PREVALENCE OF PRIMARY NON-ALCOHOLIC FATTY LIVER DISEASE IN BLACK AFRICANS WITH TYPE 2 DIABETES AT KENYATTA NATIONAL HOSPITAL.

A DISSERTATION SUBMITTED IN PART FULFILMENT OF THE REQUIREMENT FOR THE DEGREE OF MASTER OF MEDICINE IN INTERNAL MEDICINE, UNIVERSITY OF NAIROBI.

DR. KARANGA J. KIMANI
2013
DECLARATION
I hereby declare that this is my original work and has not been presented for a degree in any other university.

Signed .............................................................................Date............................................

PRINCIPAL INVESTIGATOR,

DR JAMES KARANGA KIMANI,

MBChB (UoN)

RESIDENT, DEPARTMENT OF CLINICAL MEDICINE AND THERAPEUTICS
This dissertation has been submitted for examination with our approval as supervisors.

Prof. E. O. Ogutu  
Consultant Gastroenterologist  
Associate Professor Of Medicine  
Department Of Clinical Medicine & Therapeutics  
University Of Nairobi

Signed ......................................................................................Date..........................

Dr. C. K. Onyambu  
Consultant Radiologist  
Lecturer  
Department of Imaging & Radiation Medicine  
University Of Nairobi

Signed ......................................................................................Date..........................

Prof. C. F. Otieno  
Consultant Endocrinologist  
Senior Lecturer  
Department of Clinical Medicine & Therapeutics  
University Of Nairobi

Signed ......................................................................................Date.........................
DEDICATION

I dedicate this dissertation to my loving wife and children.
ACKNOWLEDGEMENTS
I offer my gratitude to the following people without whose contribution, this work would not have been possible: My supervisors, Prof E. Ogutu, Prof. C. F. Otieno and Dr C. K. Onyambu for their supervision, guidance and patience. Extra thanks go to Dr Onyambu and her colleagues at the department of Diagnostic Imaging & Radiation Medicine for assistance with the liver ultrasonography.

I extend my appreciation to the patients at the Diabetic Clinic of Kenyatta National Hospital who agreed to participate in this study. I also do appreciate the staff at the diabetic clinic for their cooperation and for according me time and space to enrol the study subjects, Dr S. A. Amwayi for graciously assisting with data analysis, and the staff of KNH biochemistry laboratory who performed the laboratory investigations.

To my family, friends and colleagues who encouraged me and urged me on during the research period, I am grateful for the strength you gave me.
LIST OF ABBREVIATIONS

AFLP – Acute fatty liver of pregnancy
ALP – Alkaline phosphatase
ALT – Alanine transaminase
AST – Aspartate transaminase
AZT – Zidovudine
BAAT Score – BMI, Age, ALT, Serum Triglyceride score
BMI – Body mass index
CCl4 – Carbon tetrachloride
CLD – Chronic liver disease
CT – Computed tomography
ddi – Didanosine
DDT – Dichlorodiphenyltrichloroethane
DM – Diabetes mellitus
FFA – Free fatty acids
Gamma GT – Gamma glutamyl- transpeptidase
HAIR score – Hypertension, ALT, IR score
HBV – Hepatitis B virus
HCV – Hepatitis C virus
HCC – Hepatocellular carcinoma
HDL – High density lipoprotein
HOMA – Homeostatic model assessment formula
INR – International normalisation ratio
IR – Insulin resistance
KNH- Kenyatta National Hospital
LDL – Low density lipoprotein
LFT – Liver function test
MRI – Magnetic resonance imaging
NAFLD – Non-alcoholic fatty liver disease
NASH – Non-alcoholic steatohepatitis
NCEP – National Cholesterol Education Program
OGTT – Oral glucose tolerance test
OHA - Oral hypoglycaemic agents
PI – Principal Investigator
PTI – Prothrombin time index
QUICKI – Quantitative insulin sensitivity index check index
TGF beta – Tumour growth factor beta
TNF alpha – Tumour necrosis factor alpha
UON – University of Nairobi
U/S - Ultrasound
USA – United States of America
# Table of Contents

DECLARATION .................................................................................................................. iii
DEDICATION ....................................................................................................................... v
ACKNOWLEDGEMENTS .................................................................................................... vi
LIST OF ABBREVIATIONS ............................................................................................... vii
LIST OF FIGURES ............................................................................................................ xi
LIST OF TABLES ............................................................................................................... xii
ABSTRACT ....................................................................................................................... xiii

1.0 LITERATURE REVIEW ........................................................................................................ 1
1.1 INTRODUCTION ............................................................................................................. 1
1.2 NON ALCOHOLIC FATTY LIVER DISEASE (NAFLD) ...................................................... 2
1.3 PRIMARY NAFLD ............................................................................................................ 2
1.4 SECONDARY NAFLD ....................................................................................................... 3
1.5 ADDITIONAL MECHANISMS OF LIVER INFLAMMATION ........................................... 4
1.6 PREVALENCE OF NAFLD ............................................................................................... 5
1.7 DIAGNOSIS OF NAFLD .................................................................................................. 6
1.8 LABORATORY FINDINGS ................................................................................................. 7
1.9 IMAGING STUDIES ......................................................................................................... 7
1.10 LIVER BIOPSY ............................................................................................................... 8
1.11 BAAT (BMI, Age, ALT, serum Triglyceride score) ............................................................. 9
1.12 HAIR (Hypertension, ALT, IR score) ............................................................................ 9
1.13 CLINICAL COURSE ........................................................................................................ 10
1.14 TREATMENT ................................................................................................................ 11

2.0 STUDY JUSTIFICATION .................................................................................................. 12
3.0 RESEARCH QUESTION .................................................................................................... 13
4.0 STUDY OBJECTIVES ..................................................................................................... 13
4.1 BROAD OBJECTIVE ....................................................................................................... 13
4.2 SPECIFIC OBJECTIVES ................................................................................................. 13

5.0 MATERIALS AND METHODOLOGY .............................................................................. 14
5.1 STUDY DESIGN .............................................................................................................. 14
5.2 STUDY POPULATION .................................................................................................... 14
5.3 STUDY AREA ................................................................................................................ 14
5.4 SAMPLE SIZE ............................................................................................................... 14
LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure I</td>
<td>The ‘two-hit hypothesis’</td>
<td>5</td>
</tr>
<tr>
<td>Figure II</td>
<td>Proposed schema of evaluation and decision to do liver biopsy on suspected NAFLD patients</td>
<td>11</td>
</tr>
<tr>
<td>Figure III</td>
<td>Recruitment flow chart</td>
<td>19</td>
</tr>
<tr>
<td>Figure IV</td>
<td>Prevalence of NAFLD in study population</td>
<td>20</td>
</tr>
<tr>
<td>Figure V</td>
<td>Ultrasonographic grades of NAFLD in study subjects</td>
<td>21</td>
</tr>
<tr>
<td>Figure VI</td>
<td>Gender distribution of study subjects with NAFLD</td>
<td>21</td>
</tr>
<tr>
<td>Figure VII</td>
<td>Distribution by age group of NAFLD status of study subjects</td>
<td>23</td>
</tr>
<tr>
<td>Figure VIII</td>
<td>Deranged liver parameters in study subjects</td>
<td>24</td>
</tr>
<tr>
<td>Figure IX</td>
<td>BMI categories of study subjects</td>
<td>25</td>
</tr>
<tr>
<td>Figure X</td>
<td>Increased waist circumference in study subjects</td>
<td>26</td>
</tr>
<tr>
<td>Figure XI</td>
<td>Blood pressure indices of study subjects</td>
<td>27</td>
</tr>
<tr>
<td>Figure XII</td>
<td>Lipid profiles of study subjects</td>
<td>28</td>
</tr>
</tbody>
</table>
LIST OF TABLES

Table I: Gender distribution of study population..................................................19

Table II: Bivariate analysis of deranged liver parameters of study population ....23

Table III: Bivariate analysis of elevated BMI in study subjects .........................25

Table IV: Bivariate analysis of increased waist circumference in study subjects..26

Table V: Bivariate analysis of blood pressures in study subjects ......................27

Table VI: Bivariate analysis of lipid profiles in study subjects ..........................29
ABSTRACT

Background: Non-alcoholic fatty liver disease (NAFLD) is increasingly being recognized as a common disease in syndromes thought to have a patho-physiological basis in Insulin Resistance (IR), especially in patients with type 2 diabetes. There is an associated risk of progressive liver disease contributing to morbidity and mortality in such patients. Prevalence of this disease in Kenyans with type 2 diabetes remains unknown. This study aimed to determine the ultrasonographic prevalence of NAFLD in type 2 diabetics and correlate this with other known predisposing factors.

