Prevalence of Non-Alcoholic Fatty Liver Disease Among Patients with Pre-Diabetes Mellitus

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BACKGROUND OF THE STUDY

Non-alcoholic fatty liver disease (NAFLD) affects 15-30% of the general population in western countries and is increasingly becoming the most common cause of liver disease in the world.[1,2] The prevalence of NAFLD is increasing to about 15-20% in cities like Shanghai, Guangzhou and Hong Kong in China.[3] In the Philippines, the prevalence of NAFLD in a single center, hospital-based study was 12.2%.[4]One of the risk factors for NAFLD is an elevated fasting blood sugar (FBS). Early diagnosis and intervention in this patient group may be helpful in preventing progressive liver disease. In the Philippines, the prevalence of pre-diabetes is at 9.7%.[5] The increasing prevalence of pre-diabetes is a concern and could be due to a change of diet in Asian countries and the adaptation of western diets which are high in calories.

REVIEW OF LITERATURE

The incidence of NAFLD in the general population is underreported and studies vary greatly.[6] The prevalence of NAFLD in the general population worldwide is about 6.3-33%.[7]The definition of NAFLD requires that there is evidence of hepatic steatosis, either by imaging or by histology and there are no causes for secondary hepatic fat accumulation such as significant alcohol consumption, use of steatogenic medication or hereditary disorders[7]. Detection of NAFLD in the general population is a challenge because patients are asymptomatic. The gold standard for diagnosing NAFLD is still liver histology on biopsy, but application of liver biopsy to the general population, even in high risk patients could be impractical. Patients who are at high risk to have NAFLD

are patients with DM, Metabolic syndrome, obesity, and raised liver enzymes. However, this patient population is still too large to make liver biopsy for all patients who have these risk factors cost-effective.[8] Therefore, a non-invasive test that is easy, quick, and accurate is needed to detect NAFLD patients. Most epidemiologic studies on NAFLD now use ultrasound as the basis for the diagnosis of NAFLD, and this is an increasingly accepted method of diagnosis. In the study by Pathik et al they utilized Fibroscan, NAFLD Fibrosis Score (NFS), and AST/ALT Ratio (AAR) to predict severe fibrosis in patients with NAFLD.[8] They found out that these screening tools may be utilized in patients with high risk for fibrosis to determine need for liver biopsy.[8] Controlled attenuation parameter (CAP) is a novel algorithm that evaluates ultrasound attenuation. Shen et al (2014), utilized fibroscan with CAP to evaluate hepatic steatosis.[9] They found out that CAP has a high accuracy in detecting steatosis of >/= 5% and more significant steatosis.[9]

NAFLD encompasses a disease spectrum and is further categorized histologically into simple steatosis, where there is no evidence of hepatocellular injury in the form of ballooning of the hepatocytes, and non-alcoholic steatohepatitis (NASH), which is defined as the presence of hepatic steatosis and inflammation with hepatocyte injury (ballooning) with or without fibrosis (3,9). Patients with NAFLD are also at risk of developing progressive fibrosis which can eventually lead to cirrhosis and hepatocellular carcinoma. Compared to the general population, patients with NASH were observed to have a higher mortality rate and this was attributed mainly to liver and cardiovascular-related mortality (11,12).There is a strong relationship between metabolic conditions like diabetes mellitus type 2 (DM2), obesity, and metabolic syndrome with NAFLD. NAFLD in patients with elevated blood sugar such

as patients with diabetes and pre-diabetes have been noted to be more aggressive and may proceed to more serious liver disorders as reflected by the high prevalence of NASH and advanced fibrosis in this population.[10] The prevalence of NAFLD in patients with DM2 is between 30-70%.[11,12]A small single-center study in the Philippines showed a 69% prevalence of diabetes in patients with NAFLD. However, there has been no published data on the prevalence of NAFLD in patients with pre-diabetes. Given the likely significant risk of liver complications and mortality in patients with pre-diabetes, it is imperative that early diagnosis and intervention of NAFLD in this patient group is needed.

OBJECTIVES

General Objectives:

To determine the prevalence of, and risk factors for NAFLD in patients with pre-diabetes.

