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Identification of type 2 diabetics with non-alcoholic fatty liver disease who are at increased risk of progressing to advanced fibrosis: A cross-sectional study

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Manuscripts

Title Page

Title of the article - Identification of type 2 diabetics with non-alcoholic fatty liver disease who are at increased risk of progressing to advanced fibrosis: A cross-sectional study

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1986

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9 **Abstract:**

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12 **Introduction;**

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14 Identification of advanced hepatic fibrosis in non-alcoholic fatty liver disease (NAFLD) is
15 important as this may progress to cirrhosis and hepatocellular carcinoma. The risk of
16 hepatic fibrosis is especially high among diabetics with NAFLD. Annual screening of
17 hepatic fibrosis is especially high among diabetics with NAFLD. Annual screening of
18 diabetics for fatty liver and significant fibrosis with calculation of FIB-4 score and vibration
19 controlled transient elastography (VCTE) has been recommended. However, VCTE is
20 expensive and may not be freely available in resource-limited settings. We aim to identify
21 predictors of significant liver fibrosis and to develop a prediction model to prioritize referral
22 of diabetic patients with NAFLD for VCTE.
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30 **Methods and analysis;**

31 This cross-sectional study will be conducted among all consenting adults with T2DM with
32 NAFLD at the Colombo North Teaching Hospital, Ragama, Sri Lanka. All patients will have
33 the FIB-4 score calculated. Those with FIB-4 ≥ 1.3 will undergo VCTE (with FibroScan® by
34 Echosens). Risk associations for progression to advanced liver fibrosis/cirrhosis will be
35 identified by comparing patients with significant fibrosis (LSM ≥ 8 kPa) and without
36 significant fibrosis (FIB-4 < 1.3). A model to predict significant liver fibrosis will be developed
37 using logistic regression.
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45 **Ethics and dissemination.** Ethical approval has been obtained from the Ethics Committee
46 of the Faculty of Medicine, University of Kelaniya (P/66/07/2021). Results of the study will
47 be disseminated as scientific publications in reputed journals.
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52 **Keywords:** Nonalcoholic Fatty liver disease, NAFLD, Diabetes mellitus, Prediction,
53 significant liver fibrosis, cross-sectional study, vibration controlled transient elastography
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Article Summary

Strengths and limitations of this study

- We will evaluate freely available lifestyle, clinical and biochemical/haematological characteristics and FIB-4 score to identify diabetics with NAFLD at high risk of developing advanced liver fibrosis/cirrhosis.
- We plan to develop a simple, cost-effective, specific, and practical model to identify the patients at risk of developing advanced liver fibrosis /cirrhosis with more utility than the FIB-4 score to prioritize patients who need transient elastography and referral to a hepatologist.
- The new model will be user-friendly and suited for low-resource settings where VCTE is not freely available.
- As this is a cross-sectional study, the limitation will be the lack of long-term follow-up data.

Main text

Introduction

Non-alcoholic fatty liver disease (NAFLD) is the most common liver disease in the world with a global prevalence of 25%, with the highest rates in the Middle East and South America and the lowest in Africa (1-3). NAFLD is a spectrum ranging from simple steatosis, nonalcoholic steatohepatitis (NASH) to cirrhosis (Figure 1)(4). NASH is strongly associated with liver fibrosis and is defined histologically on a scale ranging from F0 to F4. Stages F0 to F1 have no or minimal fibrosis, F2 is significant fibrosis with risk of progressive NASH, F3 is advanced fibrosis and F4 cirrhosis (Figure 2) (5). Fibrosis of the liver can be measured non-invasively with vibration controlled transient elastography (VCTE) with FibroScan® by Echosens which measures liver stiffness. A liver stiffness measure (LSM) between 2. -7 kPa is suggestive of absent or mild fibrosis while LSM above 12.5 kPa, is suggestive of cirrhosis.

NAFLD is a dynamic condition that can regress to simple steatosis with liver-directed therapy, smoulder at a relatively constant level of activity, or cause progressive fibrosis and lead to cirrhosis. However, the majority of NAFLD patients have a benign course and only 10% develop progressive fibrosis leading to cirrhosis and hepatocellular carcinoma (HCC) (6, 7). There is no widely accepted pharmacological treatment for established cirrhosis and the only curative treatment is liver transplantation. However, progressive NASH and its complications such as cirrhosis could be delayed or prevented if people with increased risk of progressive fibrosis are detected early in the course of the disease (at F2-3 stage or LSM \geq 8 kPa) and liver-directed therapies are initiated. This is the key to reducing the liver-related burden of NAFLD in both high- and low-income countries (8).

