COMPARISON OF LEAN VERSUS OVERWEIGHT/OBESE PATIENTS WITH NONALCOHOLIC FATTY LIVER DISEASE

Ann Margaret C. Navarroza, M.D.
Principal Author

Dennis Martin A. Castro, M.D.
Co- Author

Stephen N. Wong, M.D.
Senior Author

1Fellow, Gastroenterology, University of Santo Tomas Hospital
2Consultant, Section of Gastroenterology, University of Santo Tomas Hospital
University of Santo Tomas Hospital, Manila, Philippines

TABLE OF CONTENTS

Introduction

Nonalcoholic fatty liver disease (NAFLD) is a chronic liver disease defined as hepatic fat accumulation seen in the absence significant alcoholic intake and other cause of steatosis such as Hepatitis C. It is well recognized that NAFLD is closely associated with metabolic risk factors like obesity, dyslipidemia, systemic hypertension, glucose intolerance, and insulin resistance. However, this may also be observed among non-obese patients (lean NALFD). Prevalence rates of NAFLD vary widely in different populations, depending on the criteria being used, De Lusong et al reported a 12.2% prevalence of NAFLD among admitted Filipino patients in a single center study.

General Objective:

This study aims to compare the characteristics of lean and overweight/obese NAFLD patients

Specific Objectives:

1. To determine the proportion of lean patients among NAFLD patients
2. To compare the clinical, biochemical and metabolic profile of lean versus overweight NAFLD patients

Review of Related Literature

Nonalcoholic fatty liver disease (NAFLD) is the accumulation of excessive fat in the liver in the absence of significant alcoholic intake and other secondary etiology. It is recognized as one of the most common liver disease in the western world[1-3] and has a reported prevalence of 6-35 % worldwide[4] NAFLD prevalence in Asian countries has been found to be around 15-30%[1] and is seen to be on the rise, which is attributed to a change in dietary habits and a sedentary lifestyle. De Lusong et al reported a 12.2% prevalence of NAFLD among admitted Filipino patients in a single center study.

The diagnosis of NAFLD requires evidence of hepatic steatosis, either by imaging or by histology, with no causes of secondary hepatic fat accumulation such as significant alcohol consumption, use of steatogenic medication or hereditary disorders[5-7]. The histologic spectrum of NAFLD extends from a relatively innocuous simple steatosis stage, to nonalcoholic steatohepatitis (NASH) where ballooning degeneration of hepatocytes and significant inflammation is a feature, to cirrhosis where there may be "burn out" or absence of histologic proof of steatosis. Liver biopsy remains the gold standard for characterizing liver histology in NAFLD, such as the presence of steatohepatitis and fibrosis. However, it is limited by cost, sampling error, and procedure-related morbidity and very rare mortality risk. The invasive nature of the test makes it an unattractive option for patients and physicians alike. Thus, it should be performed in those who would benefit from diagnostic, therapeutic guidance and prognostic perspective. As a result, most of the epidemiologic studies on NAFLD make use of ultrasound as a diagnostic tool. Depending on the study, ultrasonography has been shown to have a sensitivity and specificity of as high as 90% and 95%, respectively, in the detection of moderate to severe hepatic steatosis.[2]

Based on previous studies, comorbidities associated with NAFLD include diabetes mellitus, metabolic syndrome, and obesity as it is related to insulin resistance as well as to the development of cardiovascular diseases.[1-3,7]. The estimated prevalence of type 2 diabetes and metabolic syndrome among NAFLD patients was 70-75 % [7,8] and 60%[9] respectively. While approximately 90 % among overweight to morbidly obese patients may have NAFLD[1], it may also be observed among its non-obese (those with normal body mass index [BMI])[10] counterparts having its own identifying metabolic characteristics with an estimated prevalence of 23.4 % [11]. In a study in 2007 by Targher et al, NAFLD patients had remarkably higher age and sex-adjusted prevalence of coronary, cerebrovascular and peripheral vascular disease than their counterparts without NAFLD[7]. Domanski et al., in a retrospective study of 219 NAFLD patients, showed that the prevalence of cardiovascular disease was 6.63% among patients with NAFLD, independent of age, sex, body mass index and presence of diabetes[12]. The presence of multiple metabolic disorders mentioned above in individual is associated with a potentially progressive, severe liver disease. It can progress to a dismal outcome such as