Methods: This was a hospital-based cross-sectional study that evaluated 326 type 2 diabetic patients (47±7 years) who were insulin-naïve and on treatment with Oral Hypoglycemic Agents (OHAs), and with no history of alcohol consumption. Anthropometric measurements of Body Mass Index (BMI), Blood Pressure (BP) and Waist Circumference (WC) were taken. Abdominal (hepatic) ultrasonography, Lipid Profile Tests (LPTs) and Liver Function Tests (LFTs) were performed. Data was recorded, entered into a data base and analyzed using statistical package SPSS 17.0 program.

Results: 34.4% (n=112) of study subjects had NAFLD on Ultrasound (U/S) (95% CI of 29.3%-39.8%), the most common grade being mild (n=67), followed by moderate (n=37) and severe (n=8). Deranged LFTs, obesity, dyslipidemia and hepatomegaly were significantly associated with NAFLD.

Conclusion: There was a high prevalence of NAFLD in study subjects, which was significantly associated with deranged LFTs, obesity, dyslipidemia, and hepatomegaly.
1.0 LITERATURE REVIEW
1.1 INTRODUCTION
Fatty liver disease refers to the diffuse accumulation of neutral fat in form of triglycerides in hepatocytes, and is an important clinical and pathological finding. Aetiologically, this arises from alcohol consumption (alcoholic fatty liver disease) or from non-alcoholic causes (non-alcoholic fatty liver disease/NAFLD).
NAFLD aetiologically occurs in two forms: primary and secondary.
In primary NAFLD, the aetiology remains unclear, though there is a strong association with the metabolic syndrome or any of its components, suggesting Insulin Resistance (IR) could be key in its aetiology.
Secondary NAFLD is strongly associated with possible underlying aetiological agents that include toxins, certain medications and an array of clinical conditions.
Imaging techniques namely computed tomography (CT), ultra sound, and magnetic resonance imaging (MRI) may demonstrate alterations suggestive of increased fat in the liver. This is characteristically seen as a “bright liver” on ultrasound (1).
Fatty liver disease presents in two histological categories, macrovesicular and microvesicular. Macrovesicular fatty liver is the commonest form, characterized by replacement of the hepatocyte cytoplasm with one or two large fat globules that displace the nucleus to the periphery of the cell, as demonstrated in haematoxylin and eosin (H/E) stained liver sections (1). Some of the causes of this type of fatty liver include alcoholic liver disease, obesity, diabetes mellitus, protein calorie malnutrition, drugs and hepatotoxins.
Microvesicular fatty liver is characterised by replacement of the cytoplasm by numerous small, fat filled globules producing a foamy appearance without nuclear displacement. It is seen in disorders of the urea cycle characterized by mitochondrial abnormalities and hyper-ammonaemia (1, 2, 3). Causes include Reye’s syndrome, conditions associated with pregnancy (acute fatty liver of pregnancy, pre-eclampsia, eclampsia), drugs and Jamaican vomiting sickness.
Alcoholic fatty liver disease arises from chronic consumption of alcohol, especially in doses > 30g/day, but can occur at lower doses. Alcohol decreases free fatty acid (FFA) beta oxidation in hepatocytes leading to triglyceride accumulation and hepatic steatosis, which can then progress to chronic hepatitis and ultimately hepatic fibrosis or cirrhosis (4).
Fatty liver disease is an important entity in view of its potential to progress to chronic liver disease (CLD) (1, 2, 4).
1.2 NON ALCOHOLIC FATTY LIVER DISEASE (NAFLD)

NAFLD is a clinico-pathological entity histologically resembling alcohol induced steatosis. It encompasses a spectrum of conditions characterized histologically by macrovesicular steatosis in the absence of a significant history of alcohol consumption; (no more than 10g/ day for women and 20g/ day for men). (1, 5, 6).

The spectrum of NAFLD, as seen histologically in H/E stained liver sections, progressively includes:

i. simple steatosis without inflammation,

ii. steatosis with non specific inflammation,

iii. steatosis with inflammation including neutrophils,

iv. non-alcoholic steato-hepatitis (NASH) manifesting as balloon degeneration of hepatocytes with or without sinusoidal fibrosis and Mallory’s hyaline, scattered predominantly lobular neutrophillic or mixed inflammatory cells and pericentral pericellular fibrosis and finally,

v. Cirrhosis.

The term NASH was coined by Ludwig et al (7) in 1980. It identified a previously recognized clinico-pathological syndrome indistinguishable from alcoholic hepatitis occurring mostly in obese and/or diabetic women denying alcohol consumption (8). NASH was later increasingly recognized as part of the NAFLD spectrum, ranging from fatty liver alone to forms of cryptogenic cirrhosis in which steatosis may be inconspicuous (9,10,11,12).

NAFLD occurs in two forms: primary and secondary NAFLD. Primary NAFLD is associated with components of the metabolic syndrome, either singly or in combination and most probably has a basis in insulin resistance. Secondary NAFLD is held in association with certain medications, some industrial toxins and a range of clinical disorders.

1.3 PRIMARY NAFLD

This refers to the form of NAFLD for which no underlying aetiological cause can be identified in the presence of the histological spectrum described above. The metabolic syndrome and its components, which all share insulin resistance (IR), are the most common risk factors associated with primary NAFLD (13). Components of the metabolic syndrome include (13):

- Waist circumference: Men >102 cm, Women > 88 cm
- Fasting blood glucose: ≥ 6.1 mmol/ L
- Serum triglycerides: ≥ 1.7 mmol/ L, or under fibrates
- Serum HDL cholesterol: Men < 1 mmol/ L, Women < 1.3 mmol/ L
- Arterial blood pressure: ≥ 130/ ≥ 85 mmHg, or under pharmacologic treatment for hypertension

**Insulin Resistance**

IR is defined as the ability of insulin to clear glucose from blood, also defined as insulin sensitivity (15, 16). Innate sensitivity of any tissue to insulin is determined by genetic and environmental factors. Genetic defects in the insulin receptor have been described in different IR phenotypes (17).

Most clinical cases of IR are polygenic and may involve polymorphisms in different genes involved in either insulin secretion or in mediating its effects. The metabolic state defined by IR results from the complex interplay between pancreatic islet β cells and the tissue targets of insulin. Adipose tissue plays a key role in the genesis of IR and hepatic steatosis. Adipose tissue is highly sensitive to plasma insulin concentration. A key defect in IR is the resistance of adipose tissue to insulin mediated suppression of lipolysis. Recent evidence suggests that such a defect exists in patients with either a fatty NAFLD (18).

