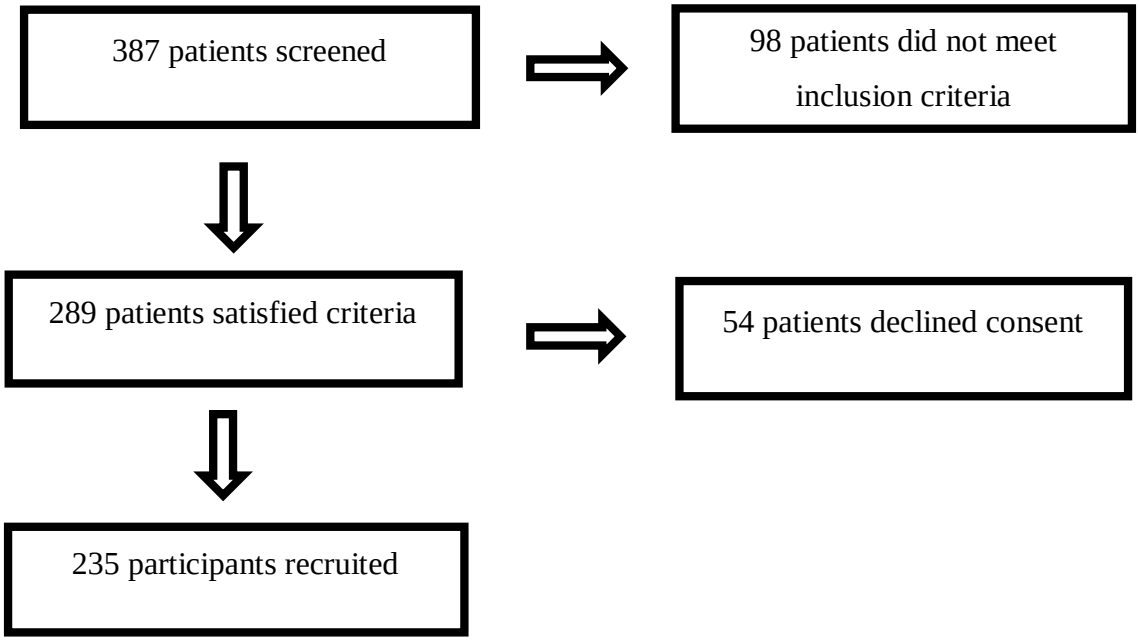


Figure 20: Recruitment flow chart

4.2 CHARACTERISTICS OF STUDY SUBJECTS

The demographic and social characteristics of all study participants are presented in Table 5. Majority of participants were female (72.8%) and the male: female ratio was 1:3.

Table 5: Socio-demographic characteristics of all study participants

Socio-demographic characteristics	Frequency, n (%)
Gender	
Male	64 (27.2)
Female	171 (72.8)
Age (years)	
≤ 30	0 (0.0)
31 – 40	6 (2.6)
41 – 50	38 (16.2)
51 – 60	80 (34.0)
61 – 70	75 (31.9)
>70	36 (15.3)
Marital Status	
Single	13 (5.5)
Married	128 (54.5)
Cohabiting	2 (0.9)
Divorced	44 (18.7)
Widowed	48 (20.4)
Ethnicity	
Akan	112 (47.6)
Ga	42 (17.9)
Ewe	32 (13.6)
Hausa	11 (4.7)
Other	38 (16.2)
Religion	
None	2 (0.9)
Christian	204 (86.8)
Moslem	28 (11.9)
Traditionalist	1 (0.4)
Highest level of education	
None	30 (12.8)
Primary	97 (41.3)
Secondary	73 (31.1)
Tertiary	27 (11.5)
Postgraduate	8 (3.4)
Residential environment	
Rural	13 (5.5)
Urban	222 (94.5)
Employment status	
Unemployed	24 (10.2)
Part-time employment	8 (3.4)
Full employment	123 (52.3)
Retired	80 (34.0)
Alcohol use	
No	193 (82.1)
Yes (insignificant)	42 (17.9)
Smoking	
Never	228 (97.0)
Current	2 (0.9)
Ex-smoker	5 (2.1)

4.2.1 Age Distribution

From Table 5, the age of all study participants ranged from 31 to 85 years with an overall mean age of 59.6 ± 10.0 years. There was no significant difference in the mean ages of males (59.7 ± 10.7 years) and females (59.5 ± 9.7 years). The age group of 51 - 60 years had the highest frequency, that is, 34.0% (80/235). Only 2.5% of the participants were below the age of 40 years. Figure 21 demonstrates that age among study participants was normally distributed.

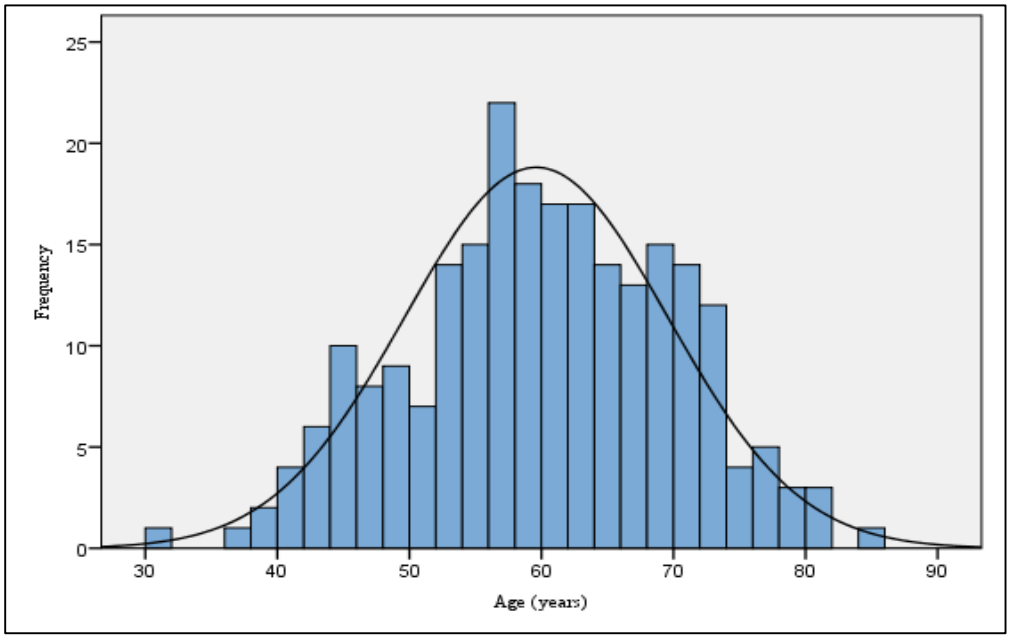


Figure 21: Age distribution of all study participants

4.2.2 Employment Status

The results of the study show that more than half of all study participants were employed and had a steady source of income. Participants who were fully employed constituted 52.3% whilst 3.4% were engaged in part-time employment (Table 5). One hundred and four (44.2%) were either retired or unemployed.

4.3 NONALCOHOLIC FATTY LIVER DISEASE IN STUDY PARTICIPANTS

4.3.1 Prevalence of Nonalcoholic Fatty Liver Disease

In this study, the prevalence of NAFLD was 38.7% (91/235) with a 95% CI of 32.5% - 45.3% (Table 6).

Table 6: Prevalence of NAFLD grades among T2DM patients

Grade of NAFLD	Frequency n, Prevalence (%)	95% CI (%)
Overall prevalence	91 (38.7)	32.5 - 45.3
Grade 1	15 (16.4)	9.5 - 25.7
Grade 2	34 (37.4)	27.4 - 48.1
Grade 3	42 (46.2)	35.6 - 56.9

Grade 3 NAFLD was the most prevalent among the NAFLD population with a prevalence of 46.2% (95% CI: 35.6 - 56.9). Prevalence of grades 1 and 2 NAFLD was 16.4% and 37.4% respectively.

4.3.2 Characteristics of Participants with Nonalcoholic Fatty Liver Disease

The sociodemographic characteristics of the 91 participants diagnosed with NAFLD were subsequently compared with the 144 participants without NAFLD to determine whether there were any significant differences between these two groups (Table 7).

Table 7: Socio-demographic characteristics of participants with and without NAFLD

Socio-demographic factors	NAFLD, n (%)				
	Yes (n = 91)	No (n = 144)	[α]*p- value*	Crude OR [95% CI]	p-value
Gender			0.402		
[ǂ]Male	22 (24.2)	42 (29.2)		-	
Female	69 (75.8)	102 (70.8)		1.29 [0.71 - 2.35]	0.403
Age (years)			***0.008***		
[ǂ]31 – 40	2 (2.2)	4 (2.8)		-	
41 – 50	21 (23.1)	17 (11.8)		2.47 [0.40 - 15.15]	0.328
51 – 60	35 (38.5)	45 (31.3)		1.56 [0.27 - 8.99]	0.621
61 – 70	27 (29.7)	48 (33.3)		1.13 [0.19 - 6.55]	0.896
>70	6 (6.6%)	30 (20.8)		0.40 [0.06 - 2.70]	0.347
Ethnicity of participants			0.146		
[ǂ]Akan	36 (39.5)	76 (52.8)		-	
Ga	16 (17.6)	26 (18.0)		1.30 [0.62 - 2.72]	0.487
Ewe	14 (15.4)	18 (12.5)		1.64 [0.74 - 3.67]	0.226
Hausa	4 (4.4)	7 (4.9)		1.21 [0.33 - 4.39]	0.776
Other	21 (23.1)	17 (11.8)		2.61 [1.23 - 5.53]	*0.013*
Employment status			***0.019***		
[ǂ]Unemployed	10 (11.0)	14 (9.7)		-	
Part-time employment	2 (2.2)	6 (4.2)		0.47 [0.078 - 2.81]	0.405
Full employment	58 (63.7)	65 (45.1)		1.25 [0.52 - 3.03]	0.622
Retired	21 (23.1)	59 (41.0)		0.50 [0.19 - 1.29]	0.152
Alcohol use			0.927		
[ǂ]No	75 (82.4)	118 (81.9)		-	
*Yes	16 (17.6)	26 (18.1)		0.97 [0.49 - 1.92]	0.927
Smoking			0.834		
[ǂ]Never	89 (97.8)	139 (96.5)		-	
Current	0 (0.0)	2 (1.4)		-	
Ex-smoker	2 (2.2)	3 (2.1)		1.04 [0.17 - 6.36]	0.965

[ǂ] Reference category [α]*p-value from X^2 or Fisher exact test* *Insignificant use

4.3.2.1 Gender distribution among participants with Nonalcoholic Fatty Liver Disease

Table 7 depicts that, out of the 91 study participants with NAFLD, there were more females than males, 75.8% versus 24.2%, giving a male to female ratio of approximately 1:3. Gender had no association with NAFLD in this study (p-value = 0.402).

4.3.2.2 Age distribution among participants with Nonalcoholic Fatty Liver Disease

Majority of the participants with NAFLD, 38.5% (35/91), were aged between 51 and 60 years (Table 7). These formed approximately 43.7% (35/80) of all participants in the 51 – 60 age group. The least proportion of participants, 2.2% (2/91), were below the age of 40 years. Figure 22 shows that the prevalence of NAFLD increased gradually with age until the 6th decade and then declined afterwards.

A significant association existed between age and NAFLD (p-value = 0.008). However, there was no significant increase in the odds of NAFLD among the various age categories compared with the reference group.

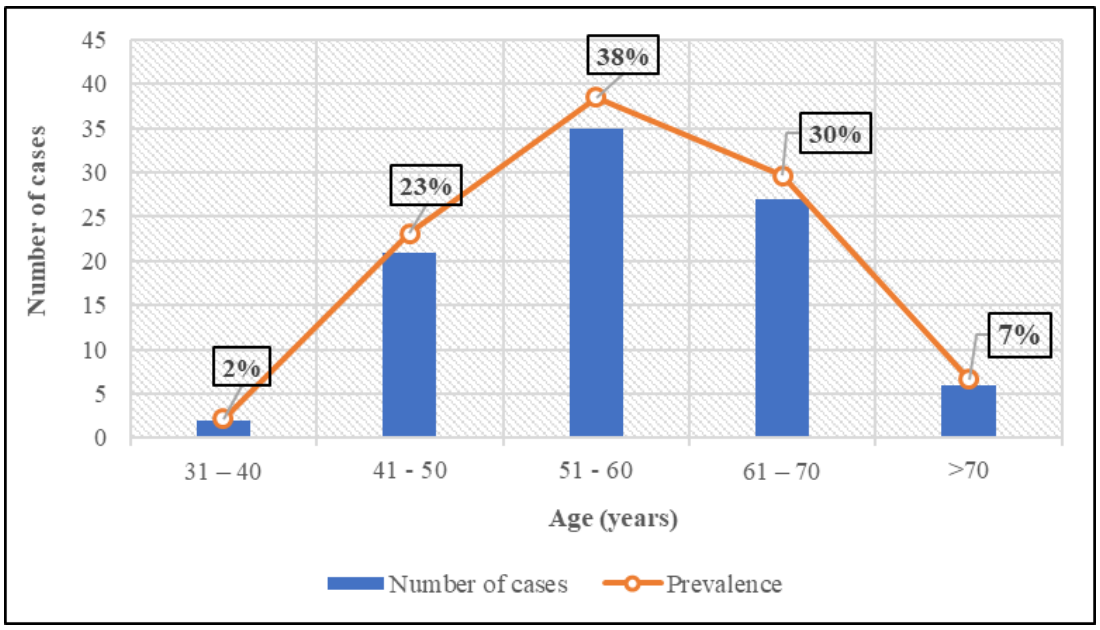


Figure 22: Age distribution among participants with NAFLD

4.3.3.3 Employment Status of Participants with Nonalcoholic Fatty Liver Disease

For this study, the occurrence of NAFLD was significantly higher in participants with full-time employment (p-value = 0.019). Fifty-eight (63.7%) of the 91 participants were fully employed (Table 7).

4.3.3 Clinical Data of Participants

These results are presented in Tables 8, 9 and 10.

4.3.3.1 Drug History

The most commonly used OHA was metformin, 84.7%, which was used singly or in combination with other oral agents or insulin. Similar proportions of participants in both the NAFLD and non-NAFLD group were on metformin-containing therapies, 84.6% versus 84.7%. In Table 8, the proportion of NAFLD participants on the metformin / TZD combination was 6.6% (6/91). None of the participants was on TZD only. There was no significant difference in DM management in the 2 groups. Prevalence of NAFLD also did not increase with DM management.

The prevalences of different grades of NAFLD did not show a significant association with the type of management received for DM (Table 9).

Table 8: Bivariate analysis of DM management

	NAFLD n (%)					
	Yes (n=91)	**No (n=144)**	***[α]p-value***	**Crude OR**	**[95% CI]**	**p-value**
DM Management			0.581			
[ǂ] Diet only	4 (4.4)	9 (6.3)		-		
Metformin	20 (22.0)	32 (22.2)		0.34	0.050 - 2.26	0.337
Metformin + other (without TZD regimen)	51 (56.0)	72 (50.0)		0.65	0.13 - 3.26	0.650
Metformin & TZD containing regimen	6 (6.6)	18 (12.5)		0.50	0.062 - 4.04	0.500
Others (SU, Insulin, Meglitinides)	10 (11.0)	13 (9.0)		0.25	0.20 - 3.07	0.278

[ǂ] reference category *[α] p-value from X^2 or Fisher exact test* *TZD – thiazolidinediones* *SU - sulphonylurea*

Table 9: NAFLD grade and DM Management

DM Management	All participants, n (%)	NAFLD Grade, n (%)				Total	p-value
		Grade 0	Grade 1	Grade 2	Grade 3		
Diet only	13 (5.5)	9 (69.2)	0 (0.0)	0 (0.0)	4 (30.8)	13	0.474
Metformin only	52 (22.1)	32 (61.5)	2 (3.8)	9 (17.3)	9 (17.3)	52	
Metformin + other (without TZD regimen)	123 (52.3)	72 (58.5)	11 (8.9)	16 (13.0)	24 (19.5)	123	
Metformin & TZD-containing regimen	24 (10.2)	18 (75.0)	0 (0.0)	4 (16.7)	2 (8.3)	24	
Others (SU, Insulin, Meglitinides)	23 (9.8)	13 (56.5)	2 (8.7)	5 (21.7)	3 (13.0)	23	
Total	235 (100)						

NB: Row percentages reported *TZD – thiazolidinediones* *SU - sulphonylurea*

4.3.3.2 Clinical Features

Out of 46 participants with symptoms suggestive of NAFLD, 41.3% belonged to the NAFLD group and 58.7% in the non-NAFLD group (Table 10). The presence of current symptoms did not show any significant association with NAFLD.

Additionally, clinical examination findings as recorded in Table 10 were grouped into non-specific findings, stigmata of chronic liver disease and features of IR. These did not show any significant association with the occurrence of NAFLD. Pedal oedema was the most prevalent finding in the 51 participants with non-specific examination findings. This had near equal numbers in both groups, 48.7% (19/39) and 51.3% (20/39) in the NAFLD and non-NAFLD groups respectively. Within the NAFLD population, only 21.9% of participants had pedal oedema. Signs of liver disease were rarely seen in participants.

Features of IR were the most common finding but showed no difference in prevalence in the two groups (p-value = 0.272). Arcus cornealis, the most prevalent feature of IR, was present in 34.1% (30/88) of the NAFLD group versus 65.9% (58/88) of the non-NAFLD group (p-value = 0.272).

