Non Alcoholic Fatty Liver Disease Patients Attending Two Metropolitan Hospitals in Melbourne, Australia; High Risk Status and Low Prevalence

Running title: NAFLD clinical prevalence and severity

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Abstract

Background: Non- alcoholic fatty liver disease (NAFLD) is the commonest liver disease globally with increased rates in high risk populations including type 2 diabetes and obesity.

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The condition increases the risk of end stage liver disease, hepatocellular carcinoma and all-cause mortality. NAFLD is asymptomatic and often remains undiagnosed as routine screening in high risk groups is not practised.

Aims: The aim of this study was to determine the rates and characteristics of NAFLD patients attending liver clinics at two Melbourne metropolitan hospitals.

Methods: Liver clinics were prospectively screened for ten consecutive months and participants with a diagnosis of NAFLD were further evaluated using pathology and imaging results obtained from medical records.

Results: Of the 2050 patients screened, 148 (7%) had NAFLD predominantly diagnosed using ultrasound (81%). NAFLD patients were obese (mean BMI 30.7 ± 5.9kg/m^2), insulin resistant (median HOMA 4.2 (3.2) mmol/L), had elevated liver enzymes (ALT median, males 47.0 (34.3), females 36.0 (28.0) U/L) and 18% of patients with liver stiffness measure >12kPa suggesting a moderate probability of cirrhosis. Patients with liver stiffness measure ≥9.6kPa had significantly higher: glucose (median 5.5 (1.2) vs. 6.2 (5.3) mmol/L, p=0.007), AST levels (median 25.5 (26.0) vs. 41.0 (62.0) u/L, p=0.0005) and HOMA (3.1 (3.0) vs. 5.4 (5.5) mmol/L, p= 0.040).

Conclusions: NAFLD constituted a minority of liver clinic patients, most were obese, insulin resistant, hypertensive and many had an elevated liver stiffness measurement. NAFLD poses added adverse health outcomes to high risk patients and therefore early detection is warranted.

Key words: Non- alcoholic fatty liver disease, non-alcoholic steatohepatitis, liver disease, prevalence, metabolic syndrome

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Introduction

Non-Alcoholic Fatty Liver Disease (NAFLD) is prevalent in approximately 20-30% of populations in developed countries [1], with rates reported as high as 40-90% in subgroups with Type 2 diabetes mellitus (T2DM) and obesity. [2, 3] Prevalence rates may be underestimated as the condition is asymptomatic and often goes undiagnosed and

untreated until it has progressed. Insulin resistance (IR) is the underlying pathological mechanism in NAFLD, often referred to as the hepatic manifestation of metabolic syndrome. NAFLD occurs alongside hyperlipidaemia, abdominal obesity and/or hypertension. [4] NAFLD is an independent risk factor for cardiovascular disease (CVD); which is the leading cause of mortality in affected patients. [5, 6] Approximately 20% of NAFLD patients will develop the progressive form of the disease, non-alcoholic steatohepatitis (NASH). Individuals with NASH and liver fibrosis are at increased risk of end stage liver disease, hepatocellular carcinoma (HCC) as well as all-cause mortality. [7-10] Despite the high prevalence and risks associated with the disease, safe and effective, evidence based prevention and management strategies are lacking.

The diagnosis and staging of NAFLD is challenging as the gold standard for NASH and NAFLD related fibrosis is liver biopsy[11], and increasingly less precise methods such as serological/biochemistry scores, ultrasound and transient elastography (TE) are used. [9, 11-13] Serological scoring systems and TE have an acceptable reliability at distinguishing cirrhosis from non-cirrhosis. [11, 14, 15,16] Ultrasound only identifies established cirrhosis and cannot be used to accurately stage disease or monitor progression. [13]

Simple steatosis in NAFLD can be reversed with lifestyle modifications, preventing the progression to NASH and cirrhosis [7, 17, 18]; however, many patients are not identified until they have advanced disease. High risk groups with T2DM have a significant prevalence of liver injury as determined by TE.[19] Earlier detection and management[20] may prevent progression, with NAFLD projected to be the leading reason for liver transplant in 2020.[21, 22]

The aim of this observational study is to determine the rates and characteristics of NAFLD patients referred to liver clinics in two metropolitan hospitals in Melbourne, Australia. We hypothesise that in the absence of established screening practices and treatments, patients with NAFLD referred to and attending liver clinics for monitoring, will represent a minority of the patient cohort and have progressed liver disease and established co morbidities.