Visceral adipose tissue is more resistant to insulin and exhibits greater lipolysis and produces more FFA than adipose tissue in other sites. Increasing FFA concentration in portal blood increases hepatic gluconeogenesis, decreases glucose utilisation and consequently increases hepatic glucose output (19). This results in increased insulin secretion by the pancreatic islet β cells thereby maintaining normoglycemia. Progressive failure of insulin mediated suppression of lipolysis raises FFA further, worsening hyperglycemia and producing a progressive fall in insulin sensitivity until overt diabetes develops (20).

**1.4 SECONDARY NAFLD**

In this form of NAFLD, a possible underlying aetiological agent or condition is implicated or held in association. Secondary NAFLD has been described in association with an array of causes that include some medications, hepatotoxins, total parenteral nutrition, rapid weight loss, protein calorie malnutrition, mitochondrialopathies, Wilson’s disease, Coeliac disease, Turner’s syndrome and endocrinopathies (1,2,3).
1.5 ADDITIONAL MECHANISMS OF LIVER INFLAMMATION
It is not clear how hepatic accumulation of triglycerides leads to inflammation and fibrosis. Multiple theories advance the possibility of a ‘second hit’ (Figure I). These theories include:

- FFA induce several cytochrome P-450 microsomal lipoxygenases, producing hepatotoxic lipid peroxides and free oxygen radical species that can deplete antioxidant enzymes, e.g. glutathione, vitamin E, beta carotene and vitamin C, exposing the hepatocyte susceptible to oxidative injury (2,21).
- Oxidative stress as a consequence of mitochondrial dysfunction (3)
- Effectors of liver injury, such as TNF-alpha, TGF-beta may mediate inflammation and injury (6)
- Increased hepatic iron may have a role in the development of NASH via unknown mechanisms (5, 22).
- Leptin, a peptide produced primarily in adipose tissue, renders hepatocytes more insulin resistant (23).
- Adiponectin, a hormone secreted exclusively by adipose tissue produces beneficial effects in lipid metabolism. Deficiency predisposes to fatty liver disease (24).
- Resistin, an adipose tissue derived protein, has been implicated in the development of IR by increasing expression of pro-inflammatory cytokines and may directly participate in inflammation (25).
- Intestinal microbes may predispose to hepatotoxic oxidative injury, probably via production of endogenous alcohol and acetaldehyde (26).

```
Causes of insulin resistance

Insulin resistance (hit no. 1)

Free fatty acids
Hepatic steatosis

Additional mechanisms (hit no. 2)
↑ROS
Oxidative stress
```

4
Hepatocyte injury
Inflammation
Fibrosis

ROS: Reactive oxygen species

Figure I: The ‘two hit’ hypothesis

1.6 PREVALENCE OF NAFLD
True prevalence of NAFLD has been underestimated. This is evidenced by its incidental discovery in patients being worked up for the metabolic syndrome or any of its components, and without symptomatology for liver disease. Similar findings have been found in autopsies of patients who died from non-hepatic causes. It is, however, likely to become more frequent worldwide given the increasing diagnosis of major insulin resistance associated metabolic disorders (27).

Most studies have used vigorous clinical and histological criteria focused on special subsets of hospital based populations, mainly those with morbid obesity and those awaiting bariatric surgery, with few general population studies to-date. Studies in these patient sub-groups are prompted by the discovery of a high prevalence of fatty liver disease during the work up of such patients (27). Currently, there is no data on prevalence of NAFLD in sub-Saharan Africa.

NAFLD has been reported in all age groups with the highest prevalence from ages 40-49. The disease occurs with equal frequency in men and women. There is familial clustering, probably reflecting clustering of type 2 DM and obesity (6).

In Britain, Underwood et al performed post-mortem examinations on healthy aircraft crew killed in aircraft accidents and found NAFLD in 21% of the victims (28). A Scandinavian post-mortem study performed on 503 consecutive road traffic accident victims by Hilden et al found NAFLD prevalence of 24% (29).

1.2 – 9% of patients undergoing liver biopsy in different centres during work-up for abnormally elevated liver enzymes and/or ultrasonographic fatty liver had NASH (30), and 15 – 39% demonstrated the whole spectrum of NAFLD (31,32).

Nonomura et al, in a Japanese study performing hepatic ultrasonography in the general population, detected hepatic steatosis in 23% of study subjects (31). A similar study by Lonardo et al in Italy found an almost similar prevalence of 20% (33). Giovanni et al found a prevalence
of 69.5% in type 2 diabetic patients, detected by ultrasonography (34). In the Lonardo study, a prevalence of 16.4% pertained to a control group, and increased to 75.8% in obese individuals. The risk for NAFLD was 4.6 fold higher in obese persons with a BMI > 30kg/m2.

Two studies of patients undergoing bariatric surgery for morbid obesity found a prevalence of NAFLD of 86% and 96%, whereas the prevalence of NASH was 24% and 25% (13,35).

A more recent meta-analysis analyzed data from the Third National Health and Nutrition Examination, evaluating 15,676 USA adult individuals. Analysis of the data approximated 9.1 million USA individuals might have NAFLD as evidenced by unexplained elevated hepatic transaminases, with significant association with the metabolic syndrome or its components (36). Mofrad et al found the entire spectrum of NAFLD in individuals with normal ALT. Despite elevated transaminases being common in NAFLD, a contrary finding does not rule out the disease (37).

A meta-analysis of several studies on NAFLD estimates an overall prevalence of about 20%. An ultrasonography study of 846 school children in Japan showed a 2.6 overall prevalence of fatty liver with a strong correlation to BMI obesity indices (38).

In view of the above, population based studies would provide more accurate data regarding the true prevalence of NAFLD.

1.7 DIAGNOSIS OF NAFLD

NAFLD is often asymptomatic. Suspicion is aroused by the incidental finding of abnormal aminotransferases, or a “bright liver” on abdominal ultrasound in patients being worked up for chronic disorders such as the metabolic syndrome or any of its components (39, 40, 41).

The most frequently encountered complaints include fatigue and malaise (40,41), as well as vague and aching right upper quadrant discomfort, especially in children (40, 42, 43).

The most common clinically encountered sign is hepatomegally, reported in up to 50% of subjects (7,40). Stigmata of chronic liver disease (CLD) are notably absent and only occur in those patients who progress to late stage or decompensated cirrhosis. Acanthosis nigricans has been described in children with NAFLD and is likely to be a cutaneous marker of IR as seen in a study in obese Japanese children (44). Physical examination should include measurements of basic anthropometry; height and weight to determine BMI, and waist circumference.
The gold standard for diagnosis of NAFLD, following clinical, biochemical and suspicious imaging, is liver biopsy

1.8 LABORATORY FINDINGS
Aminotransferase levels can be up to five times normal, with ALT:AST >1. ALT levels tend to be persistently abnormal, although fluctuations can occur, and some patients have normal ALT levels (6). AST/ALT ratio < I is considered typical of NAFLD, though it may depend on severity of the disease; inversion of this ratio is associated with fibrosis and progression of the disease (43, 45).

Gamma GT is almost always elevated. ALP may also be variably increased up to twice the upper limits of normal (7, 40).

LFTs such as serum albumin, bilirubin and prothrombin time, are usually normal unless cirrhosis and liver failure are present. Ferritin has been reported elevated in 21 – 62% of patients, and probably reflects the hepatic inflammatory process rather than increased iron stores (46, 47, 48).

In the absence of overt diabetes, evaluation of IR should be part of the diagnostic work up. This is achieved by the Euglycemic hyperinsulinemic clamp method (49), Homeostatic Model Assessment formula (HOMA)(50), Quantitative Insulin Sensitivity Check Index (QUICKI)(51) or the 120 minute Oral Glucose Tolerance Test (OGTT)(52).