Table 10: Association between clinical features and diagnosis of NAFLD

Clinical features	NAFLD, n (%)		p-value‡	Total
	Yes	No		
***NAFLD symptoms**	19 (41.3)	27 (58.7)	0.689	46
Non-specific feature				
Pallor	5 (50.0)	5 (50.0)	0.454	10
Pedal oedema	20 (51.3)	19 (48.7)	0.078	39
Cachexia	1 (50.0)	1(50.0)	0.626	2
Lymphadenopathy	0 (0.0)	0 (0.0)	-	0
Stigmata of liver disease				
Right upper quadrant tenderness	1 (20.0)	4 (80.0)	0.651	5
Jaundice	2 (100.0)	0 (0.0)	0.149	2
Spider naevi	0 (0.0)	0 (0.0)	-	0
Clubbing	0 (0.0)	0 (0.0)	-	0
Palmar erythema	0 (0.0)	0 (0.0)	-	0
Features of insulin resistance				
Arcus cornealis	30 (34.1)	58 (65.9)	0.272	88
Acanthosis nigricans	7 (38.9)	11 (61.1)	0.587	18
Xanthoma	1(25.0)	3 (75.0)	0.497	4

Row percentages reported ‡*p-value from X^2 or Fisher exact test* ** refer to appendix IV (questionnaire)*

4.3.3.3 Duration of Type 2 Diabetes Mellitus

The duration of DM was significantly shorter among the NAFLD group than the non-NAFLD group. From Table 11, the average duration of DM was 6.4 ± 5.4 years among NAFLD patients compared with 8.7 ± 6.3 years among non-NAFLD (p-value = 0.004).

Table 11: Association between T2DM duration, obesity and blood pressure and NAFLD

Variables	NAFLD, Mean (± SD)		Sig. (2-tailed)
	Yes	No	
Duration of T2DM (years)	6.4 (± 5.4)	8.7 (± 6.3)	***0.004***
BMI (kg/m2)	31.6 (± 6.8)	27.4 (± 5.5)	< ***0.0001***
Waist Circumference Average (cm)	100.8 (± 14.6)	91.9 (± 12.9)	< ***0.0001***
Waist/Hip Ratio	0.95 (± 0.08)	0.91 (± 0.06)	< ***0.0001***
Average Systolic BP	139.7 (± 20.6)	138 (± 18.7)	0.525
Average Diastolic BP	83.6 (± 11.5)	81.5 (± 10.6)	0.147

4.3.3.4 Anthropometric Findings

As shown in Table 11, the mean BMI was significantly higher among the NAFLD population than the non-NAFLD population, 31.6 ± 6.8kg/m^2 compared to 27.4 ± 5.5kg/m^2 (p-value <0.0001). Table 12 shows a significant association between NAFLD and BMI. It also shows that, compared with the reference group (normal BMI), there was a significant increase in the risk of NAFLD when BMI was ≥ 30.0kg/m^2. The odds of NAFLD in the presence of class 3 obesity was nearly 15-fold (95% CI: 2.92 - 78.22).

Central obesity (determined by waist circumference and waist to hip ratio) showed an association with NAFLD. The mean waist circumference was significantly higher in the NAFLD group, 100.8 ± 14.6cm, than in the non-NAFLD group, 91.9 ± 12.9cm, p-value <0.0001 (Table 11). A greater proportion of the NAFLD group had increased waist circumference compared with the non-NAFLD group, 82.4% versus 66.0%, p-value = 0.006 (Table 12). The odds of NAFLD was about 2-fold when waist circumference was increased (95% CI: 1.27 - 4.59).

Table 11 indicates a significantly higher mean waist to hip ratio in those with NAFLD than in those without NAFLD, 0.95 ± 0.08cm and 0.91 ± 0.06cm respectively (p-value <0.0001). The prevalence of increased waist to hip ratio was also significantly higher among the NAFLD group (87.9%) than the non-NAFLD group (69.4%), p-value = 0.001 (Table 12). There was a 3-fold increase in the likelihood of NAFLD when waist to hip ratio was above the upper limit (95% CI: 1.55 - 6.60, p-value = 0.002).

Table 12: Bivariate logistic analysis of anthropometric characteristics

	NAFLD, N (%)					
	Yes (N=91)	**No (N=144)**	***[α]p- value***	**Crude OR**	**[95% CI]**	**p-value**
BMI (kg/m^2)			***<0.0001***			
Underweight (≤ 19.9)	0 (0.0)	6 (4.2)		-		
[ǂ]Normal (20.0 – 24.9)	14 (15.4)	47 (32.6)		-		
Overweight (25.0 – 29.9)	22 (24.2)	49 (34.0)		1.57	0.69 - 3.29	0.303
Class I (30.0 – 34.9)	27 (29.7)	24 (16.7)		3.78	1.68 - 8.50	***0.001***
Class II (35.0 – 39.9)	19 (20.9)	16 (11.1)		3.99	1.63 - 9.74	***0.002***
Class III (≥ 40.0)	9 (9.9)	2 (1.4)		15.11	2.92 - 78.22	***0.001***
Waist circumference (cm)			***0.006***			
[ǂ]Normal (M ≤ 94; F ≤ 80)	16 (17.6)	49 (34.0)		-		
Increased (M > 94; F > 80)	75 (82.4)	95 (66.0)		2.42	1.27 - 4.59	***0.007***
Waist/Hip ratio			***0.001***			
[ǂ]Normal (M < 0.90; F < 0.85)	11 (12.1)	44 (30.6)		-		
Increased (M ≥ 0.90; F ≥ 0.85)	80 (87.9)	100 (69.4)		3.20	1.55 - 6.60	***0.002***

[ǂ]Reference category *[α] – p-value from X^2 or Fisher exact test* *NS - Not significant*

4.3.3.5 Liver Size and Nonalcoholic Fatty Liver Disease

Prevalence of hepatomegaly in the overall study population was determined by USG and estimated as 21.7% (51/235). Prevalence of hepatomegaly by physical examination was 4.7% (11/235). In Table 13, hepatomegaly was common in the NAFLD group than the non-NAFLD group. Using USG, this was 41.8% (38/91) and 9.0% (13/144) respectively. The likelihood of NAFLD was increased 7-fold in the presence of hepatomegaly (95% CI: 3.54 - 14.53, p-value <0.0001). A similar picture was seen when physical examination was used.

Hepatomegaly diagnosed by USG showed a significant association with the degree of steatosis, p-value <0.0001 (Table 14). The prevalence of hepatomegaly increased as the degree of steatosis increased from grade 1 (5.9% (53/51)) to grade 3 (49.0% (25/51)). Thirteen (9.0%) of those with hepatomegaly by USG had no NAFLD. With physical examination, a similar observation is seen and this was also significant (p-value <0.0001). Only 1 (9.1%) with hepatomegaly by physicial examination did not have NAFLD.

Table 13: Bivariate analysis of liver size and association of NAFLD

	NAFLD, n (%)					
	Yes (n=91)	**No (n=144)**	***[α]p- value***	**Crude OR**	**[95% CI]**	**p-value**
Liver size by examination			***<0.0001***			
[ǂ] Normal (10 - 15cm)	81 (89.0)	143 (99.3)		-		
Enlarged (>15cm)	10 (11.0)	1 (0.7)		17.65	[2.22 - 140.42]	***0.007***
Liver size by USG			***<0.0001***			
[ǂ] Normal (10 - 15cm)	53 (58.2)	131 (91.0)		-		
Enlarged (>15cm)	38 (41.8)	13 (9.0)		7.17	[3.54 - 14.53]	***<0.0001***

[ǂ]Reference category

[α] – p-value from X^2 or Fisher exact test

Table 14: NAFLD grade and its relationship with liver size

	Grade 0	Grade 1	Grade 2	Grade 3	Total	p-value
	n (%)					
Liver size by examination						***<0.0001***
ǂNormal (10 - 15cm)	143 (63.8)	15 (6.7)	32 (14.3)	34 (15.2)	224	
Enlarged (>15cm)	1 (9.1)	0 (0.0)	2 (18.2)	8 (72.7)	11	
Liver size by USG						***<0.0001***
Normal (10 - 15cm)	130 (71.0)	12 (6.6)	24 (13.1)	17 (9.3)	183	
Reduced (<10cm)	1 (100.0)	0 (0)	0 (0)	0 (0)	1	
Enlarged (>15cm)	13 (25.5)	3 (5.9)	10 (19.6)	25 (49.0)	51	

Row percentages reported

4.3.3.6 Abdominal Subcutaneous Fat Thickness and Nonalcoholic Fatty Liver Disease

About 85.7% (78/91) of respondents with NAFLD had an abdominal subcutaneous fat thickness of more than 20mm compared with 63.9% (92/144) of non-NAFLD respondents. In Table 15, the abdominal subcutaneous fat thickness measured had a strong association with NAFLD (p-value <0.0001). Increasing abdominal subcutaneous fat thickness above the reference range of ≤ 20mm was also associated with a progressive likelihood of NAFLD.

Table 16 shows a significant association between subcutaneous abdominal fat thickness and the grade of NAFLD. Prevalence of grade 3 NAFLD also increased with increasing abdominal subcutaneous fat thickness above the reference range of ≤ 20mm.

Table 15: Bivariate analysis of abdominal subcutaneous fat thickness and association of NAFLD

	NAFLD, n (%)					
	Yes (n=91)	**No (n=144)**	***[α]p- value***	**Crude OR**	**[95% CI]**	**p-value**
[‡]≤ 20mm	13 (14.3)	52 (36.1)	***<0.0001***			
>20 – 25mm	28 (30.8)	45 (31.3)		2.49	[1.15 – 5.37]	***0.020***
>25 – 30mm	25 (27.5)	33 (22.9)		3.03	[1.36 – 6.74]	***0.007***
>30mm	25 (27.5)	14 (9.7)		7.14	[2.93 – 17.45]	***<0.0001***

[‡]*Reference category* [α] *– p-value from X^2 or Fisher exact test*

Table 16: NAFLD grade and its relationship with abdominal subcutaneous fat thickness

	Grade 0	**Grade 1**	**Grade 2**	**Grade 3**	**Total**	**p-value**
	n (%)					
Subcutaneous thickness (mm)						***<0.0001***
≤ 20	52 (80.0)	4 (6.2)	5 (7.7)	4 (6.2)	65	
>20 – 25	45 (61.6)	7 (9.6)	11 (15.1)	10 (13.7)	73	
>25 – 30	33 (56.9)	3 (5.2)	10 (17.2)	12 (20.7)	58	
>30	14 (35.9)	1 (2.6)	8 (20.5)	16 (41.0)	39	

Row percentages reported

4.3.4 Laboratory Findings

Table 17 exhibited an almost equal mean Hb in the 2 groups, 13.0 ± 2.0g/dL in the NAFLD group compared to 12.7 ± 1.6g/dL in the non-NAFLD group but this was not significant (p-value = 0.289). However, there was a significantly lower platelet level in the NAFLD group than the non-NAFLD group, 247 ± 78.5 x 10^9/L versus 278 ± 90.8 x 10^9/L (p-value = 0.008). The platelet level in the 2 groups was within the normal range. The INR level in the 2 groups was almost the same, that is, 1.0 ± 0.3 in the NAFLD population and 0.9 ± 0.3 in the non-NAFLD (p-value = 0.033) respectively.

Table 17: Estimated means of haematological parameters and association of NAFLD

	NAFLD, Mean (± SD)		
Haematological parameters	**Yes**	**No**	**Sig. (2-tailed)**
Hb (g/dl)	13.0 (± 2.0)	12.7 (± 1.6)	0.289
WBC(x 10^9/L)	6.8 (± 2.2)	6.8 (± 2.6)	0.942
Neutrophils (X10^9/L)	3.7 (± 1.8)	3.6 (± 1.9)	0.611
Lymphocytes (X10^9/L)	2.5 (± 0.9)	2.6 (± 1.0)	0.587
Platelet (X10^9/L)	247.8 (± 78.5)	278.6 (± 90.8)	***0.008***
FBS	8.2 (± 2.2)	8.0 (± 2.7)	0.521
HbA1c (%)	9.0 (± 2.4)	8.1 (± 2.1)	***0.003***
INR	1.0 (± 0.3)	0.9 (± 0.3)	***0.033***
NLR	1.64 (± 1.15)	1.56 (± 1.24)	0.664

Generally, there was sub-optimal glycaemic control in both groups (Table 17). This was significantly poorer in the NAFLD group than the non-NAFLD group with mean HbA1c levels of 9.0 ± 2.4% and 8.1% ± 2.1% respectively (p-value = 0.003).

The means of all parameters of the LFT were within the normal range (Table 18). The mean GGT level was significantly higher in the NAFLD group, 46.6 ± 22.0U/L versus 37.9 ± 19.1U/L (p-value = 0.002). Regarding AST level, a similar finding was seen in the two groups, 31.4 ± 14.9U/L in the NAFLD group and 31.0 ± 26.3U/L in the non-NAFLD group (p-value = 0.005). The seven other parameters of the LFT did not show any significant difference between the 2 groups.

Table 18: Estimated means of parameters of liver function tests

	NAFLD, Mean (± SD)		
Liver Function Test	**Yes**	**No**	**Sig. (2-tailed)**
Total bilirubin (mmol/L)	14.7 (5.1)	13.6 (± 4.2)	0.050
Direct bilirubin (mmol/L)	3.9 (± 1.6)	3.6 (± 1.3)	0.218
Indirect bilirubin (mmol/L)	10.8 (± 4.2)	9.9 (± 3.7)	0.081
AST (U/L)	31.4 (± 14.9)	26.3 (± 12.2)	***0.005***
ALT (U/L)	32.1 (± 13.6)	31.0 (± 13.5)	0.557
ALP (U/L)	95.3 (± 30.0)	88.8 (± 26.3)	0.062
GGT (U/L)	46.6 (± 22.0)	37.9 (± 19.1)	***0.002***
Protein (g/L)	75.8 (± 5.0)	75.2 (± 4.9)	0.381
Albumin (g/L)	42.2 (± 5.4)	42.8 (± 4.4)	0.379

Despite the normal values observed, Table 19 shows a significantly higher triglyceride level in the NAFLD group, 1.60 ± 0.65mmol/L, than in the non-NAFLD group, 1.29 ± 0.60mmol/L (p-value < 0.0001). There was no significant difference in the other parameters of the lipid profile.

Table 19: Association between the mean lipid profile parameter and NAFLD

	NAFLD, Mean(SD)		
Lipid Profile	**Yes**	**No**	**Sig. (2-tailed)**
Total cholesterol (mmol/L)	5.29 (± 1.60)	5.04 (± 1.29)	0.193
LDL cholesterol (mmol/L)	3.10 (± 1.51)	2.90 (± 1.15)	0.242
HDL cholesterol (mmol/L)	1.45 (± 0.55)	1.54 (± 0.44)	0.184
VLDL cholesterol (mmol/L)	0.72 (± 0.40)	0.63 (± 0.32)	***0.049***
Triglycerides (mmol/L)	1.60 (± 0.65)	1.29 (± 0.60)	***<0.0001***

Table 20 shows a significant association between NAFLD and dyslipidaemia, that is, elevated total cholesterol (p-value = 0.015), elevated LDL (p-value <0.0001), elevated triglycerides (p-value 0.021) and decreased HDL (p-value = 0.004). The odds of NAFLD exhibited a 2-fold increase in the presence of elevated total cholesterol (95% CI: 1.16 - 4.27) and a 3-fold increase when LDL and triglycerides were elevated, (95% CI: 1.61 - 5.62) and (95% CI: 1.13 - 6.18) respectively. The odds of NAFLD when serum HDL was below the lower limit was 2.25 (95% CI: 1.30 - 3.91). These were statistically significant.

Table 20: Bivariate analysis of lipid profile parameters

	NAFLD, n (%)					
	Yes (n=91)	**No (n=144)**	***[a]p- value***	**Crude OR**	**[95% CI]**	**p-value**
Total Cholesterol (mmol/L)			***0.015***			
[‡]Normal (3.30 - 6.20)	66 (72.5)	123 (85.4)		-		
Elevated (> 6.20)	25 (27.5)	21 (14.6)		2.22	[1.16 - 4.27]	***0.017***
LDL cholesterol (mmol/L)			***<0.0001***			
[‡]Normal (0 - 3.90)	59 (64.8)	122 (84.7)		-		
Elevated (> 3.90)	32 (35.2)	22 (15.3)		3.01	[1.61 - 5.62]	***0.001***
HDL-C (mmol/L)			***0.004***			
[‡]Normal (> 1.55)	28 (30.8)	72 (50.0)		-		
Low (< 1.55)	63 (69.2)	72 (50.0)		2.25	[1.30 - 3.91]	***0.004***
VLDL (mmol/L)			0.387			
[‡]Normal (0.0 - 2.59)	90 (98.9)	144 (100)		-		NS
Elevated (> 2.59)	1 (1.1)	0 (0)		-		
Triglycerides (mmol/L)			***0.021***			
[‡]Normal (0.40 - 2.25)	76 (83.5)	134 (93.1)		-		
Elevated (> 2.25)	15 (16.5)	10 (6.9)		2.65	[1.13 - 6.18]	***0.025***

[‡]Reference category *[a]p-value from X^2 or Fisher exact test* *NS - Not significant*

4.4 NONALCOHOLIC STEATOHEPATITIS

In Table 21, the overall prevalence of NASH among the 91 participants with NAFLD was 23.1% (95% CI: 14.9 - 33.1).

Table 21: Prevalence of NASH among participants with NAFLD

NAFLD and NASH	Frequency, Prevalence	95% CI (%)
NAFLD (overall)	91 (38.7%)	32.5 - 45.3
NASH (overall)	21/91 (23.1%)	14.9 - 33.1
NASH (elevated NLR)	20/21 (95.2%)	76.2 - 99.9
NASH (elevated ALT)	3/21 (14.3%)	3.1 - 36.3
NASH (elevated ALT and NLR)	2/21 (9.5%)	1.2 - 30.4

Table 21 also shows that NLR was able to predict NASH in 95.2% (95% CI: 76.2 - 99.9) of affected persons whiles the prediction using ALT was only in 14.3% (3/21). Prediction by both ALT and NLR was seen in only 2 participants.