Materials and Methods

Study participants and design

Liver clinic appointment lists were prospectively screened weekly at two metropolitan hospitals in Melbourne, Australia to determine eligibility for a randomised controlled dietary intervention trial in NAFLD subjects. [23] Data was collected for ten consecutive months at each respective site between October 2014 and June 2016. All patients aged ≥18 years were included, and the primary reason for clinic attendance was recorded. NAFLD diagnosis was based on the following: (1) sonographic diagnosis of fatty liver, defined as diffusely increased liver echogenicity (>right renal parenchyma) with vascular blurring; **or** biopsy proven fatty liver disease; and (2) a negative history of alcohol consumption exceeding current Australian health guidelines [24]; and (3) exclusion of liver disease of other aetiology including drug-induced, autoimmune, viral hepatitis, cholestatic, metabolic and genetic liver disease.

Available results including liver tests, lipid profile, glucose, HbA1c, insulin, anthropometry, liver imaging, biopsies, comorbid diagnoses and medications were obtained from medical records. Records were cross checked for duplicate appointments. The most recent test

results were included for assessment. Body mass index (BMI) was calculated (kg/m^2) and obesity was defined as BMI ≥30 kg/m. [2, 25] Homeostatic Model of Assessment (HOMA), a measure of IR was calculated using the formula [glucose (mmol/l) multiplied by insulin (μU/ml)/22.5]. [26]

The study was approved by Alfred Health, Eastern Health and La Trobe University Human Research Ethics Committees.

Statistical analysis

Analysis was performed using the statistical package SPSS 23 (SPSS Inc., 1989–2004, Chicago, IL, USA). Normality of continuous variables was assessed using Kolmogorov–Smirnov test. All data are expressed as mean ± standard deviation or median (IQR), as appropriate. For continuous data, comparisons between groups were assessed using independent t-tests or one way ANOVA in parametric variables and the Wilcoxon rank sum test/Mann–Whitney U test or Kruskal–Wallis test, in non-parametric variables. Categorical variables were compared by independent χ^2 test. A two-sided p-value of <0.05 was considered to be statistically significant. Pearson and Spearman rank correlation coefficient was used to assess strength and significance of association between variables. A Bonferroni correction was used for post hoc test analysis. The relationship between LSM and correlated predictors was assessed using multiple and univariate regression analysis.

Results

There were a total of 2050 patients across two liver clinics during the 10 month period who were screened for this study regardless of clinic attendance (Figure 1). These clinics captured all NAFLD outpatients in the Liver clinics with the exception of those seen privately. Prevalence of medical diagnoses at the clinics are listed in Table 1. There were 148 (7%) patients managed for NAFLD. The commonest reason for clinic attendance was management of Chronic Hepatitis B (20%), alcoholic fatty liver disease (18%) and Chronic Hepatitis C (13%). Autoimmune hepatitis, haemochromatosis and Primary Biliary Cholangitis represented 5%, 2% and 2% of clinic attendance, respectively, with other conditions comprising ≤1%.

NAFLD was predominantly reported with ultrasound showing consistent radiologic features (81%). Thirteen (9%) patients had NAFLD diagnosed by liver biopsy, with advanced liver fibrosis (F3) on three, cirrhosis (F4) in seven. A Brunt score was only available for 9 (6%) patients and scores ranged from 4-7 (supplementary table). A large proportion of patients had TE, Fibroscan™ (79%), and various liver biochemical measures were available in 78 - 97% of patients.