Specific Objectives:

1. To determine the prevalence of NAFLD in patients with pre-diabetes.
2. To compare the demographic characteristics of pre-diabetic patients with NAFLD versus those without NAFLD.
3. To compare the laboratory characteristics of pre-diabetic patients with NAFLD versus those without NAFLD.
4. To determine the fibroscan, CAP and NAFLD fibrosis score of pre-diabetic patients diagnosed to have NAFLD.
5. To determine independent predictors of NAFLD in patients with pre-diabetes.

METHODOLOGY:

This is a prospective, descriptive, cross-sectional study that will be conducted at the University of Santo Tomas Hospital Out-Patient Department in the clinical division and selected private clinics.

Consecutive patients who are 18 years old and above diagnosed to have pre-diabetes will be included in the study. Diagnosis of pre-diabetes will be based on the American Diabetes Association (ADA) guidelines.[13], which includes patients with impaired fasting glucose (FBS: 100-125 mg/dL), impaired glucose tolerance (two-hour plasma glucose value during a 75 g oral glucose tolerance test between 140 and 199 mg/dL) or glycosylated hemoglobin (HBA1c)of 5.7% to 6.4%. All patients will undergo ultrasound of the liver and laboratory exams with alanine aminotransferase (ALT), aspartate aminotransferase (AST), triglycerides, high-density lipoprotein (HDL), low-density lipoprotein (LDL), cholesterol, complete blood count (CBC) with platelet, fasting blood sugar (FBS), and HBA1c. Patients who had these laboratory tests within 3 months of study inclusion may use these test results as baseline results. Other data such as height, weight, body mass index (BMI), waist circumference, hip circumeference and blood pressure will be recorded. BMI will be computed based on the WHO criteria for Asians. Patients who are diagnosed to have fatty liver based on the finding of increased echogenicity of the liver in relation to the kidney on ultrasound will be diagnosed to have NAFLD if secondary hepatic fat accumulation such as significant alcohol consumption (>20 and >40 grams of alcohol per day for women and men, respectively), use of steatogenic medications or hereditary disorders are ruled out. Patients diagnosed to have NAFLD will have additional laboratory exams of

HBsAg and anti-HCV. Patients who are positive for HBsAg and anti-HCV will be excluded. Baseline demographic, laboratory and anthropometric data will be compared between pre-diabetic patients with and without NAFLD.

All pre-diabetic patients diagnosed to have NAFLD will undergo fibroscan with controlled attenuation parameter (CAP) to assess the degree of liver fibrosis and steatosis, respectively. Patients will be classified as having a fibrosis score of F0-F1 (<7.1 kpa), F2 (7.1-8.8 kpa), F3 (9.5-9.6 kpa) and F4 (12.5-14.6 kpa) based on the fibroscan results.[14]. The degree of steatosis will be based on the CAP score as follows: S0 <221 dB/m, S1 222-232 dB/m, S2 233-289 dB/m, S3 290 dB/m and above.. The NAFLD fibrosis score will also be determined in all NAFLD patients, and will be computed as follows:-*1.675 + 0.037 × age (years) + 0.094 × BMI (kg/m2) + 1.13 × IFG/diabetes (yes = 1, no = 0) + 0.99 × AST/ALT ratio – 0.013 × platelet (×109/l) – 0.66 × albumin (g/dl).*A NAFLD fibrosis score < 1.455 is correlated with F0-F2 and >0.675 is correlated with F3-F4. [15]