Using the receiver operating characteristic (ROC) curve in Figure 23 and Table 23, NLR yielded an AUC of 0.928 (95% CI: 0.887 - 0.970) in the prediction of NASH as against 0.680 (95% CI: 0.566 - 0.793) with ALT. The sensitivity, specificity, positive predictive and negative predictive values of NLR were significant, 95.2%, 87.9%, 43.5% and 99.5% respectively (p-value <0.0001).

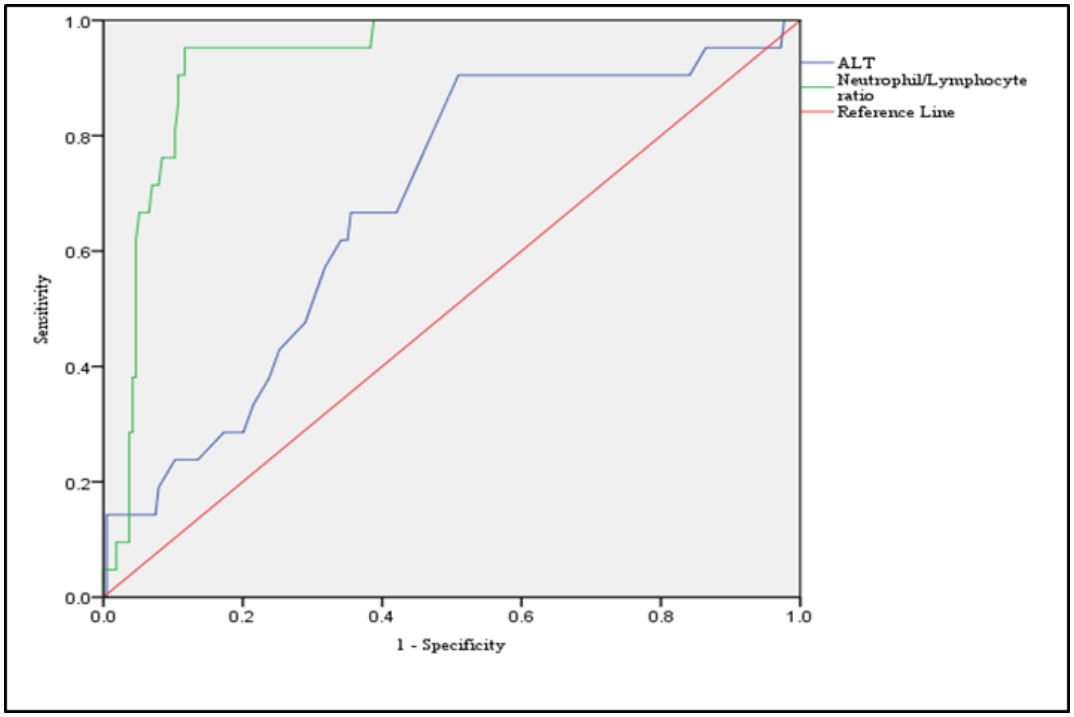


Figure 23: Receiver operating characteristic curves of the predictors of NASH

Table 22: Performance of ALT and NLR in the diagnosis of NASH

Variables	AUC	95% CI	p-value	SS (%)[1]	SP (%)[1]	PPV (%)[1]	NPV (%)[1]
ALT (cut-off point of > 69 U/L)	0.680	0.566 - 0.793	***0.007***	14	99.5	75	92.2
NLR (cut-off point of ≥ 2.0)	0.928	0.887 - 0.970	***<0.0001***	95.2	87.9	43.5	99.5

SS – Sensitivity *SP – Specificity* *PPV - Positive Predictive Value* *NPV - Negative Predictive Value*

[1] [Results based on the stated cut-off points]

Table 23 demonstrates an increasing prevalence of NASH with increasing degree of steatosis. The highest NASH prevalence was seen in participants with grade 3 NAFLD, 76.2% (16/21).

Table 23: Prevalence of NASH by NAFLD grade

NASH and Grade of NAFLD	NAFLD Grade, Frequency (%) (n=21)		
	Grade 1	Grade 2	Grade 3
NASH (elevated ALT and/or NLR)	1 (4.8)	4 (19.0)	16 (76.2)

4.5 FIBROSIS IN NONALCOHOLIC FATTY LIVER DISEASE

The distribution of advanced liver disease diagnosed using non-invasive scoring systems in T2DM patients with NAFLD is shown in Table 24. The BARD score recorded the highest prevalence of significant fibrosis, 84.6% (95% CI: 75.5 - 91.3) whiles the lowest prevalence was by the APRI score, 0.0%. However, the overall prevalence of advanced fibrosis for this study was 26.4% (24/91) with a 95% CI: 17.7 - 36.7. This estimation was done using the NAFLD fibrosis score.

Table 24: Prevalence of fibrosis among NAFLD patients using various scoring systems

	Number (n=91)	Prevalence (%)	[95% CI]
AAR Score			
No fibrosis: < 0.5	8	8.8	[3.9 - 16.6]
Indeterminate: 0.5 - 1.0	46	50.5	[39.9 - 61.2]
Fibrosis: > 1.0	37	40.7	[30.5 - 51.5]
FIB-4 Score			
No fibrosis: < 1.30	49	53.8	[43.1 - 64.4]
Indeterminate: 1.30 - 2.67	26	28.6	[19.6 - 39.0]
Significant fibrosis: > 2.67	16	17.6	[10.4 - 27.0]
APRI Score			
No fibrosis: ≤ 0.50	73	80.2	[70.6 - 87.8]
Indeterminate: 0.51 - 1.50	18	19.8	[12.2 - 29.5]
Significant fibrosis:	0	0.0	-
BARD Score			
No fibrosis: F0 - F1	14	15.4	[8.7 - 24.5]
Significant fibrosis: F2 - F4	77	84.6	[75.5 - 91.3]
NAFLD fibrosis score			
No fibrosis: < -1.455	28	30.8	[21.5 - 41.3]
Indeterminate: -1.455 - 0 .676	39	42.9	[32.5 - 53.7]
Significant fibrosis: > 0.676	24	26.4	[17.7 - 36.7]

Table 25 shows the test of agreement between the NAFLD fibrosis score and the other scoring systems. FIB-4 score had a moderate agreement with the NAFLD fibrosis score, kappa value of 0.426 (p-value < 0.0001). The BARD score and AAR had a slight agreement with the NAFLD fibrosis score, p-value < 0.0001 and 0.004 respectively. The APRI score demonstrated no or slight agreement, kappa value of 0.043 (p-value = 0.150).

Table 25: Assessment of agreement between NAFLD fibrosis score and various fibrosis scoring systems

	NAFLD fibrosis score					
Variables	**No fibrosis (n=87)**	**Intermediate (n=113)**	**Significant fibrosis (n=35)**	**Total**	**κ [95% CI]**	**p-value**
AAR Score					**0.115** [0.025 - 0.205]	***0.004***
ǂNo fibrosis: < 0.5	10	8	1	19		
Indeterminate: 0.5 - 1.0	66	69	9	144		
Cirrhosis: > 1.0	11	36	25	72		
FIB-4 Score					**0.426** [0.333 - 0.518]	***<0.0001***
ǂNo fibrosis: < 1.30	83	60	3	146		
Indeterminate: 1.30 - 2.67	4	48	12	64		
Significant fibrosis: > 2.67	0	5	20	25		
APRI Score					**0.043** [0.004 - 0.082]	0.150
ǂNo fibrosis: ≤ 0.50	87	104	19	210		
Indeterminate: 0.51 - 1.50	0	9	16	25		
BARD Score					**0.197** [0.101 - 0.293]	***<0.0001***
ǂNo fibrosis: F0 - F1	37	18	1	56		
Significant fibrosis: F2 - F4	50	95	34	179		

κ - Cohen's Kappa test of agreement

4.6 DIAGNOSTIC PERFORMANCE OF PARAMETERS

The results of independence testing of all scoring systems used in this study are seen in Table 26. From this table, there was a significant difference in the prevalence of fibrosis in the 2 groups with the NAFLD group reporting a higher prevalence with all fibrosis scores. The use of NLR to predict NASH did not show any significant difference in the 2 groups.

Using the NAFLD fibrosis score, the odds of NAFLD was significantly increased by 3.6 (95% CI: 1.96 - 10.69) when the score was >0.676 (Table 26).

Table 26: Bivariate analysis of the scoring systems used in detecting NASH and advanced fibrosis

Variables	NAFLD n (%)		[α]p- value	Crude OR	[95% CI)]	p-value
	Yes (n=91)	No (n=144)				
NLR			0.501			
[ǂ]< 2.0	71 (78.0)	118 (81.9)		-		
≥ 2.0	20 (22.0)	26 (18.1)		1.27	[0.66 - 2.46]	0.461
AAR Score			***0.021***			
[ǂ] No fibrosis: < 0.5	8 (8.8)	11 (7.6)		-		
Indeterminate: 0.5 - 1.0	46 (50.5)	98 (68.1)		0.65	[0.24 - 1.71]	0.379
Cirrhosis > 1.0	37 (40.7)	35 (24.3)		1.45	[0.52 - 4.04]	0.473
FIB-4 Score			***0.015***			
[ǂ] No fibrosis: < 1.30	49 (53.8)	97 (67.4)		-		
Indeterminate: 1.30 - 2.67	26 (28.6)	38 (26.4)		1.35	[0.74 - 2.48]	0.326
Significant fibrosis: > 2.67	16 (17.6)	9 (6.3)		3.52	[1.45 - 8.54]	***0.005***
APRI Score			***<0.0001***			
[ǂ] No fibrosis: ≤ 0.50	73 (80.2)	137 (95.1)		-	-	-
Indeterminate: 0.51 - 1.50	18 (19.8)	7 (4.9)		4.83	[1.93 - 12.09]	***0.001***
BARD Score			***0.018***			
[ǂ] No fibrosis: F0 - F1	14 (15.4)	42 (29.2)		-		
Significant fibrosis: F2 - F4	77 (84.6)	102 (70.8)		2.27	[1.16 - 4.44]	***0.017***
NAFLD fibrosis score			***<0.0001***			
[ǂ] No fibrosis: < -1.455	28 (30.8)	59 (41.0)		-		
Indeterminate: -1.455 - 0 .676	39 (42.9)	74 (51.4)		1.11	[0.61 - 2.01]	0.729
Significant fibrosis: > 0.676	24 (26.4)	11 (7.6)		4.60	[1.98 - 10.69]	***<0.0001***

[ǂ] *Reference category*

[α] *– p-value from X^2 or Fisher exact test*

The diagnostic performance of all scoring systems, liver size and abdominal subcutaneous fat thickness was compared. From Table 27 and Figure 24, liver size by USG proved to be the most sensitive tool for predicting NAFLD with an AUC of 0.752 (95% CI: 0.685 - 0.818, p-value < 0.0001). Its specificity was 91% but had a low sensitivity of 41.8%. The negative and positive predictive values were 71% and 74.5% respectively.

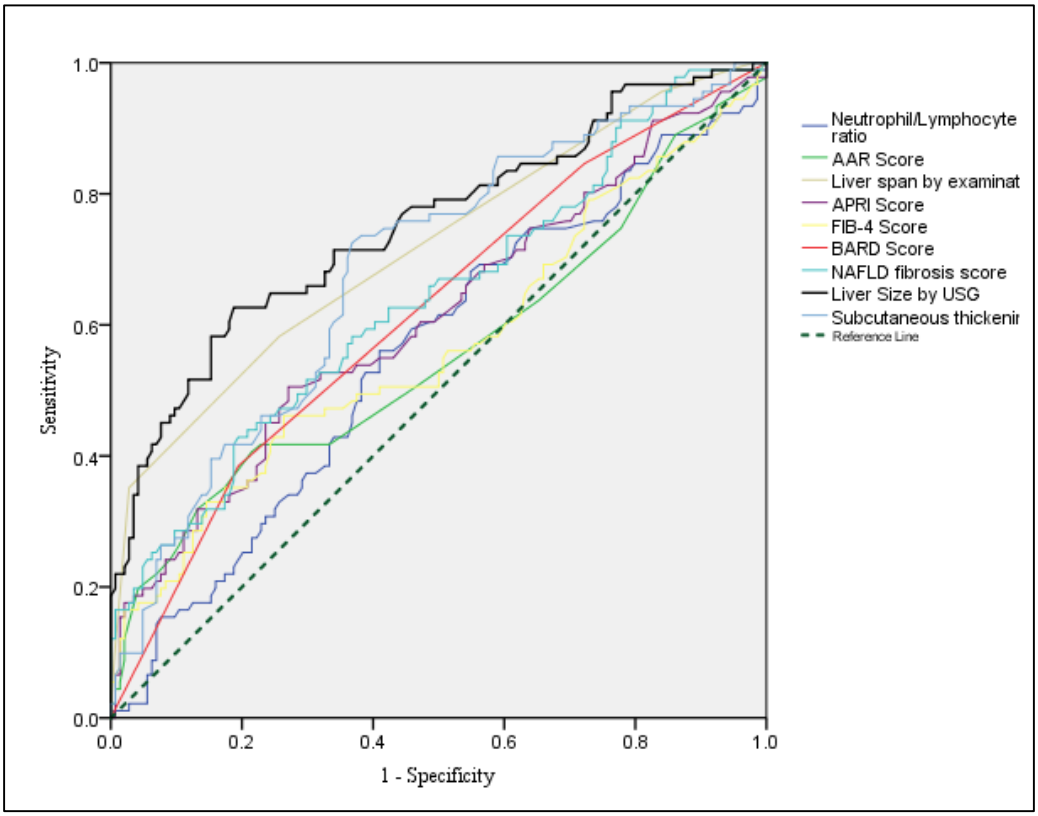


Figure 24: Receiver operating characteristic curves of the diagnostic parameters of NAFLD

Table 27: Performance of selected clinical parameters and scoring systems in the diagnosis of NAFLD

Variables	AUC	95% CI	p-value	SS (%)[2]	SP (%)[2]	PPV (%)[2]	NPV (%)[2]
NLR (≥2.0)	0.557	0.481 - 0.633	0.143	22.0	81.9	42.4	62.4
Liver size by examination (≥15cm)	0.718	0.649 - 0.787	***<0.0001***	11.0	99.3	90.9	63.8
Liver size by USG (≥15cm)	0.752	0.685 - 0.818	***<0.0001***	41.8	91.0	74.5	71.0
Subcutaneous thickness (mm) (≥20mm)	0.683	0.614 - 0.753	***<0.0001***	85.7	36.1	45.9	80.0
AAR ((≥0.5)	0.558	0.478 - 0.637	0.135	91.2	7.6	38.4	57.9
FIB-4 score (≥1.30)	0.568	0.489 - 0.646	***0.080***	46.2	67.4	47.2	66.4
APRI score (≥0.50)	0.611	0.535 - 0.687	***0.004***	19.8	95.1	72.0	65.2
BARD score (F2 - F4)	0.619	0.545 - 0.692	***0.002***	84.6	29.2	43.0	75.0
NAFLD fibrosis score (≥-1.455)	0.639	0.565 - 0.712	***<0.0001***	69.2	41.0	42.6	67.8

SS - Sensitivity *SP – Specificity* *PPV - Positive Predictive Value* *NPV - Negative Predictive Value*

[2] [Results based on the stated cut-off points]

4.7 MULTIVARIABLE REGRESSION ANALYSIS

(PREDICTORS OF NONALCOHOLIC FATTY LIVER DISEASE)

The following variables were entered into the multivariable regression analysis model: waist to hip ratio, BMI, HbA1c, GGT, triglycerides, total cholesterol, liver size determined by USG and abdominal subcutaneous fat thickness. The model was adjusted for age, sex and duration of DM and results exhibited in Table 28.

Table 28: Multivariable regression analysis for predictors of NAFLD

Variables	Adjusted Odds ratio	95% CI	p-value
Age$^{\alpha}$ (years)	0.958	0.958 - 0.922	***0.028***
Sex (female)	0.492	0.200 - 1.209	0.122
Duration of DM$^{\alpha}$ (years)	0.963	0.904 - 1.025	0.235
Liver size by USG (≥15cm)	3.318	1.496 - 7.359	***0.003***
Triglycerides (>2.25 mmol/L)	1.553	0.499 - 4.833	0.448
Total cholesterol (>6.20 mmol/L)	1.283	0.543 - 3.306	0.570
GGT (>58 U/L)	3.331	1.434 - 7.735	***0.005***
BMI$^{\alpha}$ (kg/m^2)	1.067	0.989 - 1.152	0.093
Waist/Hip ratio (M ≥0.90; F ≥0.85)	2.241	0.885 - 5.676	0.089
HbAIc$^{\alpha}$ (%)	1.055	0.901 - 1.235	0.503
Abdominal subcutaneous fat thickness$^{\alpha}$ (mm)	1.051	0.978 - 1.130	0.173

$^{\alpha}$ *– variables entered as continuous variables in the model*

The multivariable regression analysis of some anthropometric, laboratory and sonographic characteristics revealed that, age (OR = 0.958; p-value = 0.028), GGT above the upper limit of 58U/L (OR = 3.331; p-value = 0.005) and liver size of ≥15cm on USG (OR = 3.318; p-value = 0.003) significantly predicted NAFLD in T2DM. The model correctly classified 76.5% of patients who had NAFLD *($(X^2 = 3.699, p = 0.883)$*[3].

Table 29 shows the final model after subjecting the model in table 28 to a forward stepwise regression (entry = $p < 0.05$; removal = $p \geq 0.1$).