1.9 IMAGING STUDIES
Ultrasound (U/S), Computerised tomography (CT) and Magnetic Resonance Imaging (MRI) are non-invasive imaging techniques that can identify hepatic steatosis. However, fibrosis and steatosis may display similar appearance to liver fatty infiltration. Pre- and post-contrast enhanced CT images have been recommended to diagnose hepatic steatosis, as this can better discern fibrosis from steatosis (53).

However, it is impossible to differentiate the different histological forms of NAFLD on imaging techniques as none can inform on the presence and degree of inflammation, necrosis or fibrosis (54). Nevertheless, imaging, especially U/S, is a useful tool in screening, as it provides an entry point for follow up, further diagnostic work up and probable intervention.

Ultrasonographic characteristics of fatty liver
Sonography of fatty infiltration may be varied depending on whether it is diffuse or focal. Diffuse steatosis may be mild where minimal diffuse increase in hepatic echopattern is seen with normal visualisation of the portal vein radicals and diaphragm. Moderate steatosis is seen as moderate diffuse increase in hepatic echogenicity and slightly impaired visualisation of
intrahepatic vessels and diaphragm. Severe steatosis appears as marked increase in echogenicity with poor or non-visualisation of hepatic vessels and diaphragm.

Focal fatty change will be seen as focal areas of increased hepatic echogenicity especially near the porta hepatis.

CT scan can be used to confirm fatty infiltration of the liver and shows reduction in attenuation with preserved liver architecture, with reversal of the normal liver- spleen differences. The liver enhances homogenously on contrast in the presence of fatty liver disease.

MRI is the most sensitive and specific imaging modality for demonstrating hepatic steatosis. The liver shows diffuse increase in signal intensity on T1W1 and T2W1 images (55).

Of the three imaging methods mentioned above, U/S is the least sensitive. However, the advantages of U/S vis-à-vis MRI and CT scan in detection of fatty liver are:

- It is far much cheaper, and therefore more readily affordable to patients
- It is easier and much more faster to perform
- The procedure is less strenuous to the patient
- Does not expose the patient to radiation
- It is more widely available and therefore can be used in screening patients in peripheral hospitals

1.10 LIVER BIOPSY

Non invasive imaging techniques are unable to describe/analyse presence and degree of hepatocyte injury, inflammation and fibrosis. A liver biopsy is necessary to establish the diagnosis and stage of NAFLD.

Need for a liver biopsy is controversial in the absence of proven specific therapy for the NASH component of NAFLD. However, it is the only reliable method of precisely diagnosing disease in the absence of biomarkers, can grade and stage disease and provide prognostic information (56, 57, 58).

There is paucity of histological data on asymptomatic patients with persistently abnormal liver enzyme abnormalities on which to base management decisions such as liver biopsy, and hepatologists use their discretion to identify patients to biopsy. 150 asymptomatic patients with elevated amino-transferases underwent liver biopsy in a recent Scandinavian study (59). Fibrosis was observed in half the biopsies, 2% were cirrhotic and in a majority mild pericellular fibrosis was seen, the clinical significance of which was unclear. Such findings may favour considering...
performing biopsies in patients with elevated transaminases, albeit with the inclusion of a scoring system to identify those most at risk of advanced NAFLD.

Despite lack of evidence, a recent review concluded that liver histology should be obtained in such asymptomatic patients (60), and this was reinforced by a prospective study by Skelley and colleagues (61) who studied 354 patients with persistently abnormal liver enzymes (more than twice the upper limit of normal for greater than six months). Similar results obtained by Daniel and colleagues (62) found NASH/NAFLD to be the most prevalent histological finding in up to two thirds of the patients studied.

Recent evidence suggests that approximately one third of NAFLD patients progress to fibrosis, and 20% will develop cirrhosis (40, 63, 64, 65). Accurate diagnosis of fibrotic liver disease may expedite earlier intervention, which may prevent or delay progression to end stage liver disease. As it is unfeasible and unethical to biopsy all patients suspected of NAFLD, it is necessary to define groups that may benefit from a liver biopsy. These include candidates older than 45 years, diabetic or obese, as two thirds show advanced fibrosis (63). Recent data confirm that patients with NAFLD and type II diabetes mellitus are more prone to develop cirrhosis with higher mortality (64). A clinico-biological BAAT (BMI, Age, ALT, serum Triglyceride) score combining BMI, age, ALT and triglycerides has been proposed to improve overweight patient selection for liver biopsy (65).

Another clinico-biological score, HAIR, was devised for the severely obese to identify clinical and/ or biochemical risk factors that might predict advanced forms of NAFLD. This score of 0-3 is calculated by adding hypertension, ALT and IR index (65).

1.11 BAAT (BMI, Age, ALT, serum Triglyceride score)
Calculated as the sum of categorical variables; BMI kg/m2 (≥ 28=1, <28=0), age (≥ 50=1, <50=0), ALT (≥ 2XN=1, <2XN=0), and serum triglycerides (≥1.7mmol/L=1, <1.7=0).

Ranges of the score are from 0-4. Score of 0 or 1 suggests no septal fibrosis. A score of > 1 indicates possible septal fibrosis and probably the need for a liver biopsy.

1.12 HAIR (Hypertension, ALT, IR score)
Hypertension=1, ALT >40IU = 1, IR >5.0 =1; Score > 2 likely to be associated with NASH.
The following schema has also been proposed for evaluation and decision to do liver biopsy on suspected NAFLD patients:

Incidental LFT finding  Fatty liver (US or CT)

↑ ALT

Rule out co-existent or alternate liver diseases

Evaluate for the metabolic syndrome and insulin resistance

Change of life-style: exercise, diet, complete abstinence

6 months

If aminotransferases still elevated

Propose and discuss liver biopsy

**Figure II: Proposed schema of evaluation and decision to do liver biopsy on suspected NAFLD patients.**

**1.13 CLINICAL COURSE**
Natural history of NAFLD has not been fully established as few studies address long term follow up. Progression might be dependent on severity of histological damage (66). Pure steatosis seems to have the best prognosis. In one study, when followed up to 19 years, only one of 12 patients showed progression to fibrosis (11), although another study in 49 patients with fatty liver alone reported 2 (4%) who progressed to cirrhosis (69).
Compared with alcoholic liver disease, non alcoholic fatty liver disease alone seems to have a benign clinical course without excess mortality, as seen in a Danish study that followed up 109 non-alcoholic and 106 alcoholic patients (67).

Evidence to estimate histologic progression is scarce. Six pooled published series followed up 76 NAFLD patients who underwent repeated liver biopsy during a follow up of 1.4 to 15.7 years. 22 (30%) showed liver damage progression, 36 (47%) had essentially no change and 18 (23%) had improvement or resolution of liver injury. Progression from steatohepatitis to more advanced fibrosis or cirrhosis was also recognised (68, 69).

In 295 patients on a mean follow up of 7 years, there were 14 (5%) liver related deaths including one hepatocellular carcinoma (HCC). At the time of liver biopsy, cirrhosis was already present in 7-16 % of patients (68, 70).

1.14 TREATMENT
There is no proven effective therapy for NASH. Modification of risk factors such as obesity, hyperlipidemia, and poor diabetic control is generally recommended (Figure II). Trials are ongoing for potential drug treatments to reduce inflammation and necrosis, mainly specific OHA agents and some anti-oxidants (71).
2.0 STUDY JUSTIFICATION
There is an increasing global prevalence of syndromes associated with IR, namely type 2 diabetes, obesity, hypertension and dyslipidemia. These syndromes, especially type 2 diabetes, are associated with primary NAFLD. Increasing prevalence of these syndromes, and in particular the increasing prevalence of type 2 diabetes mellitus in Kenya and sub-Saharan Africa, will in all likelihood result in a rising prevalence of NAFLD and its associated morbidity and mortality. Population-based studies employing ultrasonography done so far in the Western world yield NAFLD prevalence of between 16.4 -23%.