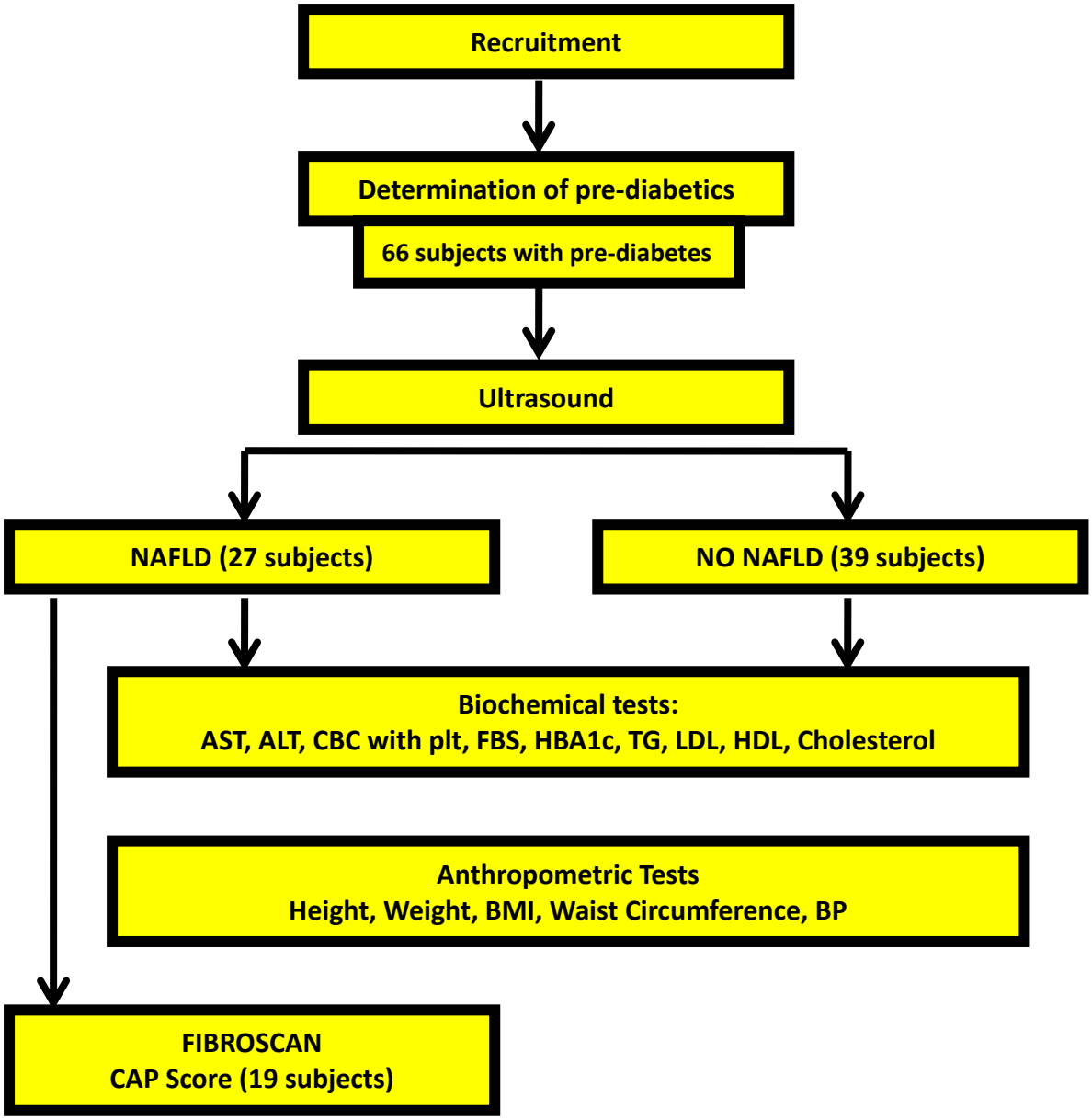
All the laboratory tests are standards of care for screening in patients with suspected metabolic diseases. Imaging tests in determining liver fibrosis are now the recommendations based on the latest European Association for the Study of Liver (EASL) in screening and evaluation of patients with chronic liver disease. The participants will be informed of this and will be included only in the study if they are able to give full consent.

Sample size:

For Sample size calculation we used the OpenEpi Ver 3 calculator. Population size for finite populations was set at 100,000 with a hypothesized frequency of outcomes for finite populations at 10% for NAFLD. The confidence level was set at 95% with a computed sample size at 139 subjects.