Table 29: Estimates of the logistic regression model for prediction of NAFLD

Variables	Adjusted Odds Ratio	95% CI	p-value
Age (years)[α]	0.949	0.918 – 0.980	***0.002***
Liver size by USG (≥15cm)	4.230	1.957 – 9.144	***<0.0001***
GGT (>58U/L)	3.591	1.631 – 7.906	***0.002***
BMI[α] (kg/m^2)	1.108	1.048 – 1.171	***<0.0001***

[α] – *variables entered as continuous variables in the model*

This final model correctly classified 73.5% of patients who had NAFLD *($(X^2 = 3.601, p = 0.891)$*[3]. Age and raised GGT had p-values of 0.002 whiles BMI and enlarged liver size had a p-value of < 0.0001 each. According to the model, every 1-year increase in age is associated with a 5% reduced odds of a diagnosis of NAFLD. Furthermore, every $1kg/m^2$ increase in BMI is associated with roughly 11% higher odds of NAFLD. The model also shows that increasing liver size and increasing GGT were also associated with an increased odd of NAFLD.

[3] Hosmer & Lemeshow goodness of fit test

CHAPTER 5

DISCUSSION

This study was structured to use USG to determine the prevalence of NAFLD among participants with confirmed T2DM. Participants were mostly female and this reflected the pattern of attendance at the DM clinic of the GARH. Almost all participants lived in the urban city of Accra.

5.1 NONALCOHOLIC FATTY LIVER DISEASE

The prevalence of NAFLD among T2DM patients in this study was 38.7%. This finding is lower than the global NAFLD prevalence among patients with T2DM, which has been documented as 55.48% (95% CI 47.26–63.67%) in a recent systematic review by Younossi et al.[17] In comparison to prevalence in all other world regions excluding Africa, it can be seen that this study's finding demonstrates a consistently lower NAFLD prevalence. For example, studies conducted in the USA and Europe identified a mean NAFLD prevalence of 51.64% and 68.82% respectively among T2DM patients.[17] In East Asia, a 2016 cross-sectional study by Nengguang and colleagues at the Shanghai First People's Hospital in China used USG and quoted the overall prevalence of NAFLD among the 541 T2DM patients as 56.6% which was a higher prevalence than what this study recorded.[101] The AASLD also in its 2018 guidelines suggested that about a third to two-thirds of all T2DM patients had NAFLD.[5] It is important to note however that the prevalence of 38.7% identified in this study is comparable to the mean prevalence of NAFLD among T2DM patients in the African region, which has been reported to be 36.29%[17] (95% CI 9.43–68.75). The disparity in the prevalence of this study and other studies in Africa, compared with other world regions such as the USA, Latin America, Europe and East Asia, may allude to the fact that Ghana and possibly Africa at large, is in the early stages of westernization whiles those other countries have progressed to advanced westernization. It is known that westernization is associated with a 'nutrition transition' in which populations undergo a change in dietary patterns that is proportional to their degree of economic and social development.[220] This in turn is associated with an increased incidence of non-communicable diseases including cardiovascular disease, obesity and NAFLD.[221] The current reduction in physical activity due to easy access to vehicular transportation also contribute to the increasing incidence of non-communicable diseases.[222] Europe, the USA and China are higher up the socioeconomic ladder compared to Ghana and other African countries.[223]

Therefore, one could postulate that though Africa lags now in terms of NAFLD prevalence, it may just be a matter of time before similar increasing trends are seen in terms of the NAFLD disease burden.

To obtain a West African perspective of NAFLD in T2DM patients, the prevalence recorded in this study is higher than that reported by Onyekwere et al[33] in 2011 and Olusanya et al[34] in 2016, in Nigeria. Prevalence in the two studies were 9.5% and 16.7% respectively. Interestingly, the most recent study by Afolabi et al[35] in 2018 reported a much higher NAFLD prevalence of 68.8% among T2DM patients. This heterogeneity of results within the same country shows why multicentre epidemiological studies in Africa are warranted. The findings in this current study fall in between the range of the reported rates in Nigeria. Ghana and Nigeria have similar cultures and diets, and the last decade has seen a gradual change in our lifestyle.[224,225] It is possible that the burden of disease may be similar, however, further studies in the region involving multiple centres and larger sample sizes would be useful to better ascertain the disease burden.

The prevalence of NAFLD is also higher than that reported by Bockarie in an unpublished study conducted at the Cape Coast Teaching Hospital, where a prevalence of 24.8% was found.[36] This current study was in a higher risk population, that is patients with T2DM, compared with the study by Bockarie, which looked at prevalence in the general population. The disparity in the reported prevalence is therefore likely a reflection of the differences in populations that were investigated. Additionally, Accra, where this study was conducted, is a much more urbanized population compared to Cape Coast, where Bockarie's study was conducted. The dietary and lifestyle patterns associated with urbanization are also likely to be related to the higher prevalence of NAFLD identified in this cohort.

Regarding the severity of the disease, there was a higher proportion of patients with a higher grade of NAFLD. It was found that grade 3 NAFLD was the most prevalent, 46.1%. The prevalence of grades 1 and 2 were 16.4% and 37.4% respectively. This finding did not corroborate with similar studies conducted in other countries. In Kenya, Kimani[114] in his study among T2DM recorded mild fatty liver (grade 1 NAFLD) as the most prevalent, 60% of the NAFLD cohort, with severe fatty liver (grade 3 NAFLD) being the least prevalent, 7% of the NAFLD cohort. The study in Nigeria by Afolabi et al[35] reported grade 2 NAFLD as the most prevalent, followed by grade 3 and then

grade 1. The recorded prevalence in his study was 32.5%, 20.0% and 16.3% in the entire cohort respectively.

5.1.1 Sociodemographic Associations with Nonalcoholic Fatty Liver Disease

5.1.1.1 Gender

In this study, NAFLD was commoner in females than in males. Although this was not statistically significant, the finding was consistent with the earliest study by Ludwig et al.[2] However, the findings of this study was in contrast with most recent studies conducted in the USA, Europe and Asia.[3,32,99] These studies observed a higher male preponderance in the premenopausal years and proposed that this may be as a result of the protective effects of the female hormones. The disparity between the findings in this current study and these recent studies is most likely because the majority of the female participants in this study were in their postmenopausal years. They, therefore, lacked the protection provided by the female hormones. The mean age of females in this study was 59.5 ± 9.7 years. Also, in this study, a higher proportion of females were recruited.

5.1.1.2 Age

Increasing age was a predictor of NAFLD in this study. Nonalcoholic fatty liver disease was common in the 5^{th} to 7^{th} decades of life with a peak in the age group of 51 - 60 years, 38.5%. This observation is similar to that cited in other studies around the world including the studies from Nigeria.[33,34] This observation in my study is worrying as this age group are in their productive years and form the working class in the country. The burden of NAFLD and T2DM which includes cardiovascular diseases, liver cirrhosis and HCC has negative implications on the economic activities of the country as affected persons may absent themselves from work due to ill-health. Also, the morbidity associated with NAFLD may overburden the already fragile health system in the country.

5.1.2 Clinical Associations with Nonalcoholic Fatty Liver Disease

Significant associations were recorded with the duration of T2DM, obesity, hepatomegaly and abdominal subcutaneous fat thickness.

5.1.2.1 Duration of T2DM

Participants with NAFLD had a shorter duration of T2DM with an average duration of 6.4 ± 5.4 years compared with 8.7 ± 6.3 years in those without NAFLD. Short DM duration was associated with NAFLD and agreed with the works of Williamson et al[99] in the Edinburgh T2DM study. In their study, the average duration of DM was 9.0 ± 6.4 years with short DM duration being an independent risk factor of NAFLD. A possible reason for this association, which was postulated in the Williamson study, is that the insidious onset of T2DM leads to its diagnosis being made many years after disease onset. This results in persistently high levels of insulin before initiation of T2DM therapy, thereby promoting hepatic steatosis. The finding in this study also supports the notion that NAFLD may precede T2DM diagnosis. This was reported by Zhang et al[92] in a longitudinal study where a bi-directional association between NAFLD and metabolic syndrome was identified. The attributable risk of DM to NAFLD and vice versa was 10.85% and 3.90% respectively.

5.1.2.2 Obesity

The association between obesity, a common feature of metabolic syndrome, and NAFLD is well documented.[6,226] Although the mechanism remains unclear, this relationship arises from their common pathogenesis involving IR. The portal and spillover hypothesis has been used to explain the link between central obesity and hepatic steatosis.[227,228] This study also found a significant association between NAFLD in T2DM patients and obesity, that is, both central obesity and high BMI. Body mass index was a significant predictor of NAFLD. This was in accordance with the findings by Hu et al[105] in their study to determine the prevalence of NAFLD by USG among employees in Shanghai, China. They revealed BMI as a better marker than waist circumference for diagnosing NAFLD.

5.1.2.3 Hypertension

Most study participants had good control of their BP. Hypertension was defined based on the blood pressure measurement at the time of data collection. No association was found between hypertension and NAFLD in this study. This was surprising as metabolic syndrome, of which hypertension is a key component, is known to be associated with NAFLD. This observation

disagreed with most studies performed worldwide.[6,23,34] The lack of association may be because most participants in this study were on treatment for hypertension which may have affected the blood pressures recorded.

5.1.2.4 Hepatomegaly

Previous data suggested that the majority of NAFLD patients had no signs on clinical examination. However, this study documented a significant association between NAFLD and hepatomegaly. Hepatomegaly by USG was a good predictor of NAFLD in this study. This correlated with the study findings of Nagaraj[127] and Kimani[114] in India and Kenya respectively. Hepatomegaly by USG also correlated well with the severity of steatosis, that is, NAFLD grade.

5.1.2.5 Abdominal subcutaneous fat thickness

Abdominal subcutaneous fat thickness showed an association with the presence and grade of NAFLD. The observation in this study agrees with that by Hegazy et al[151] where abdominal subcutaneous fat thickness measured with USG was able to predict hepatic steatosis in obese adults. The finding was however contradictory to earlier studies by Parente and Ducluzeau, who used MRI to measure the abdominal subcutaneous fat thickness.[149,150] Parente however documented an association between abdominal subcutaneous fat thickness and the presence of NASH in T2DM. For this study, the higher prevalence of grade 3 NAFLD and its positive correlation with NASH may explain this association.

5.1.3 Biochemical Associations with Nonalcoholic Fatty Liver Disease

5.1.3.1 Glycated haemoglobin

Glycated haemoglobin was unable to predict the presence of NAFLD in this study. However, participants with NAFLD had a significantly poorer DM control compared to those without NAFLD in this study. This was in keeping with the report by Atan et al.[229] The persistently high glucose level in poorly controlled DM results in hyperinsulinaemia which potentiates the process of hepatic steatosis.

5.1.3.2 Lipids

The relationship between NAFLD and dyslipidaemia and hypertriglyceridaemia is established.[226,230] This relationship is as result of their common pathogenesis of IR. Although the majority of participants were on statins which may have altered the results of the lipid profile, elevated total cholesterol, elevated LDL, elevated triglycerides and reduced HDL were significantly associated with NAFLD in this study as was seen in other studies from Africa.[34,114] However, dyslipidaemia and hypertriglyceridaemia in this study were unable to predict NAFLD.

5.1.3.3 Liver biochemistry

Gamma-glutamyltransferase was a predictor of NAFLD in this study. The means of GGT and AST showed significant association with NAFLD despite being within the laboratory range. However, no association existed between NAFLD and ALT, ALP and albumin. This is consistent with studies by Nagaraj[127] and Agarawal[29]. In those studies, elevated GGT and AST levels occurred in advanced NAFLD but not in early disease. This study could not confirm the presence of advanced disease despite observing a higher prevalence of grade 3 NAFLD which correlated well with NASH. Therefore, it is impossible to attribute this finding to the presence of advanced disease.

5.2 NONALCOHOLIC STEATOHEPATITIS

The overall prevalence of NASH as determined by a non-invasive scoring system in the population with NAFLD was 23.1%. This is lower than the 65.26% reported by Golabi et al[18] in his meta-analysis of NASH in T2DM. The differences may be attributable to differences in the methods of NASH assessment as the Golabi meta-analysis enrolled studies that confirmed NASH with histology – the gold standard for diagnosis. The prevalence of NASH in this study was higher in higher grade NAFLD.

Nonalcoholic steatohepatitis in this study was diagnosed using an elevated ALT level and NLR of more than 2.0. Comparing the two methods, NLR was a more sensitive predictor of NASH than ALT. The NLR had a better AUC of 0.928 with high sensitivity but low specificity. Elevated ALT had a high specificity and low sensitivity in the diagnosis of NASH with a lower AUC of 0.680. The poor performance of ALT was also observed in research works done by Neuschwander-Tetri et al[128] and Agarawal et al[29] These researchers recorded elevated ALT levels in the early stages of

NAFLD and subsequent normalization of ALT with the advancement of the disease. Because histology was not done in this study, the severity of the disease could not be confirmed. It is therefore difficult to conclude that ALT levels are more sensitive for predicting early NAFLD where ALT levels may be elevated.

5.3 ADVANCED FIBROSIS

Fibrosis in this study was predicted using the NAFLD fibrosis score. The recorded prevalence of fibrosis among those with NAFLD was 26.4%. Williamson and colleagues in the Edinburgh T2DM study used the NAFLD fibrosis score to record the prevalence of fibrosis among T2DM patients with NAFLD as 16.4%.[103] Worldwide studies have revealed an increasing prevalence of NAFLD and its complications in the last decade in both the general population and T2DM. The lower prevalence in their study compared with this current study can be explained by the time-setting of the Williamson study. The Edinburgh T2DM study was conducted between 2006 and 2007.

The AAR, FIB-4 and BARD were able to predict fibrosis and showed some agreement with the NAFLD fibrosis score. Among these scores, FIB-4 and BARD were preferred in the prediction of fibrosis as they showed better agreement with the NAFLD fibrosis score. The FIB-4 score however documented better specificity but had lower sensitivity and NPV among the 2 scores. The good performance of the FIB-4 score is in keeping with the study by McPherson et al.[155] The AASLD in their 2018 guidelines recommended both FIB-4 and the NAFLD fibrosis score as screening tools in high-risk patients.[5]

5.4 LIMITATIONS

The following limitations are acknowledged;

1. Some participants may have provided wrong information concerning their alcohol intake and other medical histories. This may result in an overestimation of the prevalence of primary NAFLD.

2. Data extracted from participants may be affected by recall biases. For instance, the use of steatogenic medications or a history of other conditions that may cause hepatic steatosis

could not be completely ruled out as most participants had limited knowledge of their medical information.

3. About two-thirds of study participants were on statins which may minimize the severity of steatosis and in some cases, cause liver injury. Statins may also alter the levels of cholesterol and triglycerides of the patient. The exclusion of these participants was not possible as the management of most T2DM patients involved the use of statins.

4. More than 50% of participants were on antihypertensive treatment. This may have altered the blood pressure recordings seen, hence, leading to underestimation of hypertension.

5. The inability to use histology to confirm the diagnosis of NASH and fibrosis or the unavailability of a FibroScan to confirm fibrosis may have affected the prevalence of these subtypes in this study.

6. Confirmatory tests for chronic HBV and HCV were not done due to financial constraints. Chronic viral hepatitis may have similar USG features to NAFLD and hence, lead to the overestimation of the prevalence of NAFLD.

CHAPTER 6

CONCLUSION

Conclusions made are based on this study performed in a major regional hospital in Ghana but may attest to what is happening in other hospitals in the sub-region. The conclusions of this study are;

1. The prevalence of NAFLD in T2DM patients attending the GARH is relatively high (38.7%) than reported in the general population in Ghana but lower than some data from developed countries. The prevalence of severe NAFLD (grade 3) was also high, that is, 46.1%.

2. The factors associated with NAFLD in this study were older age, short DM duration, poorly controlled DM, obesity, hepatomegaly, increased subcutaneous fat thickness dyslipidaemia, triglyceridaemia and elevated GGT and AST. Increasing age, BMI, hepatomegaly and elevated GGT were predictors of NAFLD in this study.

3. The prevalence of NASH as determined by a non-invasive method (NLR >2.0 and elevated ALT) in this study was lower than the current global prevalence.

4. The prevalence of fibrosis diagnosed using the NAFLD fibrosis score was higher than earlier studies conducted in Europe. This confirmed the worldwide increase in NAFLD.

5. The study rejected the null hypothesis that the prevalence of NAFLD-associated risk factors in T2DM patients attending the DM clinic at the GARH does not show any significant difference in those with and without NAFLD.

CHAPTER 7

RECOMMENDATION

Recommendations are as follows;

1. Clinicians taking care of T2DM must educate themselves on NAFLD because of its high prevalence in this group of patients.

2. Clinicians must have a high index of suspicion for NAFLD when managing T2DM patients.

3. Clinical features such as BMI, hepatomegaly and increasing age and basic lab parameters like elevated GGT can be used to complement identifying T2DM patients at risk of NAFLD.

4. Abnormalities on routine laboratory investigations like elevated GGT and ALT and also on USG should not be overlooked but investigated further to rule out NAFLD or advanced liver disease.

5. The use of non-invasive tools in the early diagnosis of suspected cases of NASH and advanced fibrosis is encouraged. Liver biopsies should be used in high-risk patients.