Group characteristics

The demographic, clinical and laboratory characteristics of patients with NAFLD are described in Table 2. There were slightly more females (56%) and the median BMI was 30.7 ± 5.9 kg/m^2, with 32% overweight and 40% obese. The median LSM score was 6.5 (6.0) kPa; 18% of patients with NAFLD had a LSM score greater than 12kPa, and 7% greater than 20kPa, suggestive of advanced fibrosis and cirrhosis, respectively. The median alanine

aminotransferase (ALT) level was elevated with significantly higher for males than females, (p=0.01). NAFLD patients were overall IR with a median HOMA score of 4.2 (3.2).

The presence of co-morbidities within the group are included in Table 2 Of the NAFLD patients, 44% had T2DM and an additional 10% had impaired glucose tolerance, 52% had hypertension, and 20% had a diagnosis of cardiovascular disease (CVD). Oral hypoglycaemic agents (OHAs), statins and antihypertensive medications were reported in 35%, 30% and 37% of the cohort, respectively.

Liver Stiffness Measure (LSM) Score

The cohort were further categorised according to liver disease severity based on a LSM cut-off score of < or ≥9.6kPa, a value that has a positive predictive value for advanced fibrosis of 72%.[27] (Table 2). Patients with NAFLD in the higher LSM group were older (mean age 59.9 ± 12.2 vs. 52.9 ± 14.5 years, p=0.006) and had significantly higher BMI (32.5 ± 6.4 and 29.6 ±5.5 kg/m2, p=0.02). Those with higher LSM scores also had higher median AST scores (40.0 ± 58.0 vs. 25.0 ± 33.3 u/L, p=0.0005). Median HOMA scores were significantly higher indicating that the group with higher LSM scores were more IR (5.1 ± 2.6 vs.3.1 ± 3.2, p= 0.03). Total and low density lipoprotein (LDL) cholesterol were lower in this group with higher LSM (4.6 ± 1.3 vs. 5.2 ± 1.1 mmols/L, p=0.01 and 1.1 ± 1.1 vs. 3.1 ± 1.0mmol/L, p=0.031 respectively), and there was a higher use of statins in this group (25% vs 30%, p=0.54). The group with higher LSM scores had greater rates of T2DM and hypertension;

33% vs 58%, p=0.009 and 43% vs 65%, p=0.023, respectively. Of note, there were 27 NAFLD patients with LSM scores >12kPa, a score consistent with cirrhosis. [28]

LSM scores significantly increased with increasing BMI; (mean LSM in healthy weight 5.6kPa ± 3.4, overweight 5.6kPa ± 5.3, obese 6.6kPa ± 9.4 and morbidly obese 9.9kPa ± 9.1, p= 0.032). Significantly higher LSM scores were observed in those with a diagnosis of NAFLD and T2DM (32%) compared to NAFLD patients without T2DM (47%); median 8.5 (11.6) vs. 5.9 (5.2) kPa, p=0.011, respectively. Patients with NAFLD and hypertension (52%) had significantly higher LSM scores than normotensive NAFLD patients; median of 8.2 (8.8) kPa vs. 5.7 (4.6) kPa, p=0.011, respectively.

Associations of variables with LSM scores

Correlational analysis was performed to assess the strength of associations between age, BMI and biochemical variables with LSM score. Table 3 displays the degree to which these variables correlate. The strongest correlations were reported for LSM score and HOMA (r=0.49, p=0.008), and LSM and AST; (r=0.42, p=0.0005). Correlations between BMI (r=0.27 p=0.005), age (r=0.28 p=0.002), ALT (r=0.20 p=0.03) and LDL (r=-0.33 p=0.002) showed weaker, albeit significant, associations with LSM.

Regression Analysis

A multiple regression analysis of LSM was conducted and showed that age, BMI, AST, ALT, total cholesterol and LDL cholesterol remained significant. The model indicated these variables explained 29.3% of the variance (R^2 =0.293, F(6,81)=5.59, p<0.0005). BMI, age and AST levels positively and significantly predict LSM score (β = 0.30, p=0.003; β = 0.38, p=0.0005; β = 0.23, p=0.017, respectively).

A separate univariate linear regression was conducted with HOMA as a predictor. This was not entered in the previous model as only 32 participants had a HOMA score. The regression equation with HOMA was significant, R^2 = 0.15, adjusted R^2 = 0.12, F (1, 27) = 4.89, p<0.036.