cirrhosis and or can directly develop to hepatocellular carcinoma[1,3]. It is also likely to be the most common cause of cryptogenic cirrhosis[13]. Laboratory exams such as ALT, GGT, AST are markers of liver injury and may be useful surrogate measures of NAFLD. New cut-offs for liver enzymes are warranted in order to prevent unnecessary diagnostic work-ups and early detection of NAFLD to prevent future complications [14].

Studies on NAFLD more commonly includes overweight/ obese individuals, there is however a proportion of individuals with NAFLD in context of normal or underweight body mass index (BMI), the lean individuals. Kim et al in 2004 noted that gender, waist circumference, triglyceride level, and insulin resistance were associated with normal weight NAFLD patients[11]. A similar study by Younossi et al in 2017 showed that lean NAFLD patients were more likely to be of younger age group, female sex, and a decreased likelihood of having Insulin resistance and hypercholesterolemia [15]

Aside from the aforementioned single center study in the Philippines,[3] there is paucity of data on Filipino patients with NAFLD, more so among lean NAFLD filipino patients

Materials and Methods

Study Design and Participants

This is a cross-sectional analytic study that was conducted among adult patients seen in outpatient hepatology clinic from February 2007-January 2017. Diagnosis of NAFLD was based on clinical and ultrasound findings suggestive of fatty liver. Patients who have incomplete records and those with secondary causes of steatosis such as significant alcohol drinking history (>20 G/day and >40 G/day of alcohol for women and men, respectively), hepatitis C infection, intake of drugs (such as amiodarone, estrogen, glucocorticoids, tamoxifen and valproic acid) as well as those who underwent surgical procedures (i.e., gastroplexy, jejunoileal bypass, extensive small bowel resection, biliopancreatic diversion and small bowel diverticulosis) was excluded.

Outcome Measure

Demographic data of study participants was obtained from the medical records included are age, gender, weight, and height of the patients. Body mass index (BMI) of each subject was computed and classified using the WHO criteria wherein BMI <18.5, 18.5-24.9, 25-29.9 and > 30 were considered under-weight, normal, over-weight and obese, respectively. Patients was further categorized into to lean (underweight and normal BMI) with a BMI < 25 and overweight/obese NAFLD, BMI < 25. Laboratory results within 6 -12 months of the diagnosis of NAFLD from individual patient medical records such as fasting blood glucose (FBS), uric acid, creatinine, serum cholesterol, LDL, HDL and triglyceride levels, liver enzymes such as alanine/aspartate transaminases (ALT/AST), and liver function tests such as prothrombin time, albumin and platelet count was obtained and used as metabolic markers. Serum hepatitis B surface antigen (HBsAg) and anti-HCV was also included to evaluate Hepatitis B and C status respectively. Other tests such as fibroscan and controlled attenuation parameter (CAP) were recorded if available. Diagnosis of significant comorbidities such as diabetes mellitus (DM), hypertension and coronary artery disease (CAD) and cerebrovascular disease was made according to standard criteria. Alanine aminotransferase (ALT) levels for males is 24 IU/L and 19 IU/L for females. Diagnosis of metabolic syndrome was based on ATP III which was defined as the presence of any 3 of the following 5 traits: abdominal obesity as a waist circumference over 40 inches (men) or 35 inches (women), blood pressure over 130/85 mmHg or drug treatment for elevated blood pressure, serum triglyceride (TG) >150 mg/dl, serum high-density lipoprotein (HDL) cholesterol <40 mg/dl (men) or <50 mg/dl (women) or drug treatment for low HDL cholesterol and fasting blood sugar> 100 mg/dl or drug treatment for elevated blood glucose.