6. Type 2 DM should be aggressively treated because of the association between poorly controlled DM and NAFLD.

REFERENCES

1. Ali R, Cusi K. New diagnostics and treatment approaches in non-alcoholic fatty liver disease (NAFLD). Ann Med. 2009;41:265–78.
2. Ludwig J, Viggiano TR, McGill DB, Oh BJ. Nonalcoholic steatohepatitis: Mayo Clinic experiences with a hitherto unnamed disease. Mayo Clin Proc. 1980 Jul;55(7):434–8.
3. Yu J, Marsh S, Hu J, Feng W, Wu C. The Pathogenesis of Nonalcoholic Fatty Liver Disease: Interplay between Diet, Gut Microbiota, and Genetic Background. Gastroenterol Res Pract. 2016;2016:1–13.
4. Setiawan VW, Stram DO, Porcel J, Lu SC, Le Marchand L, Noureddin M. Prevalence of chronic liver disease and cirrhosis by underlying cause in understudied ethnic groups: The multiethnic cohort. Hepatology. 2016 Dec 1;64(6):1969–77.
5. Chalasani N, Younossi Z, Lavine JE, Charlton M, Cusi K, Rinella M, et al. The diagnosis and management of nonalcoholic fatty liver disease: Practice guidance from the American Association for the Study of Liver Diseases. Hepatology. 2018;67(1):328–57.
6. Younossi ZM, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global epidemiology of nonalcoholic fatty liver disease—Meta-analytic assessment of prevalence, incidence, and outcomes. Hepatology. 2016;64(1):73–84.
7. Demir M, Lang S, Steffen H-M. Nonalcoholic fatty liver disease - current status and future directions. J Dig Dis. 2015 Oct;16(10):541–57.
8. Chalasani N, Younossi Z, Lavine JE, Diehl AM, Brunt EM, Cusi K, et al. The diagnosis and management of non-alcoholic fatty liver disease: Practice Guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association. Hepatology. 2012;55(6):2005–23.
9. Byrne CD, Targher G. EASL–EASD–EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease. Diabetologia. 2016;59(6):1141–4.
10. Suzuki A, Angulo P, Lymp J, St. Sauver J, Muto A, Okada T, et al. Chronological development of elevated aminotransferases in a nonalcoholic population. Hepatology. 2005

Jan;41(1):64–71.

11. Mccullough AJ. Epidemiology of the metabolic syndrome in the USA. J Dig Dis. 2011;12(5):333–40.

12. Lomonaco R, Chen J, Cusi K. An Endocrine Perspective of Nonalcoholic Fatty Liver Disease (NAFLD). Ther Adv Endocrinol Metab. 2011;2(5):211–25.

13. Sinn DH, Gwak GY, Park HN, Kim JE, Min YW, Kim KM, et al. Ultrasonographically detected non-alcoholic fatty liver disease is an independent predictor for identifying patients with insulin resistance in non-obese, non-diabetic middle-aged Asian adults. Am J Gastroenterol. 2012 Apr;107(4):561–7.

14. Almeda-Valdés P, Cuevas-Ramos D, Aguilar-Salinas C. Metabolic syndrome and Non alcoholic fatty liver disease. Ann Hepatol. 2009;8(1):S18–24.

15. Lonardo A, Nascimbeni F, Mantovani A, Targher G. Hypertension, diabetes, atherosclerosis and NASH: Cause or consequence? Vol. 68, Journal of Hepatology. Elsevier B.V.; 2018. p. 335–52.

16. Byrne CD, Targher G. NAFLD: A multisystem disease. J Hepatol. 2015;62(S1):S47–64.

17. Younossi ZM, Golabi P, de Avila L, Paik JM, Srishord M, Fukui N, et al. The global epidemiology of NAFLD and NASH in patients with type 2 diabetes: A systematic review and meta-analysis. J Hepatol. 2019 Oct 1;71(4):793–801.

18. Golabi P, Paik J, Deavila L, Fukui N, Srishord M, Younossi Z. The worldwide prevalence of Non-alcoholic Steatohepatitis (NASH) in patients with Type 2 Diabetes Mellitus (DM). J Hepatol. 2018 Apr;68:S841.

19. Firneisz G. Non-alcoholic fatty liver disease and type 2 diabetes mellitus: The liver disease of our age? World J Gastroenterol. 2014;20(27):9072–89.

20. Angulo P. Long-term mortality in nonalcoholic fatty liver disease: Is liver histology of any prognostic significance? Hepatology. 2010;51(2):373–5.

21. Wong RJ, Aguilar M, Cheung R, Perumpail RB, Harrison SA, Younossi ZM, et al. Clinical-Liver Nonalcoholic Steatohepatitis Is the Second Leading Etiology of Liver Disease Among

Adults Awaiting Liver Transplantation in the United States. Gastroenterology. 2015;148:547–55.

22. Younossi ZM, Gramlich T, Matteoni CA, Boparai N, McCullough AJ. Nonalcoholic fatty liver disease in patients with type 2 diabetes. Clin Gastroenterol Hepatol. 2004;2(3):262–5.

23. Armstrong MJ, Adams LA, Canbay A, Syn W-K. Extrahepatic complications of nonalcoholic fatty liver disease. Hepatology. 2014 Mar;59(3):1174–97.

24. Vanni E, Marengo A, Mezzabotta L, Bugianesi E. Systemic Complications of Nonalcoholic Fatty Liver Disease: When the Liver Is Not an Innocent Bystander. Semin Liver Dis. 2015 Sep 17;35(03):236–49.

25. Targher G, Bertolini L, Rodella S, Tessari R, Zenari L, Lippi G, et al. Nonalcoholic Fatty Liver Disease Is Independently Associated With an Increased Incidence of Cardiovascular Events in Type 2 Diabetic Patients. Diabetes Care. 2007;30:2119–21.

26. Biritwum R, Gyapong J, Mensah G. The epidemiology of obesity in ghana. Ghana Med J. 2005 Sep;39(3):82–5.

27. Ofori-Asenso R, Agyeman AA, Laar A, Boateng D. Overweight and obesity epidemic in Ghana - A systematic review and meta-analysis. Vol. 16, BMC Public Health. BioMed Central Ltd.; 2016.

28. Tracey ML, McHugh SM, Buckley CM, Canavan RJ, Fitzgerald AP, Kearney PM. The prevalence of Type 2 diabetes and related complications in a nationally representative sample of adults aged 50 and over in the Republic of Ireland. Diabet Med. 2016 Apr;33(4):441–5.

29. Agarawal J. Prevalence of elevated hepatic enzymes among North Indian patients with type 2 diabetes mellitus. Santosh Univ J Heal Sci. 2015;1(1):3–6.

30. Bellentani S, Scaglioni F, Marino M, Bedogni G. Epidemiology of Non-Alcoholic Fatty Liver Disease. Dig Dis. 2010;28(1):155–61.

31. Foster T, Anania FA, Li D, Katz R, Budoff M. The prevalence and clinical correlates of nonalcoholic fatty liver disease (NAFLD) in African Americans: the multiethnic study of atherosclerosis (MESA). Dig Dis Sci. 2013 Aug;58(8):2392–8.

32. Lazo M, Hernaez R, Eberhardt MS, Bonekamp S, Kamel I, Guallar E, et al. Prevalence of nonalcoholic fatty liver disease in the United States: the Third National Health and Nutrition Examination Survey, 1988-1994. Am J Epidemiol. 2013 Jul 1;178(1):38–45.

33. Onyekwere CA, Ogbera AO, Balogun BO. Non-alcoholic fatty liver disease and the metabolic syndrome in an urban hospital serving an African community. Ann Hepatol. 2011;10(2):119–24.

34. Olusanya TO, Lesi OA, Adeyomoye AA, Fasanmade OA. Non alcoholic fatty liver disease in a Nigerian population with type II diabetes mellitus. Pan Afr Med J. 2016;24:20.

35. Afolabi BI, Ibitoye BO, Ikem RT, Omisore AD, Idowu BM, Soyoye DO. The Relationship Between Glycaemic Control and Non-Alcoholic Fatty Liver Disease in Nigerian Type 2 Diabetic Patients. J Natl Med Assoc. 2018 Jun 1;110(3):256–64.

36. Bockarie AS. An assessment of cardiovascular risk factors among patients with nonalcoholic fatty liver disease(NAFLD) at the Cape Coast Teaching Hospital, Cape Coast, Ghana. Unpublished. KomfoAnokye Teaching Hospital; 2017.

37. Committee SE. IDF Diabetes Atlas 7th edition (2015). 2015.

38. Darkwa S. Prevalence of diabetes mellitus and resources available for its management in the Cape Coast Metropolis. ISABB J Heal Environ Sci. 2011;1(1):1–7.

39. World Health Organization. Global Report on Diabetes. Isbn. 2016;978:88.

40. Wiredu EK, Armah HB. Cancer mortality patterns in Ghana: A 10-year review of autopsies and hospital mortality. BMC Public Health. 2006 Jun 20;6:159.

41. Nartey YA, Awuku YA, Agyei-Nkansah A, Duah A, Bampoh SA, Ayawin J, et al. Ambulatory end-stage liver disease in Ghana; patient profile and utility of alpha fetoprotein and aspartate aminotransferase: platelet ratio index. BMC Gastroenterol. 2020 Dec 1;20(1):428.

42. Yang JD, Gyedu A, Afihene MY, Duduyemi BM, Micah E, Kingham TP, et al. Hepatocellular Carcinoma Occurs at an Earlier Age in Africans, Particularly in Association With Chronic Hepatitis B. Am J Gastroenterol. 2015 Nov 30;110(11):1629–31.

43. Agyei-Nkansah A, Archampong T, Tachi K. Hepatocellular carcinoma in Ghana: A retrospective analysis of a tertiary hospital data. Pan Afr Med J. 2020;36:1–8.

44. Lebovics, E.; Rubin J. Non-alcoholic fatty liver disease (NAFLD): why you should care, when you should worry, what you should do Edward. Diabetes Metab Res Rev. 2011;27:419–24.

45. Mofrad, P.S; Sanyal AJ. Nonalcoholic Fatty Liver Disease [Internet]. Medscape. 2003 [cited 2019 Jul 28]. Available from: https://www.medscape.com/viewarticle/449315

46. Contos MJ, Sanyal AJ. The clinicopathologic spectrum and management of nonalcoholic fatty liver disease. Adv Anat Pathol. 2002 Jan;9(1):37–51.

47. Brunt EM. Pathology of fatty liver disease. Mod Pathol. 2007;20 Suppl 1:S40-8.

48. Mahamid M., Safadi R., Mader M. Hepatotoxicity of tocilizumab and anakinra in rheumatoid arthritis: management decisions. Clin Pharmacol Adv Appl. 2011 Oct;3:39–43.

49. Leung K, Quezada M, Chen Z, Kanel G, Kaplowitz N. Niacin-Induced Anicteric Microvesicular Steatotic Acute Liver Failure. Hepatol Commun. 2018 Nov;2(11):1293–8.

50. Cunningham CC, Ph D, Horn CG Van. Energy Availability and Alcohol-Related Liver Pathology. Alcohol Res Heal. 2003;27(4):291–9.

51. Mendez-Sanchez, N; Almeda-Valdes, P; Uribe M. Alcoholic Liver Disease. An Update. Ann Hepatol. 2005;4(1):32–42.

52. Lelbach WK. Cirrhosis in the alcoholic and its relation to the volume of alcohol abuse. Ann N Y Acad Sci. 1975 Apr 1;252(1 Medical Conse):85–105.

53. Bellentani S, Saccoccio G, Masutti F, Crocè LS, Brandi G, Sasso F, et al. Prevalence of and risk factors for hepatic steatosis in northern Italy. Ann Intern Med. 2000 Jan 18;132(2):112–7.

54. Ratziu V, Bellentani S, Cortez-Pinto H, Day C, Marchesini G, Peter J, et al. A position statement on NAFLD/NASH based on the EASL 2009 special conference. J Hepatol. 2010 Aug;53(2):372–84.

55. Abd El-Kader SM, El-Den Ashmawy EMS. Non-alcoholic fatty liver disease: The diagnosis

and management. World J Hepatol. 2015 Apr 28;7(6):846–58.

56. Health D of. Alcohol units - A brief guide. 2008.

57. WHO. Global status report on alcohol and health - African region. Glob Status Rep Alcohol World Heal Organ. 2004;5–8.

58. Tulashie SK, Appiah AP, Torku GD, Darko AY, Wiredu A. Determination of methanol and ethanol concentrations in local and foreign alcoholic drinks and food products (Banku, Ga kenkey, Fante kenkey and Hausa koko) in Ghana. Int J Food Contam. 2017;4(1):0–4.

59. Reid AE. Nonalcoholic Fatty Liver Disease. In: Feldman, Mark; Freidman, Lawrence S.; Brandt LJ, editor. Sleisenger and Fordtran's Gastrointestinal and Liver Disease - pathophysiology/diagnosis/management. 8th ed. Saunders, Elsevier; 2006. p. 1793–805.

60. Pais R, Pascale A, Fedchuck L, Charlotte F, Poynard T, Ratziu V. Progression from isolated steatosis to steatohepatitis and fibrosis in nonalcoholic fatty liver disease. Clin Res Hepatol Gastroenterol. 2011 Jan 1;35(1):23–8.

61. Cortez-Pinto H, Camilo ME, Baptista A, De Oliveira AG, De Moura MC. Non-alcoholic fatty liver: another feature of the metabolic syndrome? Clin Nutr. 1999 Dec;18(6):353–8.

62. Margariti E, Deutsch M, Manolakopoulos S, Papatheodoridis G V. Non-alcoholic fatty liver disease may develop in individuals with normal body mass index. Ann Gastroenterol. 2012;25(1):45–51.

63. Musso G, Gambino R, Cassader M. Non-alcoholic fatty liver disease from pathogenesis to management: An update. Obes Rev. 2010;11(6):430–45.

64. Lazo M, Clark J. The Epidemiology of Nonalcoholic Fatty Liver Disease: A Global Perspective. Semin Liver Dis. 2008 Nov 27;28(04):339–50.

65. Kneeman JM, Misdraji J, Corey KE. Secondary causes of nonalcoholic fatty liver disease. Therap Adv Gastroenterol. 2012 May;5(3):199–207.

66. Bruno S, Maisonneuve P, Castellana P, Rotmensz N, Rossi S, Maggioni M, et al. Incidence and risk factors for non-alcoholic steatohepatitis: prospective study of 5408 women enrolled in Italian tamoxifen chemoprevention trial. BMJ. 2005 Apr 23;330(7497):932.

67. Price JC, Thio CL. Liver disease in the HIV-infected individual. Clin Gastroenterol Hepatol. 2010 Dec;8(12):1002–12.

68. Qureshi K, Abrams GA. Metabolic liver disease of obesity and role of adipose tissue in the pathogenesis of nonalcoholic fatty liver disease. World J Gastroenterol. 2007;13(26):3540–53.

69. Fuchs M. Non-Alcoholic Fatty Liver Disease: The Bile Acid-Activated Farnesoid X Receptor as an Emerging Treatment Target. J Lipids. 2012;2012:1–8.

70. Rui L. Energy metabolism in the liver. Compr Physiol. 2014;4(1):177–97.

71. Horton JD, Shimomura L. Sterol regulatory element-binding proteins: Activators of cholesterol and fatty acid biosynthesis. Vol. 10, Current Opinion in Lipidology. 1999. p. 143–50.

72. Yamashita H, Takenoshita M, Sakurai M, Bruick RK, Henzel WJ, Shillinglaw W, et al. A glucose-responsive transcription factor that regulates carbohydrate metabolism in the liver. Proc Natl Acad Sci U S A. 2001 Jul 31;98(16):9116–21.

73. Barrera G, Pizzimenti S, Daga M, Dianzani C, Arcaro A, Cetrangolo GP, et al. Lipid peroxidation-derived aldehydes, 4-hydroxynonenal and malondialdehyde in aging-related disorders. Vol. 7, Antioxidants. MDPI AG; 2018.

74. McGarry JD, Foster DW. Effects of exogenous fatty acid concentration on glucagon-induced changes in hepatic fatty acid metabolism. Diabetes. 1980;29(3):236–40.

75. Sanyal AJ. Mechanisms of Disease: pathogenesis of nonalcoholic fatty liver disease. Nat Clin Pract Gastroenterol Hepatol. 2005 Jan;2(1):46–53.

76. Day CP, James OFW. Steatohepatitis: A tale of two ‘Hits’? Gastroenterology. 1998;114(4 I):842–5.

77. Imajo K, Yoneda M, Kessoku T, Ogawa Y, Maeda S, Sumida Y, et al. Rodent models of nonalcoholic fatty liver disease/nonalcoholic steatohepatitis. Int J Mol Sci. 2013;14(11):21833–57.

78. Peverill W, Powell LW, Skoien R. Evolving concepts in the pathogenesis of NASH: Beyond

steatosis and inflammation. Int J Mol Sci. 2014;15(5):8591–638.

79. Buzzetti E, Pinzani M, Tsochatzis EA. The multiple-hit pathogenesis of non-alcoholic fatty liver disease (NAFLD). Metabolism. 2016;65(8):1038–48.

80. Romeo S, Kozlitina J, Xing C, Pertsemlidis A, Cox D, Pennacchio LA, et al. Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease. Nat Genet. 2008 Dec;40(12):1461–5.

81. Bruschi FV, Tardelli M, Claudel T, Trauner M. PNPLA3 expression and its impact on the liver: current perspectives. Hepatic Med Evid Res. 2017 Nov;Volume 9:55–66.

82. Sharma P, Arora A. Approach to prevention of non-alcoholic fatty liver disease after liver transplantation. Transl Gastroenterol Hepatol. 2020 Oct 5;5(0):51–51.

83. Kim CH, Kallman JB, Bai C, Pawloski L, Gewa C, Arsalla A, et al. Nutritional Assessments of Patients with Non-alcoholic Fatty Liver Disease. Obes Surg. 2010 Feb 17;20(2):154–60.

84. Wree A, Broderick L, Canbay A, Hoffman HM, Feldstein AE. From NAFLD to NASH to cirrhosis?new insights into disease mechanisms. Nat Rev Gastroenterol Hepatol. 2013 Aug 20;10(11):627–36.

85. Paradis V, Perlemuter G, Bonvoust F, Dargere D, Parfait B, Vidaud M, et al. High glucose and hyperinsulinemia stimulate connective tissue growth factor expression: A potential mechanism involved in progression to fibrosis in nonalcoholic steatohepatitis. Hepatology. 2001 Oct;34(4):738–44.