This study will serve as a baseline study. Currently, to the best of my knowledge, there is lack of local and even regional data on the prevalence of NAFLD in association with its known risk factors and hence no local guidelines in its prevention, diagnosis and management. In addition, the data obtained from this study can be compared with results of other completed or ongoing studies from other regions of the world. With increasing adoption of Western lifestyles and urbanisation, the data collected in this study is likely to change in the future.
3.0 RESEARCH QUESTION
What is the prevalence of NAFLD and its associated risk factors in black African patients with type 2 diabetes mellitus attending the Diabetic Clinic at KNH?

4.0 STUDY OBJECTIVES
4.1 BROAD OBJECTIVE
To determine the prevalence of NAFLD in type 2 diabetic patients attending Kenyatta National Hospital Diabetic Clinic.

4.2 SPECIFIC OBJECTIVES
1. To determine the ultrasonographic prevalence of NAFLD in patients with type 2 diabetes mellitus.
2. Document the prevalence of deranged liver function tests in type 2 diabetes mellitus patients with NAFLD.
3. Document the prevalence of other components of the metabolic syndrome, namely, obesity, hypertension and dyslipidemia in type 2 diabetic patients with NAFLD.
5.0 MATERIALS AND METHODOLOGY

5.1 STUDY DESIGN
This study was a hospital-based cross sectional descriptive study.

5.2 STUDY POPULATION
The study subjects were adults with type 2 DM attending the KNH Diabetic Clinic.

5.3 STUDY AREA
This study was carried out at the KNH Diabetic Clinic.

5.4 SAMPLE SIZE
The sample size (n) was determined using the following formula for prevalence study (Fisher, 1991) (72).

\[ n = \frac{Z^2 P (1 - P)}{d^2} \]

\( n = \) required minimum sample size
\( P = \) prevalence of ultrasonographic NAFLD in type 2 DM patients from the Giovanni et al study, where it was found to be 69.5% (33).
\( d = \) level of precision (set at ± 5%).
\( Z = \) standard normal deviate corresponding to 95% level of confidence (1.96).

Therefore \( n = \frac{1.96^2 \times 0.695 \times 0.305}{0.05 \times 0.05} \)

\( N = 326 \)

STUDY PERIOD

5.5 SAMPLING TECHNIQUES
Consecutive sampling was done. All patients with type 2 DM who met the inclusion criteria, agreed to participate in the study and gave informed consent were recruited by the Principal Investigator (PI) until the sample size was achieved.

CASE DEFINITION
Patients older than 30 years who had been reviewed by a consultant endocrinologist and diagnosed to have type 2 DM attending the Diabetic Clinic in KNH, and on OHA or lifestyle modification for control of elevated blood glucose, with no history of insulin therapy.

INCLUSION CRITERIA

- Informed consent to participate in the study
- Lifelong abstainer from alcohol consumption
- Adult patients with a diagnosis of type 2 DM

**EXCLUSION CRITERIA**
- Patients with prior serologic evidence of HBV or HCV in their clinic files at the time of recruitment
- Patients who had ever received insulin therapy

**5.6 SCREENING AND RECRUITMENT**
The Principal Investigator (PI) perused all the files of the patients attending the Diabetic Clinic. Patients who, from their file history, met the inclusion criteria were taken to a separate room where the PI introduced himself and explained the nature of the study. Those who agreed to participate were recruited and detailed consent obtained. Data was obtained by the PI using the following methods:

**CLINICAL METHODOLOGY**
- Socio-demographic data was obtained and a full medical history taken.
- A general physical examination was performed to look for stigmata of chronic liver disease.
- Blood pressure was measured with a mercury sphygmomanometer by applying the cuff around the left arm and readings were expressed in millimetres of mercury (mmHg).
- Waist circumference at the widest abdominal girth was measured using a tape measure and readings expressed in centimetres (cm).
- Examined for hepatomegaly, defined as a liver span in excess of 15 cm.
- Measured height in metres (m) using a tape measure and weight in kilograms (kg) without shoes and clothing using a weighing scale to determine and grade body mass index (BMI) (Appendix II).
- 4ml of venous blood was drawn aseptically from the antecubital vein for lipid profile and liver function tests and these were done at the Kenyatta National Hospital Biochemistry laboratory.
**BAAT SCORE CALCULATION**

BAAT is an abbreviation for BMI, Age, ALT and serum Triglyceride. These four parameters are used in a scoring system to identify individuals at high risk of septal fibrosis. A score of 0 and 1 indicates low risk of fibrosis, whereas a score of more than 1 suggests a high risk of septal fibrosis, and hence liver biopsy may be useful in the latter. The scores are categorized as follows: BMI kg/m² (≥28 =1, <28=0), Age (≥50=1, <50=0), ALT (≥2XN=1, <2XN=0), and Serum triglyceride (≥1.7mmol/L=1, <1.7=0)

**RADIOLOGICAL METHODOLOGY**

The supervisor radiologist or a delegated colleague, with the PI in attendance, performed B mode 2 dimensional hepatic/abdominal ultrasonography on all recruited patients using a Phillips HD II ultrasound machine at the Department of Diagnostic Radiology, University of Nairobi. Diagnosis of NAFLD was made on the basis of the criteria outlined below (54):

**DIFFUSE STEATOSIS**

**Mild:** Minimal diffuse increase in hepatic echopattern with normal visualisation of portal vein radicals and diaphragm

**Moderate:** Moderate diffuse increase in hepatic echogenicity and slightly impaired visualisation of intrahepatic vessels and diaphragm

**Severe:** Marked increase in echogenicity with poor or non-visualisation of hepatic vessels and diaphragm

**FOCAL FATTY CHANGE:** Focal areas of increased hepatic echogenicity especially near the porta hepatis.

**5.7 STATISTICAL ANALYSIS**

The data was recorded on a study proforma, entered into a data base and analysed using Statistical Package for Social Scientists (SPSS) version 17.0 program. Descriptive statistics, namely, means, standard deviations and medians were used for continuous variables i.e. ALT, AST, gamma GT, ALP and lipid profile levels. Frequency distribution was used for categorical variables i.e. sex, age, presence or absence of a fatty liver on U/S, BMI categories, BP categories. Data was presented in the form of tables, charts and graphs.
5.8 ETHICAL CONSIDERATIONS
The study was carried out after approval by the Department of Internal Medicine and Therapeutics, UON, and the Ethics and Research Committee, Kenyatta National Hospital. The procedures and purpose of the study were carefully explained to the patients verbally and in writing. Study participation was voluntary, and patients declining to participate in the study were not denied access to medical care. The costs of the study were borne by the investigator. These included cost of materials, ultrasonography and provision of travelling stipend for study subjects. The results of the investigations were availed by the PI to the patients and into their clinic files and any necessary therapeutic intervention made in accordance to accepted standards of practice by the attending physician. All patients found to have NAFLD were referred to the KNH Liver clinic for further follow up. However, they still continued with their regular Diabetic clinic follow up. The identities of the study participants were kept confidential.
6.0 RESULTS
During the study period, 760 patients with Type 2 DM attending the KNH Diabetic clinic were screened. 389 did not meet the inclusion criteria and were excluded. 371 satisfied the inclusion criteria after screening, 45 of whom declined to be enrolled into the study for various reasons: 6 were afraid of possible unfavourable hepatic U/S findings, 12 lost interest mainly due to time constraints, 16 felt they were already overwhelmed and fatigued by the burden of too many clinics associated with their medical condition and 11 gave a combination of the reasons above. 326 patients who satisfied the inclusion criteria and gave informed consent were recruited into the study. The male: female ratio of the study population was 1:1.1.