Figure 1

Figure 2

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6 NAFLD is the hepatic manifestation of insulin resistance, which is the hallmark of type 2
7 diabetes mellitus (T2DM) (9). It is three times more prevalent among diabetics than in the
8 general population (10). Globally, 55.5% of patients with T2DM and 62% of Asians with T2DM
9 have NAFLD (11) (12). On the other hand, NAFLD is considered a risk factor for T2DM (13,
10 14), and NAFLD patients have a two-fold increased risk of developing T2DM compared to
11 those without NAFLD (15). Diabetes is independently associated with the degree of steatosis,
12 NAFLD progression to NASH, advanced fibrosis, and the development of HCC (16-19). The
13 progression of NAFLD to advanced fibrosis is two times higher among diabetics than in the
14 general population. Around 20% of T2DM patients with NAFLD progress to advanced liver
15 fibrosis over a mean of 5.9 years (9)(20). Therefore, both the American Diabetes
16 Association(2020) (21) and the European Association for the Study of the Liver(2016) (22)
17 recommend screening of all T2DM patients for NAFLD to prevent liver-related complications
18 of NAFLD. There are two proposed algorithms for screening diabetic patients for NAFLD,
19 and both propose annual screening of diabetics with NAFLD but using two different screening
20 tools; one using the Fibrosis-4 score (FIB-4) (23, 24) and the other using the NAFLD fibrosis
21 score (NFS)(25, 26). The proposed algorithm involves two steps. The first is an annual
22 screening of diabetics with NAFLD and the second step is liver stiffness measurement (LSM)
23 using transient elastography (25) for those with FIB-4 ≥ 1.3 (24) or NFS between -1.455 to
24 0.676 (25). The patients at high risk of advanced fibrosis/cirrhosis with an LSM ≥ 8 kPa are
25 advised referral to specialized liver centres for further assessment and management (24, 27)
26 (28).
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43 Diabetes has become an epidemic in low-middle-income countries including those in Asia.
44 Of the world's diabetic population, 60% are Asian (29). Diabetes is on the rise among
45 Asians due to urbanization, changing to Western lifestyles and dietary habits in addition to
46 a relatively high genetic predisposition (30, 31). Asians are at higher risk of developing
47 T2DM compared with people of European ancestry with genetic predisposition through
48 PNPLA3 SNPs and polymorphisms in apolipoprotein (29). However, VCTE is expensive
49 and is not freely available for all diabetics in low-middle income countries.
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2 Therefore, we aimed to identify clinical predictors of NASH (i.e.: LSM \geq 8 kPa) (28)) in type
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4 2 diabetics who are at risk of progression to advanced liver fibrosis and cirrhosis, and
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6 develop a model to identify patients at high risk of progression to advanced fibrosis. This
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8 we hope will help to guide clinicians in primary and secondary care in resource-poor
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10 settings to prioritize referrals for VCTE and to specialized liver centres.

11 12 **Methods and analysis**

13 14 **Study design and setting**

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16 This study is an ongoing cross-sectional study to identify diabetics with NAFLD who are at
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18 risk of progression to advanced liver fibrosis and/or cirrhosis. We compare the risk
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20 associations of patients with advanced liver fibrosis diagnosed by an LSM \geq 8 kPa by
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22 transient elastography and patients with no or minimal liver fibrosis diagnosed by FIB-4
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24 score $<$ 1.3. The study is being conducted at the Colombo North Teaching Hospital,
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26 Ragama, Sri Lanka.

27 28 **Study objectives**

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30 To identify diabetics with NAFLD who are at increased risk of progression to advanced liver
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32 fibrosis/cirrhosis and to develop a prediction model for the same. We further plan to
33
34 validate the FIB-4 score among Sri Lankans.

35 36 37 **Study population and eligibility criteria**

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39 All adult patients with T2DM attending Medical/Diabetes Clinics of three Consultants at
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41 three private sector hospitals of the Gampaha District of Sri Lanka over 12 consecutive
42
43 months who have ultrasonographic evidence of NAFLD will be the study population.

44 45 **Inclusion criteria**

- 46
47 • Patients with confirmed T2DM
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49 • Patients who are aged over 18 years
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51 • Patients who have ultrasonic (US) evidence of fatty liver in a US scan done within the
52
53 previous 3 months of recruitment
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Exclusion criteria

- Patients without consent
- Patients with established cirrhosis on US scan
- Males consuming alcohol > 14 units/week and females consuming alcohol > 7 units/week
- Evidence of Hepatitis B or C infection
- Patients with diagnosed liver diseases of known aetiology other than NAFLD
- Patients on medications known to cause fatty liver or liver fibrosis e.g., tamoxifen, methotrexate etc.

Sample size

The sample size was calculated for developing a clinical prediction model (32, 33) assuming an R-squared value of 0.2 for the logistic model, 10 parameters in the model, and 0.05 acceptable difference in apparent & adjusted R-squared, 0.05 margin of error in the estimation of intercept and an anticipated prevalence of advanced liver fibrosis of 15% among T2DM with NAFLD (Events per Predictor Parameter (EPP) (9).