Discussion

This observational study showed that NAFLD constituted a minority of the liver clinic population, and NAFLD patients were predominantly obese with IR, and had elevated liver enzymes and TE LSM scores consistent with mild fibrosis. Over half of the NAFLD patient group had T2DM, impaired glucose tolerance and hypertension, with 20% of the cohort diagnosed with CVD. Therefore, while NAFLD is highly prevalent in the community, this study demonstrated that it does not make up a large proportion of patients at established liver clinics. Low representation of this disease and management of the condition in the clinical setting is likely attributed to a number of factors; particularly in relation to its asymptomatic nature, the lack of routine screening practices of high risk groups for NAFLD in the community, clinic fatigue by these patients, and low rates of referrals to hepatology

services by clinicians of those with suspected NAFLD. [29, 30] It is likely that the latter is related in part to an absence of pharmacological therapies for the disease.

Definitive diagnosis and staging are problematic, as biopsy is expensive, invasive and not without risk [12], explaining the low biopsy rate in this study (14%). Despite recommendations, liver biopsy It is not a viable screening tool for at risk patients, such as those with T2DM and NAFLD. [31] Routine biochemistry and ultrasound are of limited use in this group, as they do not necessarily correlate with the severity of the liver disease[32], and liver biochemistry elevation significantly underestimates the true prevalence of NAFLD and do not predict liver histology. [1, 33] TE using Fibroscan ™ is a rapid non-invasive technique that is able to estimate the likelihood of significant liver fibrosis using LSM. It is particularly helpful in identifying the group with probable cirrhosis, but is less reliable in lesser degrees of fibrosis. However, in NAFLD it is less reliable than other liver diseases as success rates are lower in obese individuals when compared to non-obese counterparts. [28] In this study, individuals with higher LSM scores were obese, had raised liver enzymes, were IR, and had significantly higher rates of HTN. The finding that elevated LSM was associated with decreased total cholesterol is consistent with previous literature. [34]

NAFLD characteristically exists in a setting of altered metabolism, comprising abdominal obesity, IR, dysglycaemia and atherogenic dyslipidaemia, similar to CVD [35, 36]. This study highlights the elevated risk of progression to liver related complications and CVD, in patients with these features [11] Serological scoring systems may also be a non-invasive and rapid

method to differentiate between NAFLD patients with and without advanced liver fibrosis.[14, 15] However, required data to calculate such scores was not available for this cohort.

This cross sectional study shows that referral and management of patients with NAFLD in the tertiary setting is not optimal, based on the known community prevalence of the condition. Australian data reflective of metropolitan areas for this age group (mean 55 years) reiterates this point, estimating that in both males and females prevalence is likely to be 25-30% of the population. [37, 38]

Over half of the NAFLD patients in this study had T2DM or IGT. NAFLD poses an increasing risk of overall mortality compared to the general population (standardized mortality ratio 1.34) and T2DM poses an additional risk factor for death. [7, 39 40] The presence of T2DM increases the CVD risk, and has consistently been shown to be a key predictor of NASH and advanced fibrosis. [41-43] However, it may be impractical to propose screening of all T2DM for significant liver disease. Similar to previous reports, our study showed a strong positive association with IR and LSM, consistent with other evidence of more severe liver disease in T2DM and IR. [39, 44-46]

At present there is no data available on the prevalence of NAFLD within individual Australian States and Territories. It is likely that in rural areas and/or those with higher rates of indigenous populations such as the Northern Territory and Western Australia, that prevalence of NAFLD may be even higher. Strategies to engage these patients in the

community with particular attention to people with T2DM and obesity should be considered, with an economic cost benefit analysis to ascertain better investment of resources at the subclinical level of disease. Patient access to the tertiary setting is also important as it provides increased access to new and promising therapies as part of clinical trials. Multidisciplinary clinics for high risk patients such as those with T2DM, may be one such strategy to ensure screening and management of NAFLD. This would encompass hepatologists and dietitians present during diabetes clinics.