Statistical analysis:

All continuous variables will be presented as mean $\pm$ SD while categorical variables will be presented as number (%). Comparison of baseline variables will be done between pre-diabetic patients with NAFLD versus without NAFLD. Independent t-test (or Mann Whitney U if distribution is not normal) will be done to compare continuous variables while Fisher's exact test will be used for categorical variables. Factors with a p value of $\leq$0.1 on univariate analysis will be entered into a binary logistic regression model with forward LR to determine independent predictors for the presence of NAFLD in pre-diabetic patients. A p value of $\leq$0.05 will be considered significant in all analyses.


Recruitment
Determination of pre-diabetics
66 subjects with pre-diabetes
Ultrasound
NAFLD (27 subjects)
NO NAFLD (39 subjects)
Biochemical tests:
AST, ALT, CBC with plt, FBS, HBA1c, TG, LDL, HDL, Cholesterol
Anthropometric Tests
Height, Weight, BMI, Waist Circumference, BP
FIBROSCAN
CAP Score (19 subjects)

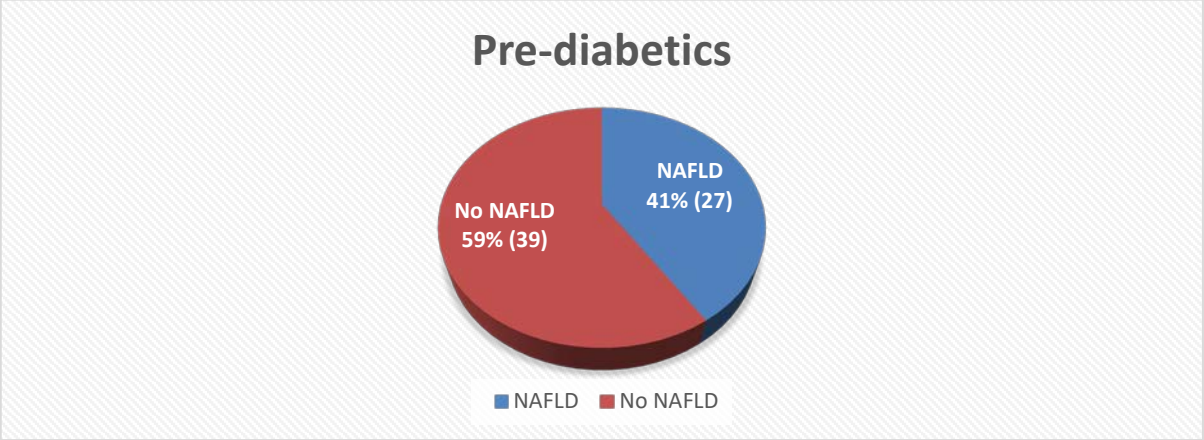
RESULTS

Table 1. Baseline Characteristics of Pre-diabetes Mellitus Patients.

	(N=66)	Standard Deviation
Age	54.9 (23-80)	+/-12.9
Gender (Male)	26 (39.4%)	
Height	1.6	+/-0.07
Weight	63.5	+/-9.92
BMI	24.6	+/-2.7
Waist Circumference	83.6	+/-16.8
Hip Circumference	92.5	+/-16.1
WHR	0.89	+/-0.24
Hypertension	26 (39.4%)	
Hemoglobin	135	+/- 12.8
Platelet	221	+/-69
Triglycerides	140	+/-147
Cholesterol	174.12	+/-94.7
LDL	109.41	+/-59.00
HDL	42.7	+/-19.8
ALT	35.80	+/-21.69
AST	33.23	+/-12.9
NAFLD Fibrosis Score	-0.84	+/-1.1

There were 66 patients enrolled in the study. The mean age of the population was 54.9 years old and 26 (39.4%) were males. The baseline characteristics of the population are described in **Table 1.**

Figure 1. Prevalence of NAFLD in Pre-diabetes Mellitus Patients.



The presence of NAFLD were determined by imaging via ultrasound. All the subjects underwent abdominal ultrasound. The prevalence of NAFLD in pre-diabetics in this study is at 40.9% **(Figure 1)**.

Table 2. Demographic and Anthropometric Characteristics of Patients with NAFLD by the Presence NAFLD.