Ethical Considerations

This study was reviewed and approved by the institutional Review Board of the University of Santo Tomas Hospital. It was conducted in accordance with the applicable International Conference on Harmonization (ICH) Guideline on Good Clinical Practice Guidelines (GCP).

Privacy and Confidentiality

The primary investigator have the access to clinic records. Each subject was given a Personal Identification Number Code. An Identifier was assigned by the investigator to each subject to protect his/her identity. An identifier was used in lieu of the subjects names when the investigator reports or make a research related data. The database generated from this research was kept in password secured computer preventing unauthorized access to it. Information of patients such as comorbidities was recoded in numerical variables to ensure confidentiality.

After the current research, the generated electronic database will be deleted using a special file deletion software to overwrite data, effectively preventing data recovery. Shredding of hard copy materials will also be done.

Benefits

The patients whose database was reviewed are not likely to benefit from this research proposal, but the investigators as well as the society will benefit from the information gained.

Risk

There is a risk of breach of confidentiality among chart review research which can be minimized with the use of Personal Identification Number Code on each patient.

Compensation and Expenses

No monetary incentives in cash or kind was provided to the patients included in this study. There was also no monetary compensation given to the investigators in any form. Financial expenses involving the writing of this study was shouldered by the investigator.

Sample Size

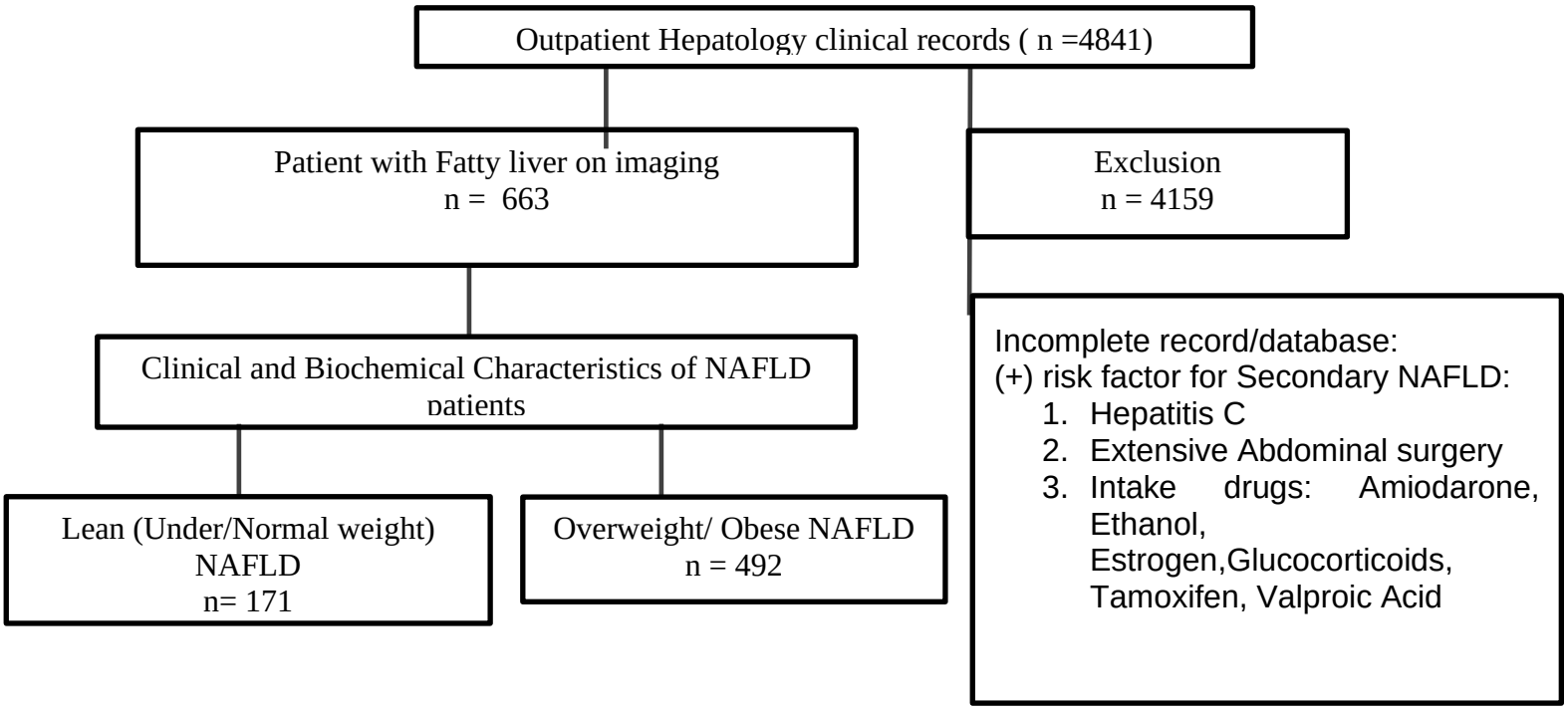
Sample size was computed at 163 subjects using a prevalence of NAFLD at 12 % [3]. The calculated sample size was set at 95% confidence interval using OpenEpi Ver 3.