Figure III: Recruitment flow chart

All study patients were on OHAs, either as a single agent or combination therapy. Metformin was the commonest OHA in use in 65% (n=212). 26.4% (n=86) were on anti-hypertensive medications and 10.4% (n=34) were on statins (28 on artovastatin and 6 on rosuvastatin). 9% (n=29) were on low dose aspirin (75mg once daily).

Gender distribution in the study population

<table>
<thead>
<tr>
<th>Sex</th>
<th>Frequency (n=326)%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>158 (48)</td>
</tr>
<tr>
<td>Female</td>
<td>168 (52)</td>
</tr>
</tbody>
</table>

Age distribution of the study population
The overall study population had a mean age of 47±7 years and a range of 31 to 69 years.
**Occupation of study participants**

The occupation with the highest proportion was businessman/women with 19.6% (n=64), followed by farmer and housewife at 15.3% (n=50) and 12.9% (n=42) respectively. Drivers and conductors accounted for 8.9% (n=29), teachers 8.2% (n=27), casual labourers 5.8% (n=19) and health care workers at 3.4% (n=12). The remaining occupations were distributed between accountants, clerical officers, secretaries, hair-dressers, security personnel and others.

**NAFLD prevalence in the study population**

Prevalence of NAFLD in the 326 study patients was 34.4% (n=112) with 95% CI of 29.3% to 39.8%.

**Figure IV: Prevalence of NAFLD in the study population**

![Pie chart showing NAFLD prevalence](image)

**Ultrasonographic grades of NAFLD in study subjects**

Mild fatty liver was the most common U/S finding at 60% (n=67) of all patients with NAFLD. Moderate fatty liver accounted for 33% (n=37) and severe fatty liver 7% (n=8).
Gender distribution of study subjects with NAFLD

For females with NAFLD, 59% (n=38), 36% (n=23) and 5% (n=3) had mild, moderate and severe NAFLD respectively. For males with NAFLD, 60% (n=29), 29% (n=14) and 10% (n=5) had mild, moderate and severe NAFLD respectively. There was no statistically significant association of gender with NAFLD (p value 0.177).
Age group distribution of study subjects by NAFLD status
The peak age group of the study subjects was 45-49 years (n=189), followed by the age group 40-44 years (n=143). The age group 30-34 (n=13) had the least number of study subjects.
Deranged liver parameters in the study population

Of the 326 study patients, 5% (n=17) had elevated ALP, 12% (n=39) had elevated ALT, 8% (n=25) had elevated AST and 10% (n=32) had gamma GT elevated above normal values. Elevated serum albumin was found in 13% (n=41) and elevated total bilirubin in 3% (n=11). 7% (n=23) of study subjects had a decreased liver span, while 13% (n=44) had an increased liver span.
Bivariate analysis of deranged liver parameters in the study population

In the bivariate analysis of deranged liver functions of the study population, seven hepatic parameters were found to be significantly associated with NAFLD, namely, elevations above normal values of ALP, ALT, AST, gamma GT, serum albumin, total bilirubin and hepatomegaly. A low serum albumin was found not to be significantly associated with NAFLD, with a p value of 0.166.
Table II: Bivariate analysis of deranged liver parameters of the study population

<table>
<thead>
<tr>
<th>CHARACTERISTIC</th>
<th>NAFLD STATUS</th>
<th>OR</th>
<th>95% CI</th>
<th>CHI SQUARE</th>
<th>P VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>YES (N=112)</td>
<td>NO (N=214)</td>
<td>TOTAL (N=326)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elevated ALP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>13(12%)</td>
<td>4(2%)</td>
<td>17(5%)</td>
<td>6.89</td>
<td>2.19-21.68</td>
</tr>
<tr>
<td>Elevated ALT</td>
<td>36(32%)</td>
<td>3(1%)</td>
<td>39(12%)</td>
<td>33.32</td>
<td>9.97-111.35</td>
</tr>
<tr>
<td>Elevated AST</td>
<td>23(21%)</td>
<td>2(1%)</td>
<td>25(8%)</td>
<td>27.39</td>
<td>6.32-118.66</td>
</tr>
<tr>
<td>Elevated GGT</td>
<td>30(27%)</td>
<td>2(1%)</td>
<td>32(10%)</td>
<td>38.78</td>
<td>9.06-165.97</td>
</tr>
<tr>
<td>Elevated serum albumin</td>
<td>7(8%)</td>
<td>34(18%)</td>
<td>41(13%)</td>
<td>0.38</td>
<td>0.16-0.91</td>
</tr>
<tr>
<td>Elevated total bilirubin</td>
<td>8(7%)</td>
<td>3(1%)</td>
<td>11(3%)</td>
<td>5.41</td>
<td>1.26-32.13</td>
</tr>
<tr>
<td>Hepatomegally</td>
<td>40(36%)</td>
<td>27(13%)</td>
<td>67(21%)</td>
<td>3.85</td>
<td>2.20-6.73</td>
</tr>
</tbody>
</table>

Obesity indices of study subjects
40% (n=130) of study patients had a normal BMI. 30% (n =99) were overweight, 26% (n=83) had class I obesity and 3% (n=11) and 1% (n=3) had class II and class III obesity respectively.

Bivariate analysis of elevated BMI in study subjects 73% (n=81) of study subjects with NAFLD had a BMI above normal, while 54% (n=115) of those without NAFLD had above
normal BMI. During bivariate analysis, above normal BMI was found to be a statistically significant risk factor associated with NAFLD with OR=2.25 (95% CI=1.37-3.68) and a p value of 0.002.

**Table III: Bivariate analysis of elevated BMI in study subjects**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>NAFLD</th>
<th>OR</th>
<th>95% CI</th>
<th>Chi square</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
<td>No</td>
<td>Total</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elevated BMI</td>
<td>81</td>
<td>115</td>
<td>196</td>
<td>2.25</td>
<td>9.83</td>
</tr>
</tbody>
</table>

**Increased waist circumference in study subjects**

64% (n=72) of study patients with NAFLD had increased waist circumferences, while 40% (n=86) of those without NAFLD had increased waist circumferences. In total, 49% (n=158) of study patients had increased waist circumferences.

**Figure X: Increased waist circumference in study patients**

**Bivariate analysis of increased waist circumference in study subjects**
An increased waist circumference was found to be significantly associated with NAFLD when compared between the study subjects with and without NAFLD, with a p value of <0.001.

**Table III: Bivariate analysis of increased waist circumference in study subjects**

<table>
<thead>
<tr>
<th>VARIABLE</th>
<th>Range</th>
<th>Mean</th>
<th>p value</th>
<th>Std Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Waist circumference</td>
<td>NAFLD (+) 76-120</td>
<td>96.8</td>
<td>&lt;0.001</td>
<td>10.2</td>
</tr>
<tr>
<td></td>
<td>NAFLD (-) 74-112</td>
<td>93.0</td>
<td></td>
<td>8.5</td>
</tr>
</tbody>
</table>

**Blood pressure indices of study subjects**

Of the 326 study patients, 26% (n=84) were normotensive and 57% (n=187) pre-hypertensive. 12% (n=40) had stage 1 hypertension and 5% (n=15) were stage 2 hypertensives.