Characteristic	NAFLD	Without NAFLD	P-Value
Age	51 +/- 12	57 +/-13.1	
Gender (Male)	14 (51.8%)	12 (30.8%)	0.110
Hypertension	10 (37 %)	16 (41 %)	0.74
Height	1.62 +/- 0.06	1.59 +/- 0.06	**0.033**
Weight	65.3 +/- 10.1	60.2 +/- 8.7	0.098
BMI	24.8 +/- 2.9	24.4 +/- 2.6	0.528
Waist Circumference	87.7 +/- 19.7	80.6 +/- 14.02	0.112
Hip Circumference	94.3 +/- 14.1	91.2 +/- 17.4	0.443
WHR	0.89 +/- 0.22	0.88 +/- 0.24	0.78

We compared the demographics and anthropometric characteristics of patients with NAFLD and without NAFLD. There were more male pre-diabetic subjects with NAFLD at 51.8 % (14). There was a significant difference with the height of the subjects with NAFLD (p-value of 0.033). There were no significant differences in the other demographic and anthropometric characteristics of the subjects **(Table 2).**

Table 3. Laboratory Characteristics and NAFLD Fibrosis Scores of Patients with Pre-diabetes Mellitus Patients by the Presence of NAFLD

	With NAFLD	Without NAFLD	P-Value
Hemoglobin	139 +/-10.2	132 +/- 13.8	**0.050**
Platelet	217 +/- 69.8	223 +/- 69.6	0.723
Triglycerides	185 +/- 206.6	109 +/- 73.1	0.076
Cholesterol	185.79+/-91.1	166.0 +/- 97.4	0.409
LDL	114.54+/-55.03	105.9 +/- 62.1	0.562
HDL	41.3 +/- 14.9	43.6 +/- 22.8	0.651
ALT	43.1 +/- 25.1	30.7 +/-17.5	**0.032**
ALT Elevation*	22 (41.5%)	31 (58.5%)	0.841
AST	37.7 +/- 15.5	30.1 +/- 9.8	**0.031**
NAFLD Fibrosis Score	-0.9 +/- 0.94	-0.78 +/- 1.2	0.559

* Above normal ALT levels, > 30 IU/L for males, >19 IU/L for females.
*Kwo, P.Y. et al. 2016.ACG Practice Guideline: Evaluation of Abnormal Liver Chemistries. American Journal of Gastroenterology.

The laboratory characteristics of pre-diabetic patients with NAFLD are seen in **Table 3**. The hemoglobin, ALT and AST levels were noted to be significantly higher in patients with NAFLD **(p-value 0.050, 0.032, 0.031, respectively)**.

Table 4. Fibroscan Scores and CAP Scores of Pre-diabetes Mellitus Patients with NAFLD.

	Fibroscan	**CAP Scores**
Pre-diabetics with NAFLD	6.13 +/- 2.39	251.6 +/- 38.3

Table 5. NAFLD Fibrosis Categories and CAP Score Categories in Pre-diabetic Patients with NAFLD

N= 19	**Fibroscan N (%)**
F0-F1	14 (73.7%)
F2	2 (10.5%)
F3	2 (10.5 %)
F4	1 (5.3%)
N = 19	**CAP Score N= 19**
S0	5 (26.3%)
S1	3 (15.8%)
S2	7 (36.8 %)
S3	4 (21.1 %)

There were 19 subjects with NAFLD who had Fibroscan and CAP. The mean Fibroscan level was 6.13 +/- 2.39 kpa (F0-1 to F2) and the mean CAP score was 251.6 +/- 38.3 (S0-S3) **(Table 4)**. Majority of the patients who had Fibroscan were at F0-F1 category (14[73.7%]) with 1 subject with an F4 category. While the majority of the subjects with CAP scores were at S2 (36.8%) followed by S0 (26.3%) and S3 (21.1%) **(Table 5)**.

Table 6. Indicators of NAFLD in Pre-diabetic Patients.

Variable	OR	CI
AST	1.1	1.015-1.123
Hemoglobin	1.1	1.010-1.127

The factors that were identified to have significance were analyzed using binary logistic regression with forward LR. The independent indicators for NAFLD in pre-diabetics that were identified were hemoglobin and AST levels with and odds ratio of 1.1 **(Table 6)**.