The strengths of this cross sectional study were that two major metropolitan hospitals were screened weekly for 10 months capturing a cohort of over 2,000 liver clinic patients, a generalizable subsample of the Melbourne clinical NAFLD patient group. However biopsy data was infrequent, and diagnosis and staging relied on biochemistry and TE, with their previously mentioned limitations[28].

The main limitation of this study is that the overall sample size of patients with NAFLD was small, with missing laboratory values affecting some outcomes. The observational design also meant there was unavailable data for assessment of disease severity using serological scoring systems. For most patients, liver injury was staged using TE which has limitations in obese patients and those with less advanced liver disease. [9,11]

Conclusion

In conclusion, patients with NAFLD referred and managed in metropolitan liver clinics from two major hospitals constitute a minority of the Liver Disease population engaged in tertiary level health care, despite high rates of NAFLD in Australia. Participants in this study with

NAFLD had multiple components of the metabolic syndrome including being overweight, IR, and with almost 20% having TE levels suggestive of cirrhosis. Given the large proportion of the population with NAFLD and the small numbers captured in these clinical settings, screening of high risk patients such as those with diagnosed diabetes, cardiovascular risk factors or who are obese, is warranted to ensure earlier detection of the disease. As biopsy is invasive, surrogate markers such as TE and HOMA scores which are rapid and easily attainable should be considered.

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Table 1: Liver Diagnoses at each clinic site

	Site 1	Site 2	Total
Patients screened (n)	892	1158	**2050**
NAFLD n (%)	44 (4.9)	104 (9.0)	148 (7.2)
Hepatitis B n (%)	29 (3.3)	379 (32.7)	408 (19.9)
History ETOH n (%)	223 (25)	145 (12.5)	368 (18)
Hepatitis C n (%)	83 (9.3)	178 (15.4)	261 (12.7)

Abbreviations: NAFLD: Non Alcoholic Fatty liver disease, ETOH: ethanol

Supplementary Table : A breakdown of the histological staging using Brunt score for a subset of patients with NAFLD diagnosis.

Case number	Steatosis (0-3)	Lobular inflammation (0-3)	Ballooning (0-2)	Brunt score (0-8)
1	3	2	2	7
2	3	1	2	6
3	3	1-2	1	6
4	2	1	2	4
5	3	1	0	4
6	3	0	0	3
7	2	2	2	6
8	2	1	2	5
9	2	1	2	5

Table 2: NAFLD patient characteristics for overall cohort and LSM score categories by risk of cirrhosis [25]

			Overall NAFLD cohort	**<9.6kPa** n=77		**≥9.6kPa** n=40		
	Reference Range	**n**	**Mean ± SD**	**n**	**Mean ± SD**	**n**	**Mean ± SD**	**p value**
Age (yrs)	-	148	55.8 ± 14.5	77	52.6 ± 14.1	40	62.1 ± 11.5	0.0005*
Height (cm) †	-	124	163.0 (16.5)	69	165.0 (10.0)	35	162.0 (18.5	0.455
Weight (kg)	-	130	85.0 ± 20.4	72	84.9 ± 21.1	37	86.4 ± 20.7	0.565
BMI (kg/m2)	18.5-25 kg/m2	124	30.7 ± 5.9	69	30.1 ± 5.9	36	32.0 ± 6.1	0.077
LSM Score (kPa) †		117	6.5 (6.0)					
Glucose (mmol/L) †	3.5-6.0	114	5.9 (1.7)	61	5.5 (1.2)	29	6.2 (5.3)	0.007*
HbA1c (%)	<6.0	33	6.3 ± 0.8	21	6.2 ± 0.8	11	6.4 ± 0.8	0.350
ALT (U/L) †	M: < 30 F: < 20	M: 64 F: 79 143	M:47.0(34.3) F: 36.0 (28.0) 42.0(38.0)	74	41.0 (33.0)	39	46.0 (31.5)	0.354
ALP (U/L) †	30-110	136	86.5 (19.3)	71	85.0 (19.0)	36	92.0 (75.0)	0.294
AST (U/L) †	M: <35 F: <30	M: 57 F: 58	M: 33 (18.8) F: 27 (20.3)	58	25.5 (26.0)	33	41.0 (62.0)	0.0005*