**Figure XI: Blood pressure indices of study subjects**

**Bivariate analysis of blood pressure indices**

16% (n=18) of study patients with NAFLD were hypertensive. 18% (n=37) of study subjects without NAFLD were also hypertensive. There was no statistically significant association between blood pressure and NAFLD.

**Table IV: Bivariate analysis of blood pressures of study subjects**
Lipid profiles of study subjects

61% (n=199) of study subjects had serum cholesterol within the desirable range. 68% (n=222) had a desirable serum LDL-cholesterol. 50% and 22% of study subjects had desirable HDL-cholesterol and serum triglyceride respectively. It is notable that 11% (n=36) of study subjects had markedly elevated LDL-cholesterol levels.

Figure XII: Lipid profiles of the study population

Bivariate analysis of lipid profiles of study subjects
There was a statistically significant association between high serum triglyceride and high serum HDL-cholesterol with NAFLD. There was no association of statistical significance of NAFLD to serum LDL-cholesterol and Total Cholesterol levels.

**Table V: Bivariate analysis of lipid profile**

<table>
<thead>
<tr>
<th></th>
<th>NAFLD (+) (n=112)</th>
<th>NAFLD (-) (n=214)</th>
<th>TOTAL (N=326)</th>
<th>OR</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Total Cholesterol</td>
<td>77 (69%)</td>
<td>123 (58%)</td>
<td>200 (61%)</td>
<td>1.63</td>
<td>1.00-2.64</td>
<td>0.062</td>
</tr>
<tr>
<td>High total cholesterol</td>
<td>24 (21%)</td>
<td>34 (16%)</td>
<td>58 (18%)</td>
<td>1.44</td>
<td>0.81-2.58</td>
<td>0.276</td>
</tr>
<tr>
<td>Low HDL</td>
<td>21 (19%)</td>
<td>24 (11%)</td>
<td>45 (14%)</td>
<td>1.83</td>
<td>0.97-3.45</td>
<td>0.088</td>
</tr>
<tr>
<td>High HDL</td>
<td>26 (23%)</td>
<td>89 (42%)</td>
<td>115 (36%)</td>
<td>0.42</td>
<td>0.25-0.71</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Normal HDL</td>
<td>65 (58%)</td>
<td>97 (45%)</td>
<td>162 (50%)</td>
<td>1.67</td>
<td>1.05-2.64</td>
<td>0.039</td>
</tr>
<tr>
<td>Normal LDL</td>
<td>72 (64%)</td>
<td>150 (70%)</td>
<td>222 (68%)</td>
<td>0.77</td>
<td>0.47-1.25</td>
<td>0.356</td>
</tr>
<tr>
<td>High Triglyceride</td>
<td>98 (88%)</td>
<td>156 (73%)</td>
<td>254 (78%)</td>
<td>2.60</td>
<td>1.38-4.92</td>
<td>0.004</td>
</tr>
</tbody>
</table>

**BAAT scores of study subjects with NAFLD**

- Ranged from 0-4 with mean of 1.6+/-1.1, median of 2 and mode of 2.
- 57/112 (51%) of patients had score > 1 suggestive of possible fibrosis.
- BAAT > 1: 50% (n=33) in mild fatty liver disease, 51% (n=19) in moderate disease and 57%(n=4) in severe disease.
**7.0 DISCUSSION**

This study was designed to determine the ultrasonographic prevalence of NAFLD in subjects with type 2 diabetes who were on OHA treatment for blood sugar control and were insulin-naive. U/S is a validated surrogate tool for screening for NAFLD in the absence of liver biopsy (1, 31, 33, 34, 38).

Of the five components of the metabolic syndrome, diabetes is the risk factor most frequently associated with NAFLD. This study further documented the prevalence of the other components of the metabolic syndrome, namely, obesity, elevated blood pressure, elevated triglyceride and low HDL-cholesterol and we sought to determine if there was significant association of these factors to NAFLD in the study subjects. Derangements of liver function tests and hepatic spans were also analysed.

The study population was mostly urban, living and working in Nairobi and its suburbs, and from diverse occupational backgrounds. The age range of the study subjects was 31-69 years, with a mean age of 47±7 years. The majority of all subjects studied were in the age group of 40-49 years.

The prevalence of ultrasonographic NAFLD among type 2 diabetic subjects in this study was 34.4%, the majority being in the age group 45-49 years, followed by the age group 40-44 years. Our findings were comparable with those of a study carried out by Matteoni et al that found the highest prevalence of NAFLD in a similar age group (5). The most common sonographic grade of NAFLD was mild fatty liver (60%), followed by moderate (33%) and then severe fatty liver (7%). A similar Italian study by Giovanni et al, also employing U/S as a screening tool, found a much higher prevalence of 69.5%. 38% (n=64) of the female study subjects had NAFLD, while 30% (n=48) of male study subjects had NAFLD. There was no statistically significant association of gender with NAFLD. This finding was comparable to that of Ludwig et al (7), who found no statistically significant association between NAFLD and gender, with the disease occurring in similar proportion among males and females.

We found significant association of NAFLD with deranged liver function tests, of which some components were found to be markedly elevated. This finding was comparable to similar liver function derangements from other studies described by Bacon et al, Cortez-Pinto et al and Reid et al (69, 70, 76). 12.5% (n=14) of study patients with NAFLD had ALT: AST < 1, suggestive of presence of cirrhosis, which is the terminal progression of NAFLD several years after the onset.
Hepatomegaly was also significantly associated with NAFLD in this study, and this was comparative with the study findings of Cortez-Pinto et al and Sheth et al (70, 73). Hepatomegaly on bedside examination or right upper abdominal quadrant discomfort or tenderness are indeed the commonest clinical findings in patients with symptomatic NAFLD.

73% of study patients with NAFLD had a BMI that was above normal, compared to 54% of patients without NAFLD that had an elevated BMI. This was statistically significant with a p value of 0.002, making obesity an important association. This was further affirmed by a significant association of increased waist circumference with NAFLD. This finding was consistent with the findings of Adler et al, Caldwell et al and Marceau et al, who also found significant association of NAFLD and obesity (8, 9, 13).

In our study, no association was found between hypertension and NAFLD. The metabolic syndrome and the resultant IR have been associated with NAFLD, as shown by Marceau et al and Cortez-Pinto et al (13, 14). It is therefore of interest to note that hypertension, which is one of the components of the metabolic syndrome, was not significantly associated with NAFLD in this study.

Analysis of the lipid profiles showed hyper-triglyceridemia to be significantly associated with NAFLD. Hyper-triglyceridemia has been shown to be associated with NAFLD (13, 14).

51% (57/112) of study subjects with NAFLD patients had a BAAT score of more than 2, translating to an increased risk of hepatic fibrosis. It is significant that more than half of study subjects with NAFLD were categorised into this high risk category.

Most of the NAFLD prevalence studies done to date are not representative of the general population. They have focused mainly on sub-sets of patients (27), and mostly obese patients, especially those undergoing bariatric surgery for morbid obesity (35). Other studies have mainly been based on post-mortem liver histology findings (28, 29).

In Africa, there is lack of data on prevalence of NAFLD, either in the general population or in specific groups of patients. It would be of interest to compare prevalence of NAFLD in the general population in this region to that found in Japanese and Italian general population studies, where the prevalence was almost similar, 23% and 20% respectively (31, 33). No other studies on NAFLD in the general population are documented. As more data becomes available from future studies on prevalence of NAFLD in type 2 diabetic patients from different regions, more
meaningful comparisons can be made. NAFLD therefore remains an understudied subject, with few studies to compare to (58).

7.1 CONCLUSION
We found a 34.4% (95% CI 29.3-39.8%) prevalence of NAFLD in type 2 diabetic study subjects with significant association to obesity and hyper-triglyceridemia.