86. Chen J, Montagner A, Tan NS, Wahli W. Insights into the role of PPARβ/δ in NAFLD. Vol. 19, International Journal of Molecular Sciences. MDPI AG; 2018.

87. Harrison SA, Kadakia S, Lang KA, Schenker S. Nonalcoholic steatohepatitis: what we know in the new millennium1. Am J Gastroenterol. 2002 Nov;97(11):2714–24.

88. Tilg H, Moschen AR. Evolution of inflammation in nonalcoholic fatty liver disease: The multiple parallel hits hypothesis. Hepatology. 2010 Nov;52(5):1836–46.

89. Hui JM, Hodge A, Farrell GC, Kench JG, Kriketos A, George J. Beyond insulin resistance in NASH: TNF-α or adiponectin? Hepatology. 2004 Jul;40(1):46–54.

90. Kirpich IA, Marsano LS, McClain CJ. Gut-liver axis, nutrition, and non-alcoholic fatty liver disease. Clin Biochem. 2015 Sep;48(13–14):923–30.

91. Yilmaz Y. Review article: is non-alcoholic fatty liver disease a spectrum, or are steatosis and non-alcoholic steatohepatitis distinct conditions? Aliment Pharmacol Ther. 2012 Nov 1;36(9):815–23.

92. Zhang Y, Zhang T, Zhang C, Tang F, Zhong N, Li H, et al. Identification of reciprocal causality between non-alcoholic fatty liver disease and metabolic syndrome by a simplified Bayesian network in a Chinese population. BMJ Open. 2015;5(9).

93. Musso G, Gambino R, Cassader M, Pagano G. Meta-analysis: Natural history of non-alcoholic fatty liver disease (NAFLD) and diagnostic accuracy of non-invasive tests for liver disease severity. Ann Med. 2011 Dec;43(8):617–49.

94. Ballestri S, Zona S, Targher G, Romagnoli D, Baldelli E, Nascimbeni F, et al. Nonalcoholic fatty liver disease is associated with an almost twofold increased risk of incident type 2 diabetes and metabolic syndrome. Evidence from a systematic review and meta-analysis. J Gastroenterol Hepatol. 2016 May 1;31(5):936–44.

95. Bedogni G, Miglioli L, Masutti F, Castiglione A, Crocè LS, Tiribelli C, et al. Incidence and natural course of fatty liver in the general population: The dionysos study. Hepatology. 2007;46(5):1387–91.

96. Whalley S, Puvanachandra P, Desai A, Kennedy H. Hepatology outpatient service provision in secondary care: a study of liver disease incidence and resource costs. Clin Med. 2007 Apr;7(2):119–24.

97. Williams CD, Stengel J, Asike MI, Torres DM, Shaw J, Contreras M, et al. Prevalence of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis among a largely middle-aged population utilizing ultrasound and liver biopsy: A prospective study. Gastroenterology. 2011;140(1):124–31.

98. Rinella M, Charlton M. The globalization of nonalcoholic fatty liver disease: Prevalence and impact on world health. Hepatology. 2016;64(1):19–22.

99. Williamson RM, Price JF, Glancy S, Perry E, Nee LD, Hayes PC, et al. Prevalence of and

Risk Factors for Hepatic Steatosis and Nonalcoholic Fatty Liver Disease in People With Type 2 Diabetes: the Edinburgh Type 2 Diabetes Study. Diabetes Care. 2011 May 1;34(5):1139–44.

100. Portillo-Sanchez P, Bril F, Maximos M, Lomonaco R, Biernacki D, Orsak B, et al. High Prevalence of Nonalcoholic Fatty Liver Disease in Patients With Type 2 Diabetes Mellitus and Normal Plasma Aminotransferase Levels. J Clin Endocrinol Metab. 2015 Jun;100(6):2231–8.

101. Fan N, Zhang L, Xia Z, Peng L, Wang Y, Peng Y. Sex-Specific Association between Serum Uric Acid and Nonalcoholic Fatty Liver Disease in Type 2 Diabetic Patients. J Diabetes Res. 2016;2016:1–6.

102. Younossi ZM. The epidemiology of nonalcoholic steatohepatitis. Clin Liver Dis. 2018;11(4):92–4.

103. Williamson RM, Price JF, Hayes PC, Glancy S, Frier BM, Johnston GI, et al. Prevalence and markers of advanced liver disease in type 2 diabetes. Q J Med. 2012 May 1;105(5):425–32.

104. Kumar NA, Das S. Fibroscan of Liver in Type 2 Diabetes Mellitus and Its Correlation with Risk Factors. J Diabetes Mellit. 2019 May 31;09(02):62–8.

105. Hu X, Huang Y, Bao Z, Wang Y, Shi D, Liu F, et al. Prevalence and factors associated with nonalcoholic fatty liver disease in shanghai work-units. BMC Gastroenterol. 2012 Nov 14;12(1):1–9.

106. Hashimoto E, Tokushige K. Prevalence, gender, ethnic variations, and prognosis of NASH. J Gastroenterol. 2011 Jan 16;46(SUPPL. 1):63–9.

107. Sheth SG, Gordon FD, Chopra S. Nonalcoholic steatohepatitis. Vol. 126, Annals of Internal Medicine. American College of Physicians; 1997. p. 137–45.

108. ANGULO P. GI Epidemiology: nonalcoholic fatty liver disease. Aliment Pharmacol Ther. 2007 Mar 30;25(8):883–9.

109. Kalia HS, Gaglio PJ. The Prevalence and Pathobiology of Nonalcoholic Fatty Liver Disease in Patients of Different Races or Ethnicities. Vol. 20, Clinics in Liver Disease. W.B.

Saunders; 2016. p. 215–24.

110. Nail A, Gadour M, Khair M, Salma B. Fatty liver disease in Sudan is not alcohol related. Sudan J Med Sci. 2007 Jan 17;1(2):97–102.

111. Kruger FC, Daniels C, Kidd M, Swart G, Brundyn K, Van Rensburg C, et al. Non-alcoholic fatty liver disease (NAFLD) in the Western Cape: A descriptive analysis. South African Med J. 2010;100(3):168–71.

112. Ezzat WM, Ragab S, Ismail NA, Elhosary YA, Nour AM, Elbaky EA, et al. Frequency of non-alcoholic fatty liver disease in overweight/obese children and adults: Clinical, sonographic picture and biochemical assessment. J Genet Eng Biotechnol. 2012;10:221–7.

113. Almobarak AO, Barakat S, Khalifa MH, Elhoweris MH, Elhassan TM, Ahmed MH. Non alcoholic fatty liver disease (NAFLD) in a Sudanese population: What is the prevalence and risk factors? Arab J Gastroenterol. 2014;15(1):12–5.

114. Kimani KJ. Prevalence of Primary Non-Alcoholic Fatty Liver Disease in Black Africans With Type 2 Diabetes At Kenyatta National Hospital . University of Nairobi; 2013.

115. Hübscher SG. Histological assessment of non-alcoholic fatty liver disease. Histopathology. 2006;49(5):450–65.

116. Dowman JK, Tomlinson JW, Newsome PN. Systematic review: the diagnosis and staging of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis. Aliment Pharmacol Ther. 2011 Mar;33(5):525–40.

117. Oh MK, Winn J, Poordad F. Review article: Diagnosis and treatment of non-alcoholic fatty liver disease. Aliment Pharmacol Ther. 2008;28(5):503–22.

118. Kunming Biomed International. Non-alcoholic Fatty Liver Disease (NAFLD) and Nonalcoholic Steatohepatitis (NASH) [Internet]. Kunming Biomed International. 2018 [cited 2019 Jul 28]. Available from: http://www.kbimed.com/NewsDis-abd0058bb6de48f3870aa89fa6434755.htm

119. Matteoni CA, Younossi ZM, Gramlich T, Boparai N, Yao Chang Liu, McCullough AJ. Nonalcoholic fatty liver disease: A spectrum of clinical and pathological severity. Gastroenterology. 1999;116(6):1413–9.

120. Rafiq N, Bai C, Fang Y, Srishord M, McCullough A, Gramlich T, et al. Long-Term Follow-Up of Patients With Nonalcoholic Fatty Liver. Clin Gastroenterol Hepatol. 2009 Feb;7(2):234–8.

121. Al Knawy B, Shiffman M. Percutaneous liver biopsy in clinical practice. Liver Int. 2007 Oct 3;27(9):1166–73.

122. Younossi ZM, Gramlich T, Yao CL, Matteoni C, Petrelli M, Goldblum J, et al. Nonalcoholic fatty liver disease: Assessment of variability in pathologic interpretations. Mod Pathol. 1998 Jun;11(6):560–5.

123. Ratziu V, Charlotte F, Heurtier A, Gombert S, Giral P, Bruckert E, et al. Sampling Variability of Liver Biopsy in Nonalcoholic Fatty Liver Disease. Gastroenterology. 2005 Jun;128(7):1898–906.

124. Rath MM, Panigrahi MK, Pattnaik K, Bhuyan P, Kar SK, Misra B, et al. Histological Evaluation of Non-alcoholic Fatty Liver Disease and Its Correlation with Different Noninvasive Scoring Systems with Special Reference to Fibrosis: A Single Center Experience. J Clin Exp Hepatol. 2016 Dec;6(4):291–6.

125. Wieckowska A, Feldstein A. Diagnosis of Nonalcoholic Fatty Liver Disease: Invasive versus Noninvasive. Semin Liver Dis. 2008 Nov 27;28(04):386–95.

126. Obika M, Noguchi H. Diagnosis and evaluation of nonalcoholic fatty liver disease. Exp Diabetes Res. 2012;2012:1–12.

127. Nagaraj S, Kiran SS, Gandham R, R WDSC, Nagaraja MR, S AN, et al. Study of prevalence of non alcoholic fatty liver disease in type 2 diabetes mellitus patients and variations in liver function tests , lipid profile and mean platelet volume in patients with fatty liver in comparison with patients without fatty liver. Int J Res Med Sci. 2016;4(3):871–6.

128. Neuschwander-Tetri BA, Clark JM, Bass NM, Van Natta ML, Unalp-Arida A, Tonascia J, et al. Clinical, laboratory and histological associations in adults with nonalcoholic fatty liver disease. Hepatology. 2010;52(3):913–24.

129. Tahan V, Canbakan B, Balci H, Dane F, Akin H, Can G, et al. Serum gamma-glutamyltranspeptidase distinguishes non-alcoholic fatty liver disease at high risk.

Hepatogastroenterology. 2008 Jul;55(85):1433–8.

130. Rinella ME. Nonalcoholic fatty liver disease a systematic review. Vol. 313, JAMA - Journal of the American Medical Association. American Medical Association; 2015. p. 2263–73.

131. Smith BW, Adams LA. Nonalcoholic fatty liver disease and diabetes mellitus: pathogenesis and treatment. Nat Rev Endocrinol. 2011;7(8):456–65.

132. Kowdley K V, Belt P, Wilson LA, Yeh MM, Neuschwander-Tetri BA, Chalasani N, et al. Serum ferritin is an independent predictor of histologic severity and advanced fibrosis in patients with nonalcoholic fatty liver disease. Hepatology. 2012 Jan;55(1):77–85.

133. Angulo P, Keach JC, Batts KP, Lindor KD. Independent predictors of liver fibrosis in patients with nonalcoholic steatohepatitis. Hepatology. 1999 Dec 1;30(6):1356–62.

134. Sumida Y, Yoshikawa T, Okanoue T. Role of hepatic iron in non-alcoholic steatohepatitis. Hepatol Res. 2009 Mar 1;39(3):213–22.

135. W. W, N. G, M.-A. K, J. A. Concise Review: Current Status and Future Directions on Research Related to Nonalcoholic Fatty Liver Disease. Stem Cells. 2017;35(1):89–96.

136. Lee SS, Park SH. Radiologic evaluation of nonalcoholic fatty liver disease. World J Gastroenterol. 2014 Jun 21;20(23):7392–402.

137. Adibi A, Maleki S, Adibi P, Etminani R, Hovsepian S. Prevalence of Nonalcoholic Fatty Liver Disease and its Related Metabolic Risk Factors in Isfahan, Iran. Adv Biomed Res. 2017;6(47):1–7.

138. Khov N, Sharma A, Riley TR. Bedside ultrasound in the diagnosis of nonalcoholic fatty liver disease. World J Gastroenterol. 2014 Jun 14;20(22):6821–5.

139. Lee JY, Kim KM, Lee SG, Yu E, Lim Y-S, Lee HC, et al. Prevalence and risk factors of non-alcoholic fatty liver disease in potential living liver donors in Korea: A review of 589 consecutive liver biopsies in a single center. J Hepatol. 2007 Aug;47(2):239–44.

140. Saadeh S, Younossi ZM, Remer EM, Gramlich T, Ong JP, Hurley M, et al. The Utility of Radiological Imaging in Nonalcoholic Fatty Liver Disease. Gastroenterology. 2002;123(3):745–50.

141. Mottin CC, Moretto M, Padoin A V., Swarowsky AM, Toneto MG, Glock L, et al. The Role of Ultrasound in the Diagnosis of Hepatic Steatosis in Morbidly Obese Patients. Obes Surg. 2004 May 1;14(5):635–7.

142. Fierbinteanu-Braticevici C., Dina I., Petrisor A., Tribus L., Negreanu L., Carstoiu C. Noninvasive investigations for non alcoholic fatty liver disease and liver fibrosis. World J Gastroenterol. 2010 Oct 14;16(38):4784–91.

143. Fishbein M, Castro F, Cheruku S, Jain S, Webb B, Gleason T, et al. Hepatic MRI for fat quantitation: Its relationship to fat morphology, diagnosis, and ultrasound. J Clin Gastroenterol. 2005 Aug 1;39(7):619–25.

144. de Lédinghen V, Vergniol J. Transient elastography (FibroScan). Gastroentérologie Clin Biol. 2008 Sep;32(6):58–67.

145. Yoneda M, Yoneda M, Yoneda M, Fujita K, Fujita K, Inamori M, et al. Transient elastography in patients with non-alcoholic fatty liver disease (NAFLD). Gut. 2007 Sep;56(9):1330–1.

146. Wong VWS, Vergniol J, Wong GLH, Foucher J, Chan HLY, Le Bail B, et al. Diagnosis of fibrosis and cirrhosis using liver stiffness measurement in nonalcoholic fatty liver disease. Hepatology. 2010;51(2):454–62.

147. De Lédinghen V, Vergniol J, Foucher J, El-Hajbi F, Merrouche W, Rigalleau V. Feasibility of liver transient elastography with FibroScan® using a new probe for obese patients. Liver Int. 2010 Feb 5;30(7):1043–8.

148. Yin M, Talwalkar JA, Glaser KJ, Manduca A, Grimm RC, Rossman PJ, et al. Assessment of Hepatic Fibrosis With Magnetic Resonance Elastography. Clin Gastroenterol Hepatol. 2007;5(10):1207–13.

149. Ducluzeau PH, Manchec-Poilblanc P, Roullier V, Cesbron E, Lebigot J, Bertrais S, et al. Distribution of abdominal adipose tissue as a predictor of hepatic steatosis assessed by MRI. Clin Radiol. 2010 Sep;65(9):695–700.

150. Parente DB, Oliveira Neto JA, Brasil PEAA, Paiva FF, Ravani JPR, Gomes MB, et al. Preperitoneal fat as a non-invasive marker of increased risk of severe non-alcoholic fatty

liver disease in patients with type 2 diabetes. J Gastroenterol Hepatol. 2018 Feb 1;33(2):511–7.

151. Hegazy MA, Samy MA, Tawfik A, Naguib MM, Ezzat A, Behiry ME. Abdominal subcutaneous fat thickness and homeostasis model assessment of insulin resistance as simple predictors of nonalcoholic steatohepatitis. Diabetes, Metab Syndr Obes Targets Ther. 2019;12:1105–11.

152. Lee SH, Kim D, Baek MY, Tchah H, Kim YS, Ryoo E, et al. Abdominal subcutaneous fat thickness measured by ultrasonography correlates with hyperlipidemia and steatohepatitis in obese children. Pediatr Gastroenterol Hepatol Nutr. 2015;18(2):108–14.

153. Pearce SG, Thosani NC, Pan J-J. Noninvasive biomarkers for the diagnosis of steatohepatitis and advanced fibrosis in NAFLD. Biomark Res. 2013;1(7).

154. Cichoż-lach H, Celiński K, Prozorow-król B, Swatek J, Słomka M, Lach T. The BARD score and the NAFLD fibrosis score in the assessment of advanced liver fibrosis in nonalcoholic fatty liver disease. Med Sci Monit. 2012;18(12):735–40.

155. McPherson S, Stewart SF, Henderson E, Burt AD, Day CP. Simple non-invasive fibrosis scoring systems can reliably exclude advanced fibrosis in patients with non-alcoholic fatty liver disease. Gut. 2010;59(9):1265–9.

156. Angulo P, Hui JM, Marchesini G, Bugianesi E, George J, Farrell GC, et al. The NAFLD fibrosis score: A noninvasive system that identifies liver fibrosis in patients with NAFLD. Hepatology. 2007;45(4):846–54.

157. Fujii H, Enomoto M, Fukushima W, Tamori A, Sakaguchi H, Kawada N. Applicability of BARD score to Japanese patients with NAFLD. Vol. 58, Gut. 2009. p. 1566–7.

158. Wong VWS, Wong GLH, Chim AML, Tse AML, Tsang SWC, Hui AY, et al. Validation of the NAFLD fibrosis score in a Chinese population with low prevalence of advanced fibrosis. Am J Gastroenterol. 2008 Jul;103(7):1682–8.

159. Calès P, Lainé F, Boursier J, Deugnier Y, Moal V, Oberti F, et al. Comparison of blood tests for liver fibrosis specific or not to NAFLD. J Hepatol. 2009 Jan;50(1):165–73.