		115	32.0 (43.5)					
GGT (U/L) †	M: <62 F: <38	M: 62 F: 75 137	M: 67.5 (112.3) F: 72.0 (90.0) 69.0 (99.0)	71	66.0 (72.0)	37	86.0 (131.0)	0.10
Bilirubin (umol/L) †	<21	137	10.0 (6.8)	71	10.0 (7.0)	37	12.0 (8.5)	0.114
Insulin (mIU/L) †	< 25	38	13.8 (12.4)	23	13.0 (14.1)	10	16.5 (16.0)	0.346
Total Cholesterol (mmol/L)	< 5.5	127	4.9 ± 1.2	67	5.1 ± 1.1	34	4.6 ± 1.4	0.009*
HDL (mmol/L)	> 1.0	113	1.3 ± 0.4	60	1.3 ± 0.4	31	1.2 ± 0.4	0.618
LDL (mmol/L)	< 3.5	110	2.8 ± 1.1	57	3.1 ± 1.0	31	2.6 ± 1.2	0.015*
TGs (mmol/L) †	< 2.0	124	1.5 (0.7)	65	1.6 (0.6)	33	1.5 (1.5)	0.643
HOMA-IR (mmol/L) †	< 2.0	32	4.2 (3.2)	21	3.1 (3.0)	8	5.4 (5.5)	0.040*
Co morbidities by Site				**Co-morbidities by LSM**				
	Total %	**Site 1 %**	**Site 2 %**		**n (%)**	**n (%)**	**p value**	
T2DM	43.9	55	39		25 (33)	23 (58)	0.009*	
HTN	52	55	51		33 (43)	26 (65)	0.023*	
CVD	19.6**	9	24		10 (13)	9 (23)	0.19	
Depression	22.3	20	23		-	-	-	
IGT	10.1	16	8		12 (16)	1 (3)	0.033*	
HCC	2.7	4.5	1.9		-	-	-	
Deranged Lipids	33.1**	18	7		27 (35)	13 (33)	0.78	

Obstructive Sleep Apnoea	10.1	50	29	-	-	-
Medications						
OHAs	50**	29	35.1	20 (26)	19 (48)	0.019*
Statins	36	28	30.4	19 (25)	12 (30)	0.54
Antihypertensives	36	38	37.2	22 (29)	19 (18)	0.042*

† Non parametric data presented as median (IQR).

* $p<0.05$ statistically significant **Between site comparison using χ^2, $p<0.05$

Abbreviations: BMI: Body Mass Index, ALT: alanine aminotransferase, ALP: alkaline phosphatase, AST: aspartate aminotransferase, GGT: Gamma-glutamyltransferase, HDL: High Density Lipoproteins, LDL: Low density Lipoproteins, TG: Triglycerides, HOMA-IR: Homeostatic Model of Assessment- IR, T2DM: Type 2 Diabetes Mellitus, HTN: Hypertension, CVD: CVD, IGT: Impaired Glucose tolerance, HCC: hepatocellular carcinoma, OHA's: Oral Hypoglycaemic agents

Table 3: Correlations between LSM score and other variables

Variables	Glucose	BMI	Age	Cholesterol	LDL	HDL	TG	ALT	ALP	AST	GGT	HOMA	Insulin
LSM Score													
r	0.19	0.27	0.28	-0.27	-0.33	-0.02	-0.11	0.20	0.08	0.42	0.17	0.49	0.30
p	0.07	0.005*	0.002*	0.007	0.002*	0.85	0.29	0.03*	0.44	0.0005*	0.09	0.008*	0.09

*significance indicated with a $p<0.05$.

Abbreviations: BMI: Body Mass Index, ALT: alanine aminotransferase, ALP: alkaline phosphatase, AST: aspartate aminotransferase, GGT: Gamma-glutamyltransferase, HDL: High Density Lipoproteins, LDL: Low density Lipoproteins, TG: Triglycerides, HOMA-IR: Homeostatic Model of Assessment- IR.

Figure 1: *Screening and recruitment process for characterising patient diagnoses*