7.2 STUDY LIMITATIONS
HBV and HCV markers were not done owing to financial constraints, and viral hepatitides may occasionally mimic NAFLD.

10.4% (n=34) of the study patients were on statins. Statins are known to cause fatty liver disease. However, it was not practical to exclude these patients from the study as statins are widely used in the management of diabetic patients.

It was not possible to completely rule out previous use of medications that can cause secondary fatty liver disease owing to limited patient recall and poor medical record keeping by most study subjects.

Finally, some study patients may not have been truthful pertaining to alcohol consumption.

7.3 RECOMMENDATIONS
We recommend liver biopsy in diabetic patients with ultrasonographic evidence of this disease and a BAAT score > 2 for histological profiling, as proposed by Laurin et al and Talwalkar et al (56, 57). Intensive modification of associated risk factors has been shown to improve hepatic histology in affected patients, hence the need for vigilance and stringent management of prevalent risk factors for NAFLD in patients with type 2 DM, as this may delay onset or progression of the disease.

Further, we recommend routine hepatic ultrasonography and LFTs monitoring in type 2 DM patients. Subsequently, any patients found to have NAFLD should be referred to a hepatologist for follow up.
8. REFERENCES


15 Himsworth H. Diabetes mellitus: a differentiation into insulin sensitive and insulin insensitive sub-types. Lancet 1936; i: 127-130.


27 Cortez-Pinto H, Camilo ME. Non-alcoholic fatty liver disease/non-alcoholic steatohepatitis: diagnosis and clinical course. Best practice and research clinical gastroenterology 2004; 18: 1090-1099.


45 Reid AE. Nonalcoholic steatohepatitis. Gastroenterology 2001; 121: 710- 723.


47 George DK, Goldwurm S, McDonald GA et al. Increased hepatic iron concentration in non-alcoholic steatohepatitis is associated with increased fibrosis. Gastroenterology 1998; 114: 311- 318.


56 Laurin J. Motion- all patients with NASH need to have a liver biopsy: arguments against the motion. Can J Gastroenterol 2002; 16: 722- 726.

57 Talwalkar JA. Motion- all patients with NASH need to have a liver biopsy: arguments for the motion. Can J Gastroenterol 2002: 16: 718- 721.


APPENDIX I: STUDY PROFORMA

DATE OF INTERVIEW ..........................................................

NAME ..............................................................................

HOSPITAL NUMBER .........................................................

SEX .........................

AGE .........................

OCCUPATION .................................................................

RESIDENCE ......................................................................

CONTACTS .................................................................

DRUG HISTORY ............................................................... 

MEDICAL HISTORY .........................................................

PHYSICAL EXAMINATION

BLOOD PRESSURE ............mmHg. □ HIGH □ NORMAL □ LOW

LIVER SPAN ..........cm. (12-15cm) □ INCREASED □ NORMAL □ DECREASED

WEIGHT .................................................................kg

HEIGHT .................................................................cm

WAIST CIRCUMFERENCE ...... cm (men < 102 cm, women <88 cm) (□ NORMAL □ INCREASED)

CALCULATED BMI ......kg/m². ( □ LOW □ NORMAL □ HIGH)

ULTRASONOGRAPHIC FINDINGS ........................................

LABORATORY FINDINGS


38
LIVER FUNCTION TESTS

- SERUM ALBUMIN ..........g/L. (35-52g/L) □ NORMAL □ HIGH □ LOW
- SERUM BILIRUBIN: DIRECT ..........umol/L. (0-4.3umol/L). □ NORMAL □ HIGH
  TOTAL ..........umol/L. (<17umol/L). □ NORMAL □ HIGH
- ALT ............U/L. (0-37 U/L) □ NORMAL □ HIGH
- AST .............U/L. (0-42U/L) □ NORMAL □ HIGH
  - ALT/AST RATIO........................
- GAMMA GT ........U/L. (5-45) □ NORMAL □ HIGH
- ALP ...........U/L. (98-279) □ NORMAL □ HIGH

FASTING LIPID PROFILE

- TOTAL CHOLESTEROL ........mmol/L. □ NORMAL □ HIGH □ LOW
- LDL- CHOLESTEROL ........mmol/L. □ NORMAL □ HIGH □ LOW
- HDL- CHOLESTEROL ........mmol/L. □ NORMAL □ HIGH □ LOW
- SERUM TRIGLYCERIDES ........mmol/L. □ NORMAL □ HIGH □ LOW

BAAT score: ............ 0-1: suggests no septal fibrosis, >2 indicates possible fibrosis
APPENDIX II: CALCULATION AND GRADING OF BODY MASS INDEX (BMI) (kg/m²):

\[
\text{BMI} = \frac{\text{WEIGHT (KG)}}{\text{HEIGHT} \times \text{HEIGHT (M²)}}
\]

- 18.5- 24.9: NORMAL
- 25- 29.9: OVERWEIGHT
- 30- 34.9: CLASS I OBESITY
- 35- 39.9: CLASS II OBESITY
- 40: > CLASS III OBESITY
APPENDIX III : CONSENT EXPLANATION

My name is Dr. Karanga J K. I am a postgraduate student pursuing a Masters degree in internal medicine at the University of Nairobi. The program requires me to write a thesis. My research is on non-alcoholic fatty liver disease in patients with type 2 diabetes.

I will require you to take a detailed history and perform a thorough physical examination on you. A radiologist will perform a liver ultrasound on you to screen for fatty liver disease.

Benefits of the study
The study aims to establish the prevalence of non-alcoholic fatty liver disease in type 2 diabetic patients like you. This will assist in planning for follow up and intervention measures to alleviate risk factors for those found to have this liver disease or any of its associated risk factors.

Ultrasonography is a painless procedure.

Your participation is absolutely voluntary and an informed written consent will be required from you before participation. All information obtained will be confidential. You can withdraw from the study at any stage should you desire without jeopardy to your current treatment. The results obtained shall be availed and discussed with you. I will bear the costs of all the investigations.

You can contact me on 0733235142 in case of any queries.
APPENDIX IV: CONSENT FORM

I, …………………………………………………………………………………………………………, do hereby consent to participate in the above study, the nature of which has been fully explained to me by Dr ………………………………………….. I understand the results of this study shall be used for research work and strict confidentiality shall be maintained at all times.

Date………………………………. Signed………………………………………

I confirm that I have explained to the patient the nature of the study and tests to be done

Date………………………………. Signed………………………………………
# APPENDIX V: STUDY BUDGET

<table>
<thead>
<tr>
<th>Item</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stationery</td>
<td>5,000</td>
</tr>
<tr>
<td>Secretarial services</td>
<td>5,000</td>
</tr>
<tr>
<td>Ultrasound</td>
<td>326,000</td>
</tr>
<tr>
<td>Travelling allowance</td>
<td>65,000</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>401,000</td>
</tr>
</tbody>
</table>
APPENDIX VI: ATP III CLASSIFICATION OF LDL, TOTAL AND HDL CHOLESTEROL AND SERUM TRIGLYCERIDES (MMOL/L)

**LDL cholesterol**
- <2.6  optimal
- 2.6-3.3 near optimal/above optimal
- 3.4-4.1 borderline high
- 4.1-4.9 high
- ≥4.9 very high

**Total cholesterol**
- <5.2  desirable
- 5.2-6.2 borderline high
- ≥ 6.2  high

**HDL cholesterol**
- <1.03  low
- >1.55  high

**Triglycerides**
- <1.7  normal
- 1.7-2.2 borderline high
- 2.2-5.6 high
- ≥5.6  very high
ETHICS APPROVAL LETTER