160. Wai C-T, Greenson JK, Fontana RJ, Kalbfleisch JD, Marrero JA, Conjeevaram HS, et al. A

simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. Hepatology. 2003 Aug;38(2):518–26.

161. Ruffillo G, Fassio E, Alvarez E, Landeira G, Longo C, Domínguez N, et al. Comparison of NAFLD fibrosis score and BARD score in predicting fibrosis in nonalcoholic fatty liver disease. J Hepatol. 2011 Jan 1;54(1):160–3.

162. Imajo K, Kessoku T, Honda Y, Tomeno W, Ogawa Y, Mawatari H, et al. Magnetic Resonance Imaging More Accurately Classifies Steatosis and Fibrosis in Patients with Nonalcoholic Fatty Liver Disease Than Transient Elastography. Gastroenterology. 2016;150(3):626–37.

163. Shah AG, Lydecker A, Murray K, Tetri BN, Contos MJ, Sanyal AJ, et al. Comparison of noninvasive markers of fibrosis in patients with nonalcoholic fatty liver disease. Clin Gastroenterol Hepatol. 2009 Oct 1;7(10):1104–12.

164. Alkhouri N, Morris-Stiff G, Campbell C, Lopez R, Tamimi TA-R, Yerian L, et al. Neutrophil to lymphocyte ratio: a new marker for predicting steatohepatitis and fibrosis in patients with nonalcoholic fatty liver disease. Liver Int. 2012 Feb 1;32(2):297–302.

165. Tanoglu A, Karagoz E. Neutrophil-to-lymphocyte ratio: an emerging prognostic factor of cirrhosis? Eur J Gastroenterol Hepatol. 2014 Mar;26(3):362.

166. Abdel-Razik A, Mousa N, Shabana W, Refaey M, ElMahdy Y, Elhelaly R, et al. A novel model using mean platelet volume and neutrophil to lymphocyte ratio as a marker of nonalcoholic steatohepatitis in NAFLD patients: Multicentric study. Eur J Gastroenterol Hepatol. 2016;28(1):e1–9.

167. Singh S, Allen AM, Wang Z, Prokop LJ, Murad MH, Loomba R. Fibrosis progression in nonalcoholic fatty liver vs nonalcoholic steatohepatitis: a systematic review and meta-analysis of paired-biopsy studies. Clin Gastroenterol Hepatol. 2015 Apr;13(4):643-54.e1-9; quiz e39-40.

168. Pais R, Charlotte F, Fedchuk L, Bedossa P, Lebray P, Poynard T, et al. A systematic review of follow-up biopsies reveals disease progression in patients with non-alcoholic fatty liver. J Hepatol. 2013 Sep 1;59(3):550–6.

169. Adams LA, Sanderson S, Lindor KD, Angulo P. The histological course of nonalcoholic fatty liver disease: A longitudinal study of 103 patients with sequential liver biopsies. J Hepatol. 2005;42(1):132–8.

170. Cholankeril G, Patel R, Khurana S, Satapathy SK. Hepatocellular carcinoma in non-alcoholic steatohepatitis: Current knowledge and implications for management. World J Hepatol. 2017 Apr 18;9(11):533–43.

171. Bhala N, Ibrahim Kamal Jouness R, Bugianesi E. Epidemiology and Natural History of Patients with NAFLD.

172. Wideroff L, Gridley G, Mellemkjaer L, Chow WH, Linet M, Keehn S, et al. Cancer incidence in a population-based cohort of patients hospitalized with diabetes mellitus in Denmark. J Natl Cancer Inst. 1997;89(18):1360–5.

173. Musso G, Gambino R, Tabibian JH, Ekstedt M, Kechagias S, Hamaguchi M, et al. Association of Non-alcoholic Fatty Liver Disease with Chronic Kidney Disease: A Systematic Review and Meta-analysis. PLoS Med. 2014;11(7).

174. Sayki Arslan M, Turhan S, Dincer I, Mizrak D, Corapcioglu D, Idilman R. A potential link between endothelial function, cardiovascular risk, and metabolic syndrome in patients with Non-alcoholic fatty liver disease. Diabetol Metab Syndr. 2014;6(1):1–6.

175. Hazlehurst JM, Woods C, Marjot T, Cobbold JF, Tomlinson JW. Non-alcoholic fatty liver disease and diabetes. Metabolism. 2016;65(8):1096–108.

176. Barb D, Portillo-Sanchez P, Cusi K. Pharmacological management of nonalcoholic fatty liver disease. Metabolism. 2016;65(8):1183–95.

177. Paredes AH, Torres DM, Harrison SA. Treatment of nonalcoholic fatty liver disease: Role of dietary modification and exercise. Clin Liver Dis. 2012 Sep;1(4):117.

178. Hallsworth K, Fattakhova G, Hollingsworth KG, Thoma C, Moore S, Taylor R, et al. Resistance exercise reduces liver fat and its mediators in non-alcoholic fatty liver disease independent of weight loss. Gut. 2011 Sep;60(9):1278–83.

179. Lazo M, Solga SF, Horska A, Bonekamp S, Diehl AM, Brancati FL, et al. Effect of a 12-month intensive lifestyle intervention on hepatic steatosis in adults with type 2 diabetes.

Diabetes Care. 2010;33(10):2156–63.

180. Haufe S, Engeli S, Kast P, Böhnke J, Utz W, Haas V, et al. Randomized comparison of reduced fat and reduced carbohydrate hypocaloric diets on intrahepatic fat in overweight and obese human subjects. Hepatology. 2011 May;53(5):1504–14.

181. Belfort R, Harrison SA, Brown K, Darland C, Finch J, Hardies J, et al. A Placebo-Controlled Trial of Pioglitazone in Subjects with Nonalcoholic Steatohepatitis. 2006;2297–307.

182. Aithal GP, Thomas JA, Kaye P V, Lawson A, Ryder SD, Spendlove I, et al. Randomized, placebo-controlled trial of pioglitazone in nondiabetic subjects with nonalcoholic steatohepatitis. Gastroenterology. 2008 Oct 1;135(4):1176–84.

183. Sanyal AJ, Chalasani N, Kowdley K V., McCullough A, Diehl AM, Bass NM, et al. Pioglitazone, vitamin E, or placebo for nonalcoholic steatohepatitis. N Engl J Med. 2010;362(18):1675–85.

184. Younossi ZM, Loomba R, Rinella ME, Bugianesi E, Marchesini G, Neuschwander-Tetri BA, et al. Current and future therapeutic regimens for nonalcoholic fatty liver disease and nonalcoholic steatohepatitis. Vol. 68, Hepatology. John Wiley and Sons Inc.; 2018. p. 361–71.

185. Pacana T, Sanyal AJ. Vitamin e and nonalcoholic fatty liver disease. Vol. 15, Current Opinion in Clinical Nutrition and Metabolic Care. NIH Public Access; 2012. p. 641–8.

186. Lomonaco R, Ortiz-Lopez C, Orsak B, Finch J, Webb A, Bril F, et al. Role of ethnicity in overweight and obese patients with nonalcoholic steatohepatitis. Hepatology. 2011;54(3):837–45.

187. Luisa Vonghia SF. Pharmacological Treatment for Non-alcoholic Fatty Liver Disease. 2019;

188. Ratziu V, Harrison SA, Francque S, Bedossa P, Lehert P, Serfaty L, et al. Elafibranor, an Agonist of the Peroxisome Proliferator-Activated Receptor-α and -δ, Induces Resolution of Nonalcoholic Steatohepatitis Without Fibrosis Worsening. Gastroenterology. 2016 May 1;150(5):1147-1159.e5.

189. Neuschwander-Tetri BA, Loomba R, Sanyal AJ, Lavine JE, Van Natta ML, Abdelmalek

MF, et al. Farnesoid X nuclear receptor ligand obeticholic acid for non-cirrhotic, non-alcoholic steatohepatitis (FLINT): A multicentre, randomised, placebo-controlled trial. Lancet. 2015 Mar 14;385(9972):956–65.

190. Cusi, K., Consoli A., Defronzo A. Metabolic effects of metformin on glucose and lactate metabolism in noninsulin-dependent diabetes mellitus. J Clin Endocrinol Metab. 1996;81(11):4059–67.

191. Loomba R., Lutchman G., Kleiner DE., Ricks M., Feld JJ., Borg BB., et al. Clinical trial: pilot study of metformin for the treatment of non- alcoholic steatohepatitis. Aliment Pharmacol Ther. 2009;29(2):172–82.

192. LI Y, LIU L, WANG B, WANG J, CHEN D. Metformin in non-alcoholic fatty liver disease: A systematic review and meta-analysis. Biomed Reports. 2013;1(1):57–64.

193. Musso G, Gambino R, Cassader M, Pagano G. A meta-analysis of randomized trials for the treatment of nonalcoholic fatty liver disease. Hepatology. 2010 Jul 1;52(1):79–104.

194. Neuschwander-Tetri BA. Non-alcoholic fatty liver disease. BMC Med. 2017;15(45):1–6.

195. Hossain N, Kanwar P, Mohanty SR. A Comprehensive Updated Review of Pharmaceutical and Nonpharmaceutical Treatment for NAFLD. Gastroenterol Res Pract. 2016;2016:1–17.

196. Chavez-Tapia NC, Tellez-Avila FI, Barrientos-Gutierrez T, Mendez-Sanchez N, Lizardi-Cervera J, Uribe M. Bariatric surgery for non-alcoholic steatohepatitis in obese patients. Cochrane Database Syst Rev. 2010 Jan 20;(1):CD007340.

197. Belfort R, Berria R, Cornell J, Cusi K. Fenofibrate Reduces Systemic Inflammation Markers Independent of Its Effects on Lipid and Glucose Metabolism in Patients with the Metabolic Syndrome. J Clin Endocrinol Metab. 2010 Feb 1;95(2):829–36.

198. Fernández-Miranda C, Pérez-Carreras M, Colina F, López-Alonso G, Vargas C, Solís-Herruzo JA. A pilot trial of fenofibrate for the treatment of non-alcoholic fatty liver disease. Dig Liver Dis. 2008 Mar;40(3):200–5.

199. Nakamuta M, Morizono S, Soejima Y, Yoshizumi T, Aishima S, Takasugi S, et al. Short-Term Intensive Treatment for Donors with Hepatic Steatosis in Living-Donor Liver Transplantation. Transplantation. 2005 Sep 15;80(5):608–12.

200. Laurin J, Lindor KD, Crippin JS, Gossard A, Gores GJ, Ludwig J, et al. Ursodeoxycholic acid or clofibrate in the treatment of non-alcohol-induced steatohepatitis: A pilot study. Hepatology. 1996 Jun;23(6):1464–7.

201. Van Der Veen JN, Lingrell S, Gao X, Takawale A, Kassiri Z, Vance DE, et al. Fenofibrate, but not ezetimibe, prevents fatty liver disease in mice lacking phosphatidylethanolamine N-methyltransferase. J Lipid Res. 2017;58(4):656–67.

202. Zamor PJ, Russo MW. Liver function tests and statins. Curr Opin Cardiol. 2011 Jul;26(4):338–41.

203. Cohen DE, Anania FA, Chalasani N, National Lipid Association Statin Safety Task Force Liver Expert Panel. An Assessment of Statin Safety by Hepatologists. Am J Cardiol. 2006 Apr 17;97(8):S77–81.

204. Ginsberg HN., Elam MB., Lovato LC., Crouse IJR., Leiter LA., Linz P. Effects of combination lipid therapy in type 2 diabetes mellitus. N. New Engl J Med. 2010;17(362):1563–74.

205. Trovato FM, Castrogiovanni P, Malatino L, Musumeci G. Nonalcoholic fatty liver disease (NAFLD) prevention: role of Mediterranean diet and physical activity. HepatoBiliary Surg Nutr. 2019 Apr;8(2):167–9.

206. Li B, Zhang C, Zhan YT. Nonalcoholic Fatty Liver Disease Cirrhosis: A Review of Its Epidemiology, Risk Factors, Clinical Presentation, Diagnosis, Management, and Prognosis. Vol. 2018, Canadian Journal of Gastroenterology and Hepatology. Hindawi Limited; 2018.

207. Zipprich A, Garcia-Tsao G, Rogowski S, Fleig WE, Seufferlein T, Dollinger MM. Prognostic indicators of survival in patients with compensated and decompensated cirrhosis. Liver Int. 2012 Oct;32(9):1407–14.

208. Benhammou JN, Aby ES, Shirvanian G, Manansala K, Hussain SK, Tong MJ. Improved survival after treatments of patients with nonalcoholic fatty liver disease associated hepatocellular carcinoma. Sci Rep. 2020;10(1):1–9.

209. Ghana Statistical Service. 2010 Population and Housing Census: National Analytical Report. 2013;430.

210. International Labour Office. International Standard Classification of Occupations. Vol. I. Geneva; 2012.

211. Nuttall FQ. Body mass index: Obesity, BMI, and health: A critical review. Nutr Today. 2015;50(3):117–28.

212. World Health Organization. Waist Circumference and Waist-Hip Ratio Report of a WHO Expert Consultation. Geneva; 2008.

213. Alberti G., Zimmet P, Shaw J., Grundy SM. The IDF consensus worldwide defi nition of the Metabolic Syndrome. IDF Commun. 2006;1:1–23.

214. Council ES, Guidelines EC for P. 2013 ESH / ESC Guidelines for the management of arterial hypertension The Task Force for the management of arterial hypertension of the European Society of Hypertension (ESH) and of the European Society. Eur Heart J. 2013;34:2159–219.

215. Glynn M. Gastrointestinal System. In: Glynn M, Drake W., editors. Hutchison's Clinical Methods. 23rd ed. International: Saunders Elsevier; 2012. p. 217–48.

216. American Diabetes Association (ADA). Standard of medical care in diabetes - 2017. Diabetes Care. 2017;40 (sup 1)(January):s4–128.

217. Patzak M, Porzner M, Oeztuerk S, Mason RA, Wilhelm M, Graeter T, et al. Assessment of liver size by ultrasonography. J Clin Ultrasound. 2014;42(7):399–404.

218. Özmen Z, Aktaş F, Özmen Z, Almus E, Demir O. Ultrasound measurement of liver longitudinal length in a North Anatolian population: A community-based study. Niger J Clin Pract. 2018;21(5):653–7.

219. Singh T, Singla B. Clinical and Sonographic Estimation of Liver Span in Normal Healthy Adults. www.ijmrhs.com Int J Med Res Heal Sci. 2017;6(1):94–7.

220. Vorster HH, Kruger A, Margetts BM. The nutrition transition in Africa: can it be steered into a more positive direction? Vol. 3, Nutrients. Multidisciplinary Digital Publishing Institute (MDPI); 2011. p. 429–41.

221. Popkin BM, Gordon-Larsen P. The nutrition transition: Worldwide obesity dynamics and

their determinants. Int J Obes. 2004;28:S2–9.

222. Lee IM, Shiroma EJ, Lobelo F, Puska P, Blair SN, Katzmarzyk PT, et al. Effect of physical inactivity on major non-communicable diseases worldwide: An analysis of burden of disease and life expectancy. Lancet. 2012 Jul 21;380(9838):219–29.

223. United Nations. Inequality in a Rapidly Changing World. World social report 2020. 2020. 216 p.

224. Food and Agriculture Organization. The Rising Middle Class and Evolving Food Demand in Ghana and Nigeria.

225. Mosahab R, Mahamad O, Ramayah T, RA Nur Amalina, Ekonomi F, Diponegoro U, et al. THE CULTURAL POLICIES OF GHANA AND NIGERIA: A COMPARATIVE STUDY. Vol. 4, knust.edu. 2011.

226. Cuenza LR, Razon TLJ, Dayrit JC. Correlation between severity of ultrasonographic nonalcoholic fatty liver disease and cardiometabolic risk among Filipino wellness patients. J Cardiovasc Thorac Res. 2017 Jun 8;9(2):85–9.

227. Bjorntorp P. 'Portal' adipose tissue as a generator of risk factors for cardiovascular disease and diabetes. Vol. 10, Arteriosclerosis. 1990. p. 493–6.

228. Item F, Konrad D. Visceral fat and metabolic inflammation: The portal theory revisited. Obes Rev. 2012 Dec;13(SUPPL.2):30–9.

229. Atan NAD, Koushki M, Motedayen M, Dousti M, Sayehmiri F, Vafaee R, et al. Type 2 diabetes mellitus and non-alcoholic fatty liver disease: A systematic review and meta-analysis. Vol. 10, Gastroenterology and Hepatology from Bed to Bench. Research Institute for Gastroenterology and Liver Diseases; 2017. p. S1–7.

230. Paschos P, Paletas K. Non alcoholic fatty liver disease and metabolic syndrome. Hippokratia. 2009 Jan;13(1):9–19.

APPENDICES

APPENDIX I

GHANA HEALTH SERVICE ETHICS REVIEW COMMITTEE

In case of reply the number and date of this Letter should be quoted.

MyRef. GHS/RDD/ERC/Admin/App/
Your Ref. No. 11/5/18

Research & Development Division
Ghana Health Service
P. O. Box MB 190
Accra
Tel: +233-302-681109
Fax + 233-302-685424
Email: ghserc@gmail.com
14th May, 2018

Dr. Sally Afua Bampoh
Department of Internal Medicine
Greater Accra Regional Hospital
P.O. Box 473,
Accra

The Ghana Health Service Ethics Review Committee has reviewed and given approval for the implementation of your Study Protocol.