Statistical Analysis

All continuous variables was presented as mean SD while categorical variables was presented as number (%). Statistical Analysis was carried out using SPSS version 25, data was converted into variables, which was analyzed using frequency and cross tabulation functions. Independent t-test was used for continuous data, while chi-square was used for categorical variables. The test was considered as significant if p is < 0.05

Data Collection Process

Figure 1. Details of the study design



RESULTS

Table 1. Baseline characteristics of the study participants

Variables	f (%) ± SD
Age (yrs)	51.8 ± 14.46
Gender (m: f)	385 (58.1%): 278 (41.9%)
BMI	
Underweight	5 (0.8%)
Normal	166 (25%)
Overweight	331 (49.9%)
Obese	161 (24.3%)
Metabolic syndrome	376 (56.7%)
Diabetes Mellitus)	303 (45.7%)
Hypertension	366 (55.2%)
CAD/ CVD	112 (16.9%)
Dyslipidemia	470 (70.9%)
Elevated ALT status	544 (82.1%)
Hbsag status	117 (17.65 %)
HCC	39 (5.9%)
Cirrhosis	29 (4.4%)
Asymptomatic	428 (64.6 %)
alt (u/L)	53.67 ± 49.4
Ast (u/L)	40.3 ± 29.52
Albumin (g/dl)	4.3 ± 0.46
PT	12.12 ±1.4
FBS (mg/dl)	118 ±38.84
Cholesterol (mg/dl)	204. 7 ± 45.2
TG (mg/dl)	164. 7 ± 100.2
LDL (mg/dl)	123.1 ± 40
HDL (mg/dl)	48.3 ± 24.06
Creatinine (mg/dl)	1.06 ± 4.3
Uric acid (mg/dl)	6.3 ± 8.7

Data are means SD or proportions. N= 663

Of the 4,841 clinic records reviewed, 682 patients was diagnosed with fatty liver by imaging who met the criteria for review (Table 1). Results showed that 58.1% of the subjects were male and 41.9% were female. Mean patient age was 51.8 ± 14.46 years. Mean BMI was 27.5 ± 4.12 with 74.52% of patients of patients had a BMI of > 25 being overweight/obese NAFLD of having while 25.8 % have lean NAFLD (underweight/ normal). Cirrhosis (5.9%) or hepatocellular carcinoma (HCC) (4.4%) were already present in a proportion of patients on initial consult.