The calculated minimum sample size is 398 patients with T2DM with NAFLD (among whom 60 are expected to have significant fibrosis, i.e.: LSM \geq 8 kPa).

Patient recruitment -

Consecutive adult patients (>18 years) with T2DM attending Medical/Diabetes Clinics of three Consultants at three private sector hospitals of the Gampaha District of Sri Lanka over 12 consecutive months starting from November 2021 are being screened and the eligible patients are recruited to this study after being referred to the Gastroenterology and Hepatology clinic of the Colombo North Teaching Hospital Ragama until the sample size is achieved (Figure 3). All eligible patients are given a patient information sheet to read and time to clarify doubts with investigators before consenting. The participants will be informed about the study including the data collection procedure and that a subgroup of participants will undergo a non-invasive VCTE of the liver free of charge. The patients will be made aware of the ability to withdraw consent at any point without having to give reasons. Informed written consent from all participants will be obtained before recruiting into the study. Permission has been obtained from the director of the North Colombo Teaching

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2 Hospital and the consultant in charge of the Diabetic and Endocrine clinics to carry out the
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4 study.
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16 Study procedure

17 Patients with diabetes mellitus and complying with inclusion and exclusion criteria will be
18 interviewed and medical records will be assessed. Data will be collected using an
19 interviewer-administered questionnaire. Information on demography, history of metabolic
20 risk factors, medications, diet, and exercise will be recorded. Haematological and
21 biochemical investigations done within 3 months before recruitment will be extracted from
22 clinic records. Anthropometry: height, weight and waist circumference will be measured at
23 recruitment. FIB-4 score will be calculated for all patients using age, sex, and the most
24 recent AST, ALT and platelet count done within 3 months of recruitment to the study. All
25 patients with a FIB-4 score ≥ 1.3 will undergo VCTE of the liver free of charge by a single,
26 trained medical officer. LSM and CAP measures will be recorded. A subset of patients with
27 a FIB-4 score < 1.3 will also undergo VCTE to confirm the validity of the FIB-4 score to
28 exclude significant liver fibrosis.
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40 Statistical analysis

41 IBM SPSS 22.0 software will be used for data management and analyses. Multiple logistic
42 regression will be used to identify factors associated with significant liver fibrosis. A model
43 will be developed using the identified risk factors to predict “significant liver fibrosis”
44 according to their weighted scores (β -coefficient). Cut-off points and the sensitivity and
45 specificity of predictions will be determined using ROC curves. The predictions of the new
46 model will be compared with the predictions of the simple FIB-4 score.
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54 Data management and monitoring

55 All completed questionnaires and VCTE reports will be stored securely. An electronic
56 screening log and database will be maintained as a password-protected file.
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Ethical considerations

This is not an interventional study and is associated with no risks to the patients. Selected patients will undergo non-invasive VCTE of the liver free of charge. There are no risks associated with this scan. The results of the scan will only be divulged to the treating physician of the patients for initiation of relevant treatment options. The findings of the study could be beneficial to all diabetics in the early diagnosis/prediction of significant liver fibrosis in individuals. Participants will have the right to withdraw from the study at any point without providing explanations. Ethical approval for the study has been obtained from the Ethics Committee of the Faculty of Medicine, University of Kelaniya, Sri Lanka (Ref. P/66/07/2021).

Termination of the study

The study will be terminated if:

- A new and cost-effective tool to predict significant liver fibrosis that changes current guidelines become available.
- Significant violation of good clinical practice that compromises the ability to achieve study objectives or compromises subject safety occurs.

Study status

The trial commenced in November 2021 according to the protocol version 2.0, 06 August 2021 and is currently open for recruitment. We have recruited 220 patients for the study so far.

Patient and public involvement

Reports of the VCTE (LSM) done as part of the trial will be available to all participants who request it and will be used in the standard management when required. All patients are given a health education leaflet on Fatty Liver and secondary prevention. Results of the VCTE are notified to the treating physician for necessary action. The results of the study will be disseminated to study participants and other patients with diabetes and fatty liver using patient education leaflets and lectures after completion of the study.

Discussion

In this study, we aim to develop a practical, cost-effective model to predict diabetics with NAFLD who are at increased risk of progressing to advanced liver fibrosis and/or cirrhosis to target those that require transient elastography. Furthermore, even though there are non-invasive markers of liver fibrosis such as FIB-4 (23), BRAD (34), and NFS (26), none of these has been developed specifically for diabetics and has been validated in Asian except among Japanese (35).