GHS-ERC Number	**GHS-ERC004/03/18**
Project Title	Nonalcoholic Fatty Liver Disease in Type 2 Diabetes Mellitus Patients Attending the Diabetes Clinic at the Greater Accra Regional Hospital
Approval Date	8th May,2018
Expiry Date	7th May, 2019
GHS-ERC Decision	**Approved**

This approval requires the following from the Principal Investigator

- Submission of yearly progress report of the study to the Ethics Review Committee (ERC)
- Renewal of ethical approval if the study lasts for more than 12 months,
- Reporting of all serious adverse events related to this study to the ERC within three days verbally and seven days in writing.
- Submission of a final report **after completion** of the study
- Informing ERC if study cannot be implemented or is discontinued and reasons why
- Informing the ERC and your sponsor (where applicable) before any publication of the research findings.

Please note that any modification of the study without ERC approval of the amendment is invalid.

The ERC may observe or cause to be observed procedures and records of the study during and after implementation.

Kindly quote the protocol identification number in all future correspondence in relation to this approved protocol

SIGNED.....
DR. CYNTHIA BANNERMAN
(GHS-ERC CHAIRPERSON)

Cc: The Director, Research & Development Division, Ghana Health Service, Accra

APPENDIX II

In case of reply the number and date of this letter should be quoted

My Ref. No : GHS/GARH/GA/G-02
Your Ref. No....

CORE VALUES:
People-Centered
Professionalism
Team Work
Innovation/Excellence,
Discipline
Integrity
Pacesetters

THE GREATER ACCRA REGIONAL HOSPITAL, RIDGE
GHANA HEALTH SERVICE
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6TH FEBRUARY, 2018

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THE CHAIRMAN
GHANA HEALTH SERVICE ETHICAL REVIEW COMMITTEE
RESEARCH & DEVELOPMENT DIVISION
ACCRA

PERMISSION LETTER
DR. SALLY AFUA BAMPOH – PHYSICIAN SPECIALIST

This serves to introduce to you the above-named person who is pursuing a fellowship in Gastroenterology and Hepatology under the Ghana college of physician and surgeons.

As part of her fellowship, she is required to submit a dissertation which will be on the topic **"Nonalcoholic fatty liver disease in type 2 mellitus patients attending the diabetes clinic at The Greater Accra Regional Hospital, Ridge".**

Management of the hospital has granted her the permission to use the facility as her case study.

Thank you.

DR. EMMANUEL K. SROFENYOH
Ag. MEDICAL DIRECTOR

APPENDIX III

Consent Form

Participant ID: **Date:**

Title of Project: Nonalcoholic fatty liver disease in type 2 diabetes mellitus patients attending the diabetes clinic at the Greater Accra Regional Hospital

Researcher: Dr Sally Afua Bampoh

Address of Investigator: Department of Medicine, Korle Bu Teaching Hospital, Accra

Information: This consent form contains information about the study entitled "Nonalcoholic fatty liver disease in type 2 diabetes mellitus patients attending the diabetes clinic at the Greater Accra Regional Hospital". To be well informed about this research and make an informed decision about your participation or not, we ask that you carefully read this consent form or have it read and explained to you. You can ask for further clarification. You will be required to sign or thumb print this form in the presence of a witness.

Purpose of the study: Nonalcoholic fatty liver disease is a common cause of chronic liver disease which occurs when there is excessive deposition of fat in the liver in the absence of significant alcohol intake. It may occur in combination with type 2 diabetes mellitus or obesity. It can result in complications like liver cirrhosis and hepatocellular carcinoma. Currently, there are no effective treatment modalities for this condition but early detection with lifestyle modification decreases risk of complications. Despite the worldwide increase in the prevalence of this disease, little is known about it in Ghana. The current increase in type 2 diabetes mellitus and chronic liver disease in the country has precipitated the need for this study. This study aims at determining the prevalence and factors associated with nonalcoholic fatty liver disease in these patients. The findings will guide healthcare professionals to detect condition early and initiate effective monitoring and management strategies in affected patients. Guidelines can be formulated to decrease the incidence of complications.

General information and your part in the study: To be part of this study, you should be a type 2 diabetes mellitus patient and attend the diabetes clinic at the Greater Accra Regional Hospital. You must test negative to a rapid diagnostic test for chronic hepatitis B and hepatitis C virus. As

a participant, a study questionnaire will be used to ask questions about yourself and your condition and also extract data from your medical records if you readily do not have answers to some of these questions. A physical examination will then be performed to assess your weight and height. About 15mls of blood will be drawn for analysis. You will undergo an abdominal ultrasonography to assess your liver. All this information will be kept confidential as provided by law. There will be no remuneration and you will not incur additional cost by participating in this study.

Possible benefits: The direct benefit of this study to you is the early detection of the condition if present to enable early management to decrease the risk of complications. Your participation will also give a better understanding of this condition in type 2 diabetes mellitus patients and help improve its surveillance and management. This can be extended to the general population especially those with other lifestyle-related diseases.

Possible risks: During this study, blood samples will be drawn. The amount of blood drawn is harmless. The risks of taking blood samples include discomfort or pain at the site, bleeding and infections. To minimize these risks, well-trained health personnel will draw all blood samples and only disposable syringes, gloves and single-use equipment will be used. You may experience some discomfort at the site and also some bleeding or bruising but these will disappear after a few minutes. In the event of severe bleeding, the necessary emergency management will be offered.

Withdrawal from study: Please be informed that, taking part in the study is entirely voluntary and your refusal to be part of this study is your right. Also, you have a right to withdraw from this study at any given time without having to give reasons despite your initial consent. Any such decision will be respected and will not alter the care you receive from the hospital.

Confidentiality: Information collected during this study would be treated as strictly confidential and protected from all persons not associated with the study. Also, your name will not be identified with any reports or publications. If you ever have any questions about the research study or seek further clarification later on, you may contact me, **Dr Sally Afua Bampoh** on **0206301486**.

Ethical review: This research has been reviewed and approved by the Ethics Review Committee of the Ghana Health Service. This committee is a group of scientists and lay people who look at research to be undertaken to ensure that the research is safe and that, no deliberate harm comes to individuals who partakes in it. For questions about the ethical aspects of this study or your rights

as a volunteer, you may contact the administrator of the Ethics Review Committee of the Ghana Health Service during working hours via email on **ghserc@gmail.com** or on **+233507041223**.

Volunteer Agreement

The above document describing the benefits, risks and procedures for the research titled "Nonalcoholic fatty liver disease in type 2 diabetes mellitus patients attending the diabetes clinic at the Greater Accra Regional Hospital" has been read and explained to me. I have been given an opportunity to ask any question about the research and these questions answered to my satisfaction. I agree to participate as a volunteer.

_ _

Name of participant **Signature / Thumbprint** **Date**

If volunteers cannot read the form themselves, a witness must sign here:

I was present while the benefits, risks and procedures were read to the volunteer. All questions were answered and the volunteer has agreed to take part in the research.

_ _

Name of witness **Signature / Thumbprint** **Date**

I certify that the nature and purpose, the potential benefits, and possible risks associated with participating in this research have been explained to the above individual.

_ _

Name of person who obtained consent **Signature** **Date**

A copy of the signed informed consent form must be given to the subject / patient.

APPENDIX IV

Questionnaire

Date of Interview: .....

You will be asked some questions about yourself and also medical information about yourself and your family. I assure you that any information you give will be treated as confidential.

Thank you for your cooperation.

(i) <u>Identifier Information</u>

Patient's ID number: ..... **Patient's Initials:** ..... **Folder number:** .....

Residential Address: …..... **Contact No:** .....

Environment (residence): Urban□ Rural□

(ii) <u>Demography</u>

Sex:	Male□		Female□
Age (years):	18 - 30□	31 - 40□	41 - 50□
	51 - 60□	61 - 70□	> 70□
Ethnicity:	Akan□	Ga□	Ewe□
	Hausa□	Other□….....	
Religion:	None□	Christian□	Moslem □
	Traditionalist□	Other□.....	
Marital status:	Single□	Married□	Cohabiting□
	Divorced□		Widowed□
Education level:	None□	Primary□	Secondary□
	Tertiary□		Postgraduate□

Employment Status: Unemployed□ Part-time□

Fully employed□ Retired□

Occupation: …….

Occupation Category: None□ Major group 0□ Major group 1□

Major group 2□ Major group 3□ Major group 4□

Major group 5□ Major group 6□ Major group 7□

Major group 8□ Major group 9□

(iii)<u>Current Symptoms of NAFLD:</u> Asymptomatic □ Symptomatic□

If Symptomatic;

Fever:	Yes□	No□
Malaise:	Yes□	No□
Fatigue:	Yes□	No□
Weight loss:	Yes□	No□
Nausea:	Yes□	No□
Vomiting:	Yes□	No□
Haematemesis:	Yes□	No□
Melaena Stools:	Yes□	No□
Jaundice:	Yes□	No□
Right Upper Quadrant pain:	Yes□	No□
Abdominal distension:	Yes□	No□
Pedal swelling:	Yes□	No□

Others □ ……

(iv)Medical History

T2DM Duration: …. (yrs)	< 1□	1 - 5□	6 - 10□
	11 - 15□	16 - 20□	> 20□
Compliance:	No□		Yes (diet only) □
	Yes (medication only) □		Yes (both)□
Personal History:	Hypertension□	Dyslipidaemia□	Obesity□
Family History:	T2DM□	Hypertension□	Dyslipidaemia□
	Obesity□	NAFLD□	Liver Disease□

(i) Drug History

1. .....
2. .....
3. .....
4. .....
5. .....
6. .....
7. .....
8. .....
9. ……
10. ……

(ii) Exercise History

How many days do you exercise in a week?

Never□ 1 day / week□

2-3 days / week□ >3 days / week□

If exercising;

What exercises do you do?

Walking□ Jogging□ Skipping□

Aerobics□ Other□……..

What is the duration of each session?

< 30mins□ 30mins – 1 hour□ >1 hour□

(iii)<u>Social History</u>

Alcohol: Yes□ No□

If yes;

Duration (years): <1□ 1 - 5□ 5 - 10□ > 10□

Type of alcohol: Beer (1.3 IU / 330ml) □ Wine (1 IU / glass) □

Spirits (1 IU / pub) □ Other: ..... (...IU/…) □

Quantity (IU per week): ≤ 7□ > 7 - 14□ > 14 - 21□

Smoking: Never□ Current smoker□ Ex-smoker□

If current smoker;

Number of pack per years: < 5□ 5 - 10□ ≥10□

Substance smoked? Tobacco□ Marijuana□ Other□……

If ex-smoker;

How many years after quitting? <1□ 1 - 5□ 5 - 10□ >10□

Herbal medication: Yes□ No□

If yes,

Duration (years): … <1□ 1 - 5□ 5 - 10□ >10□

(iv)<u>Examination Findings</u>

Anthropometry:

Weight (Kg): 1st ..... 2nd..... Average.....

Height (m): 1st..... 2nd..... Average.....

BMI (kg/m^2): ..... Underweight□ Normal□ Overweight□

Class I□ Class II□ Class III□

Waist Circumference (cm): 1st..... 2nd..... Average.....

(M: ≤94cm; F: ≤80cm): Normal□ Increased□

Hip Circumference (cm): 1st..... 2nd..... Average.....

Waist-Hip ratio: .... (M: <0.90, F: <0.85) Normal□ Increased□

BP (mmHg): 1st..... 2nd..... Average.....

BP Class: Optimal□ Normal□ High Normal□

Grade I□ Grade II□ Grade III□

Isolated Systolic Hypertension□

General Examination: Normal□ Abnormal□

If Abnormal, specify;

Fever: Yes□ No□

Pallor: Yes□ No□

Jaundice: Yes□ No□

Lymph nodes: Yes□ No□

Spider Naevi: Yes□ No□

Clubbing: Yes□ No□

Palmar Erythema: Yes□ No□

Pedal Oedema: Yes□ No□

Arcus cornealis: Yes□ No□

Acanthosis nigricans: Yes□ No□

Xanthoma: Yes□ No□

Abdominal Exam: Normal□ Abnormal□

Inspection: Distended□ Dilated veins□

Liver Span (10-14cm): .... Shrunken□ Normal□ Enlarge□

Spleen enlarged? Yes□ No□

Abdominal mass? Yes□ No□

If yes, specify.....

Ascites: None□ Mild□ Moderate□ Massive□

Others: .....

(v) <u>Laboratory tests</u>:

FBC:

Hb (M: 13 - 18g/dL; F: 11 - 16 g/dL):	Normal□		Mild Anaemia□
	Mod-Severe Anaemia□		Polycythaemia□
WBC (3.50 - 9.50 x 10^3/L): …….	Low□	Normal□	Increased□
Neutrophils (1.80 - 6.30 x 10^3/L): ….	Low□	Normal□	Increased□
Lymphocyte (1.10 - 3.20 x 10^3/L): …	Low□	Normal□	Increased□
Platelets (150 - 400 x 10^3/L): …..…...	Low□	Normal□	Increased□
FBS (4.0 - 6.0mmol/L):	Controlled□		Uncontrolled□
HbA1c (< 7.0%): ……	Controlled□		Uncontrolled□
INR: …….....	≤ 1.2□		> 1.2□

LFT:

Total Bilirubin (3 - 22umol/L):	Normal□		Elevated□
Direct Bilirubin (0.0 - 5.0umol/L): ……	Normal□		Elevated□
Indirect Bilirubin (3 - 17umol/L): ……..	Normal□		Elevated□
AST (15 - 46 U/L): ……	Normal□		Elevated□
ALT (13 - 69 U/L): ……	Normal□		Elevated□
ALP (38 - 126 U/L): …….	Normal□		Elevated□
GGT (12 - 58 U/L): ……...	Normal□		Elevated□
Total Protein (63 - 82 g/L): ..…….....	Decreased□	Normal□	Elevated□
Albumin (35 - 50 g/L): ……...	Decreased□	Normal□	Elevated□

Lipids:

Total Cholesterol (3.30 - 6.20 mmol/L): …	Normal□	Elevated□
Triglycerides (0.40 - 2.25 mmol/L): …..…	Normal□	Elevated□
HDL (> 1.03mmol/L): …….....	Normal□	Reduced□
VLDL (0.00 - 2.59 mmol/L): …….	Normal□	Elevated□
LDL (0.00 - 3.90 mmol/L): …….	Normal□	Elevated□

(vi) <u>Scores:</u>

NLR: ……...…	< 2.0□		≤ 2.0□
APRI Score: ……...	≤ 0.5□	> 0.5 - 1.5□	≥ 1.5□
FIB-4 Score: ……...	<1.30□	1.30 - 2.67□	> 2.67□
AST / ALT Ratio: …….	≤ 0.5□	> 0.5 - 1.0□	> 1.0□
NAFLD Fibrosis Score: ……	<-1.455□	-1.455-0.676□	>0.676□
BARD Score: …….	0 - 1□		2 - 4□

(vii) <u>Ultrasonography:</u>

Liver:

Size (10cm - 15cm): …….	Shrunken□	Normal□		Enlarged□
Impaired hepato-renal contrast:	None□	Minimal□	Moderate□	Marked□
Impaired diaphragm visualization:	No□	Slight□		Marked□
Blurred hepatic vessels:	No□	Slight□		Marked□
Visible edges:	No□			Yes□
Focal Lesion:	No□			Yes□
If yes, **Number?**	Single□		2 - 5□	> 5□
Size?	< 3cm□		3 - 5cm□	> 5cm□
Spleen (≤ 12cm): ……	Not Enlarged□			Enlarged□
Ascites:	None□	Mild□	Moderate□	Massive□
Subcutaneous thickening: ……	≤ 20mm□			> 20 - 25mm□
	> 25 - 30mm□			>30□

APPENDIX V

FULL BLOOD COUNT				
	Reduced		**Normal**	**Elevated**
Hb (g/dL)	**Moderate-Severe**	**Mild**		
	<8.0	M = ≥8.0 - 13.0 F = ≥8.0 - 11.0	M = 13.0 - 18.0 F = 11.0 - 16.0	>18.0 >16.0
WBC (x10^3/L)	<3.50		3.50 - 9.50	>9.50
Neutrophils (x10^3/L)	<1.80		1.80 - 6.30	>6.30
Lymphocytes (x10^3/L)	<1.10		1.10 - 3.20	>3.20
Platelets (x10^3/L)	<150		150 - 400	>400

BIOCHEMISTRY			
Liver Function Test:			
	Low	**Normal**	**Elevated**
Total Bilirubin (umol/L)	<3.0	3.0 - 22.0	>22.0
Direct Bilirubin (umol/L)	-	0.0 - 5.0	>5.0
Indirect Bilirubin (umol/L)	<3.0	3.0 - 17.0	>17.0
AST (U/L)	<15.0	15.0 - 46.0	>46.0
ALT (U/L)	<13.0	13.0 - 69.0	>69.0
ALP (U/L)	<38.0	38.0 - 126.0	>126.0
GGT (U/L)	<12.0	12.0 - 58.0	>58.0
Total Protein (g/L)	<63.0	63.0 - 82.0	>82.0
Albumin (g/L)	<35.0	35.0 - 50.0	>50.0

Lipids:			
Total Cholesterol (mmol/L)	<3.30	3.30 - 6.20	>6.20
Triglycerides (mmol/L)	<0.40	0.40 - 2.25	>2.25
HDL (mmol/L)	≤1.03	>1.03	-
VLDL (mmol/L)	-	0.00 - 2.59	>2.59
LDL (mmol/L)	-	0.00 - 3.90	>3.90

	Controlled	**Uncontrolled**
FBS (mmol/L)	≤6.1	>6.1
HbA1c (%)	≤7.0	>7.0
INR	≤1.2	>1.2

SCORING SYSTEMS			
	Insignificant	**Indeterminate**	**Significant**
AAR	≤0.5	>0.5 - 1.0	>1.0
APRI score	≤0.5	>0.5 - 1.5	>1.5
BARD score	0 - 1		2 - 4
FIB-4 score	<1.30	1.30 - 2.67	>2.67
NAFLD fibrosis score	<-1.455	-1.455 - 0.676	> 0.676
NLR	<2.0		≥2.0