Table 2. Demographics and Clinical features of NAFLD patients, grouped according to BMI check values and put labels

Variables	BMI Classification		P-value
N	Underweight/Normal 171	Overweight/Obese 492	
Gender Male	85 (49.7%)	300 (61%)	0.012
Female	86 (50.3%)	192 (39%)	
Age (years)	54.6 ±14.2	50.9 ± 14.2	0.004
Metabolic syndrome	63(36.8%)	313 (63.6%)	<0.0001
Diabetes Mellitus)	58 (33.9%)	245(49.8%)	<0.0001
Hypertension	79 (46.2%)	287 (58.3%)	0.007
CAD/ CVD	21 (12.3)	91(18.5 %)	0.075
Dyslipidemia	108 (63.2%)	362 (73.6 %)	0.011
Elevated ALT status	125 (73.1 %)	435 (85.2%)	<0.0001
Hbsag status	37 (21.6%)	80 (16.3%)	0.130
Presence of Complication on first visit	17 (9.9%)	35 (7.1 %)	0.249
HCC	10 (5.8%)	29 (5.9 %)	0.982
Cirrhosis	13 (7.6%)	16 (3.3%)	0.027
Asymptomatic	104 (60.8 %)	324 (65.9%)	0.265

Most overweight/obese patients had an with an elevated alanine aminotransferase (ALT) at 82.1%. Compared to Lean NAFLD patients, overweight/obese patients were more likely to have diabetes mellitus (33.9 % vs 49.8%; p= <0.0001), hypertension (46.2% vs 58.3%; p= 0.007), CAD/CVD (12.3% vs 18.5 %;p= 0.075), dyslipidemia (63.2% vs 73.6%;p=0.011) and metabolic syndrome (36.8% vs. 63.6%;p=<0.0001). Most of these patients are asymptomatic at initial presentation (60.8 % vs 65.9 %; p= 0.236), however among symptomatic patients, upper abdominal discomfort (30.2%) accounts for the most common reason for consult. Lean NAFLD patients were also older as compared to their older counter part (54.6 ±14.2 vs 50.9 ± 14.2, p= 0.004. Cirrhosis or hepatocellular carcinoma (HCC) were already present in 4.4% and 5.9%, respectively, on initial consult. Concomitant hepatitis B was equally common in patients with and without cirrhosis (20.7%vs17.5%;p=0.660) or HCC (17.9% vs 12.8%, p = 0.415).None of the patients were taking medication known to be associated with the development of hepatic steatosis. Details are shown in table 2.

Table 3. Biochemical Profile of NAFLD patients, grouped according to BMI

Variables	BMI Classification		P-value
N	Underweight Normal 171	Overweight/Obese 492	
Alt (u/L)	43.5 ± 36.4	57 ± 52.8	<0.0001
Ast (u/L)	37.24 ± 25.3	41.4 ± 30.79	0.105
Albumin (g/L)	4.3 ± 0.45	4.3 ± 0.46	0.330
INR	0.98 ± 0.12	1.9 ± 9.3	0.025
FBS (mg/dl)	109.4 ± 26	122.01 ±41.9	<0.0001
Cholesterol (mg/dl)	204.73 ± 47.7	204.6 ± 44.43	0.992
TG (mg/dl)	154.76 ± 83.3	168.1 ± 105.2	0.132
LDL (mg/dl)	121.99 ± 43.5	123.49 ± 39.3	0.691
HDL (mg/dl)	54.59 ± 39.6	46.12 ±14.7	<0.0001
Creatinine (mg/dl)	0.87 ± 0.27	1.13 ± 5	0.491
Uric acid (mg/dl)	5.8 ± 1.73	6.4 ± 10	0.404

Compared to Lean NAFLD patients, overweight/obese patients were more likely to be younger (50.9± 14.2vs54.6 ±14.2;p=0.004), higher ALT (57±52.8 vs 43.5±36.4,p=<0.0001) and higher INR(1.9±9.3vs0.98 ±0.12,p=0.025). Patients with elevated ALT had higher aspartate aminotransferase (44.40±30.96 u/L vs 21.8±11.4u/L; <0.0001) compared to those with normal ALT. However there is no significant difference in the level of AST, albumin, Cholesterol, Trigylceride, LDL, creatinine and uric acid among these two groups. Glutamyltransferase, hba1c and fibroscan results were not included due to scarcity of available data from reviewed records.