DISCUSSION

Metabolic conditions like pre-diabetes, diabetes, and metabolic syndromes have been known to have strong relationships to the development of NAFLD. The prevalence of NAFLD in pre-diabetics in this study was high compared to the global prevalence of NAFLD at 6.3-33 % (1,2) and the local prevalence at 12.2% (4). However, there has been an increasing trend of NAFLD in neighboring Asian countries like China (3). In diabetics the prevalence of NAFLD is at 30-70% (11, 12) with a local prevalence of NAFLD in diabetics at 69% (4). There are no current published data regarding the prevalence of NAFLD in pre-diabetics but it can be surmised from this study that even early on in this metabolic condition the development of fatty liver is already evident. The BMI of the patients were not significantly different in patients with NAFLD (24.8 +/- 2.9) and without NAFLD (24.4 +/- 2.6) however, these patients were at normal to overweight categories using the WHO classification and at the overweight and pre-obese categories using the Asian classification.

In the study, majority of patients who had Fibroscan were at F0-F1 category but it was noted that 1 patient already had advanced fibrosis category of F4. The degree of steatosis using CAP in these patients are varied but is predominantly at S2 category.

Pre-diabetic patients may benefit in early screening for NAFLD and detection of fibrosis and steatosis through non-invasive tools like Fibroscan and CAP. If detected early, physicians will be able to intervene and provide the appropriate intervention and impede the progression of fibrosis and steatosis.

CONCLUSION

The prevalence of NAFLD in pre-diabetic patients was high at 40.9%. The anthropometric measurements of the subjects were not significant except for the height. The laboratory characteristics that were significant were AST, ALT, and hemoglobin levels. The independent predictors of NAFLD identified were hemoglobin levels and AST. The degree of fibrosis of these patients using Fibroscan were predominantly at the F0-F1 category, while the degree of steatosis of these patients using CAP were predominantly at S2 category. The utilization of non-invasive tools to detect fibrosis and steatosis may be beneficial in these patients as it can appropriate early intervention and halt progress of the liver disease.

RECOMMENDATIONS

Our recommendations for this study is to get a larger population and to have all pre-diabetics subjects with NAFLD undergo Fibroscan and CAP to get a better picture of the degree of fibrosis and steatosis in this population. The limitation of our study was the availability and access to the Fibroscan and CAP facilities.

Budget

This is a self-funded research. The bulk of financial requirements for this study will be allocated to statistical analysis and office supplies. Blood tests and ancillary procedures will be shouldered by participants once they have given consent.

Statistical Analysis	**Php 8,000.00**
Office Supplies	**4,000.00**
TOTAL	**Php 12,000.00**

ETHICAL CONSIDERATIONS

Compensation and Expenses

No monetary incentives in cash or kind will be provided to patient's included in this study. The investigators will likewise not receive any compensation for the study. All ancillary procedures and biochemical tests will be shouldered by the patients. Financial expenses involving the writing of this study will be shouldered by the investigator.

Informed Consent

Physicians will be informed of the enrolment of their patients to the study. A formal letter stating the nature and purpose of the study will be forwarded to the Physicians whose patients will participate in the study. The study will be explained and discussed by the physician. All data and information involving the patient will be kept

confidential. Voluntary participation from the subjects through informed and written consents will be obtained by the physicians prior to participating in the study. The participants may be excluded from the study anytime that they would decide to withdraw for any reason. This is in accordance to the Declaration of Helsinki 2013 where it is stated that the duty of the physician who are involved in medical research is to protect the life, health, dignity, integrity, right to self-determination, privacy, and confidentiality of personal information of research subjects.

Benefits

The patient would be able to determine the state of their liver in terms of fibrosis and scores. Data obtained from this paper will be used to improve current knowledge and management of patients with pre-diabetes and NAFLD. Moreover, this would be eventually helpful in formulating effective preventive measures that will be applicable in our own local setting.

Conflicts of Interests

There are no disclosures or any sponsors for this study with no conflict of interests with regard to doing this study.

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