Table 4. Predictors of Overweight/ Obese NAFLD

Variable	OR	CI	P value
Age	1.02	1.02- 1.003	0.014
HDL	1.02	1.029- 1.005	0.005
Metabolic Syndrome	0.358	0.5232 – 0.245	<0.0001
Cirrhosis	2.493	5.586-1.116	0.026

The factors that were identified to have significance were analyzed using binary logistic regression with forward LR. (Table 4). Independent factors associated with overweightedness and obesity in NAFLD patients were younger age (OR=1.02 95% CI=1.02- 1.003;p= 0.014, lower HDL (OR= 1.02 95% CI=1.029-1.005;p=0.005), metabolic syndrome OR= 0.358 95% CI=0.5232 – 0.245; p=<0.000),absence of cirrhosis (OR= 2.493 95% CI=5.586-1.116;p= 0.026).

Discussion

In the current study, we identified two groups according to subjects' BMI and NAFLD status which are the lean (underweight and normal BMI – NAFLD) and overweight/ obese BMI– NAFLD. The demographic, clinical features and biochemical profile of NAFLD were analyzed in this study. As reported by Cho et al[16] in 2016 wherein the prevalence of lean NAFLD is at 12-42 % among asian general population similar to this study at 25.8 %, which is significantly lower than the prevalence among overweight/ obese NAFLD patients. While the age of NAFLD patients from this study was similar from the previous studies with a mean age of 51.8 $\pm$ 14.46 years old, lean NAFLD patients were mostly older than that their counterparts contrary to the findings in this study likely due to diversity of the population being included.

Previous studies have proven a role for metabolic syndrome and its components such as diabetes mellitus, hypertension, CAD/CVD and dyslipidemia in development of NAFLD. These findings were also consistent in the findings of this study which shows that the previously mentioned factors were independently and significantly associated with among obese/overweight NAFLD while inversely associated with lean NAFLD.

The biochemical profile of these patients such as lower HDL, elevated alt and FBS level, were associated with overweight NAFLD patients (p= <0.001) similar to the findings of Cantero et, al in 2018.

Ratziu et al. suggested that high ALT level is a marker of the progression of NAFLD to steatosis and hepatic fibrosis. In this study, higher ALT level where seen among obese/overweight NAFLD patients which may be a consequence of fatty deposition that might cause oxidative damage to the liver parenchyma.

Conclusion

Majority of NAFLD patients are overweight/obese and with elevated ALT, with a significant proportion (7.8%) already with cirrhosis/HCC on initial presentation. Overweight/obese NAFLD patients are more likely to have metabolic derangements and its consequences compared to lean patients.

Gantt Chart

Activities	Nov-Dec 2017	May-Sept 2018	Nov 2018	Dec 2018	Feb 2019
Writing of Research Proposal					
Institutional Review Board Approval					
Study Proper					
Data Collection					
Statistical Analysis					
Writing of Final Paper					
Paper Presentation					

BUDGET

This is a self-funded research. The bulk of financial requirements for this study will be allocated to statistical analysis and office supplies.

Statistical analysis	PhP 6,000.00
Office supplies (folders, papers, ink)	4,000.00
Total	PhP 10,000.0

REFERENCES:

1. Ahmed M. Non-alcoholic fatty liver disease in 2015. World journal of hepatology 2015;7:1450-9.
2. Angulo P. Nonalcoholic fatty liver disease. The New England journal of medicine 2002;346:1221-31.
3. De Lusong MA, Labio E, Daez L, Gloria V. Non-alcoholic fatty liver disease in the Philippines: comparable with other nations? World journal of gastroenterology 2008;14:913-7.
4. Bellentani S. The epidemiology of non-alcoholic fatty liver disease. Liver international : official journal of the International Association for the Study of the Liver 2017;37 Suppl 1:81-4.
5. Chalasani N, Younossi Z, Lavine JE, et al. The diagnosis and management of non-alcoholic fatty liver disease: practice guideline by the American Gastroenterological Association, American Association for the Study of Liver Diseases, and American College of Gastroenterology. Gastroenterology 2012;142:1592-609.
6. Day CP. Non-alcoholic fatty liver disease: current concepts and management strategies. Clinical medicine 2006;6:19-25.
7. Targher G, Bertolini L, Padovani R, et al. Prevalence of nonalcoholic fatty liver disease and its association with cardiovascular disease among type 2 diabetic patients. Diabetes care 2007;30:1212-8.
8. Medina J, Fernandez-Salazar LI, Garcia-Buey L, Moreno-Otero R. Approach to the pathogenesis and treatment of nonalcoholic steatohepatitis. Diabetes care 2004;27:2057-66.
9. Fattahi MR, Niknam R, Safarpour A, Sepehrimanesh M, Lotfi M. The Prevalence of Metabolic Syndrome In Non-alcoholic Fatty Liver Disease; A Population-Based Study. Middle East journal of digestive diseases 2016;8:131-7.
10. Vendhan R, Amutha A, Anjana RM, Unnikrishnan R, Deepa M, Mohan V. Comparison of characteristics between nonobese and overweight/obese subjects with nonalcoholic fatty liver disease in a South Indian population. Diabetes technology & therapeutics 2014;16:48-55.
11. Kim HJ, Kim HJ, Lee KE, et al. Metabolic significance of nonalcoholic fatty liver disease in nonobese, nondiabetic adults. Archives of internal medicine 2004;164:2169-75.
12. Domanski JP, Park SJ, Harrison SA. Cardiovascular disease and nonalcoholic fatty liver disease: does histologic severity matter? Journal of clinical gastroenterology 2012;46:427-30.
13. Caldwell SH, Oelsner DH, Iezzoni JC, Hespenheide EE, Battle EH, Driscoll CJ. Cryptogenic cirrhosis: clinical characterization and risk factors for underlying disease. Hepatology 1999;29:664-9.
14. Sanyal D, Mukherjee P, Raychaudhuri M, Ghosh S, Mukherjee S, Chowdhury S. Profile of liver enzymes in non-alcoholic fatty liver disease in patients with impaired glucose tolerance and newly detected untreated type 2 diabetes. Indian journal of endocrinology and metabolism 2015;19:597-601.
15. Zobair M. Younossi M, MPH, Maria Stepanova, PhD, Francesco Negro, MD, Shareh Hallaji, BS,, Youssef Younossi BL, MS, and Manirath Srishord, RN. Nonalcoholic Fatty Liver Disease in Lean Individuals in the United States. 2017.

16. Cho HC. Prevalence and factors associated with nonalcoholic fatty liver disease in a nonobese Korean population. Gut Liver. 2016;10(1):117-125. doi:10.5009/gnl14444

Appendix A.
Data Collection Tool

A. Demographics
Patient number: _____
Age: _____ Gender: _____
Height: _____ Weight: _____BMI: _____BMI class_____
Chief Complaint: _____
Date of first visit: _____
Date of last visit: _____
Number of follow-up: _____
Date of first Diagnosis: _____
Alcohol intake (g/day): _____
B. Comorbidity: (According to standard criteria)

() HPN
() DM
() CAD/CVD
() Dyslipidemia
() Metabolic syndrome
() HCC
() Cirrhosis

B. Laboratories/ Diagnostics:
ALT: ___u/L
AST: ___ u/L
Albumin: ____g/dl
PT w/ INR: _____
FBS: _____mg/dl
Cholesterol: _____ mg/dl
Triglyceride: _____g/dl
HDL: _____ mg/dl
LDL: _____ mg/dl
uric acid: _____ mg/dl
Creatinine: _____ mg/dl