Apart from a few studies from North India (36) and Vietnam (37), there are no reports of VCTE data in diabetics from Asia. There is no data on VCTE from Sri Lanka. Furthermore, the FIB-4 score has not been validated among Sri Lankans. Through our study, we hope to provide a low-cost, practical model to identify the patients with type 2 diabetes and NAFLD who are at increased risk of progression to advanced fibrosis/cirrhosis.

Limitations

Liver biopsy is the gold standard for staging liver fibrosis but in our study, VCTE will be used to diagnose significant liver fibrosis (38). However, liver biopsy is an invasive procedure and current practice guidelines recommend VCTE as a surrogate to exclude advanced fibrosis, with liver biopsy reserved for those with equivocal VCTE results (24, 25).

Author Contributions: CM conceived the study. CM,TE, CD, RF,NL, LR, CR, DK, AP JdeS and AD contributed to the study design. CM drafted the manuscript and AD, and JdeS significantly edited the manuscript. All authors assisted in developing the protocol and have read, reviewed, edited, and approved the final manuscript.

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Competing interests: None declared.

Patient consent for publication: Not required.

Data statement : Technical appendix, statistical code, and dataset available from the corresponding author on a valid request

Provenance and peer review: Not commissioned

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40 Figures -

41 Figure 4 Natural history of nonalcoholic fatty liver disease

42 NAFLD - nonalcoholic fatty liver disease, NASH - nonalcoholic steatohepatitis, HCC –
43 hepatocellular carcinoma
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48 Figure 5 Liver stiffness cut-offs in chronic liver diseases

49 kPa - kilo Pascal
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54 Figure 6 Study design and participant flow through the study

55 FIB-4 score - fibrosis 4 score, LSM - liver stiffness measure, kPa - kilo Pascal
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For peer review only

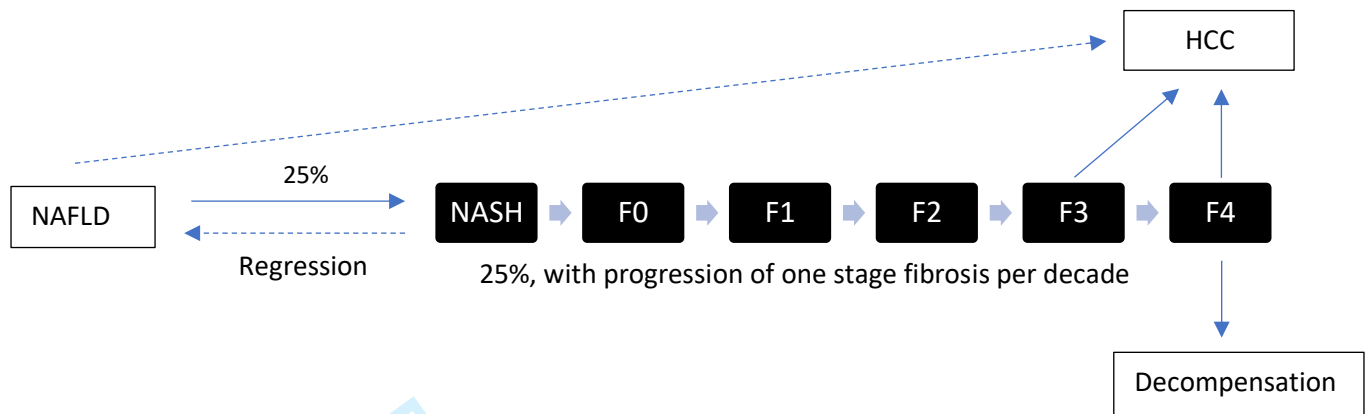


Figure 1 Natural history of nonalcoholic fatty liver disease

NAFLD - nonalcoholic fatty liver disease, NASH - nonalcoholic steatohepatitis, HCC – hepatocellular carcinoma

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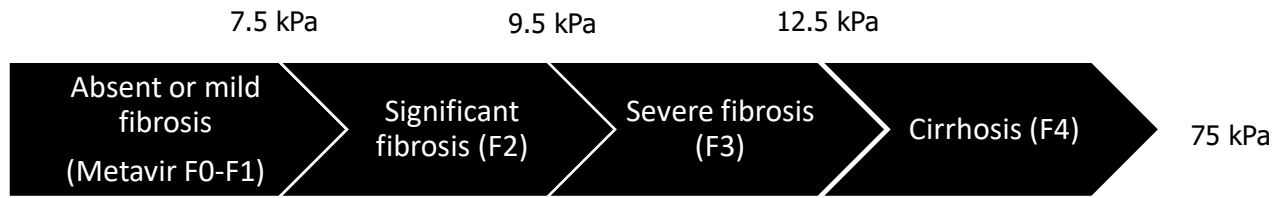


Figure 2 Liver stiffness cut-offs in chronic liver diseases

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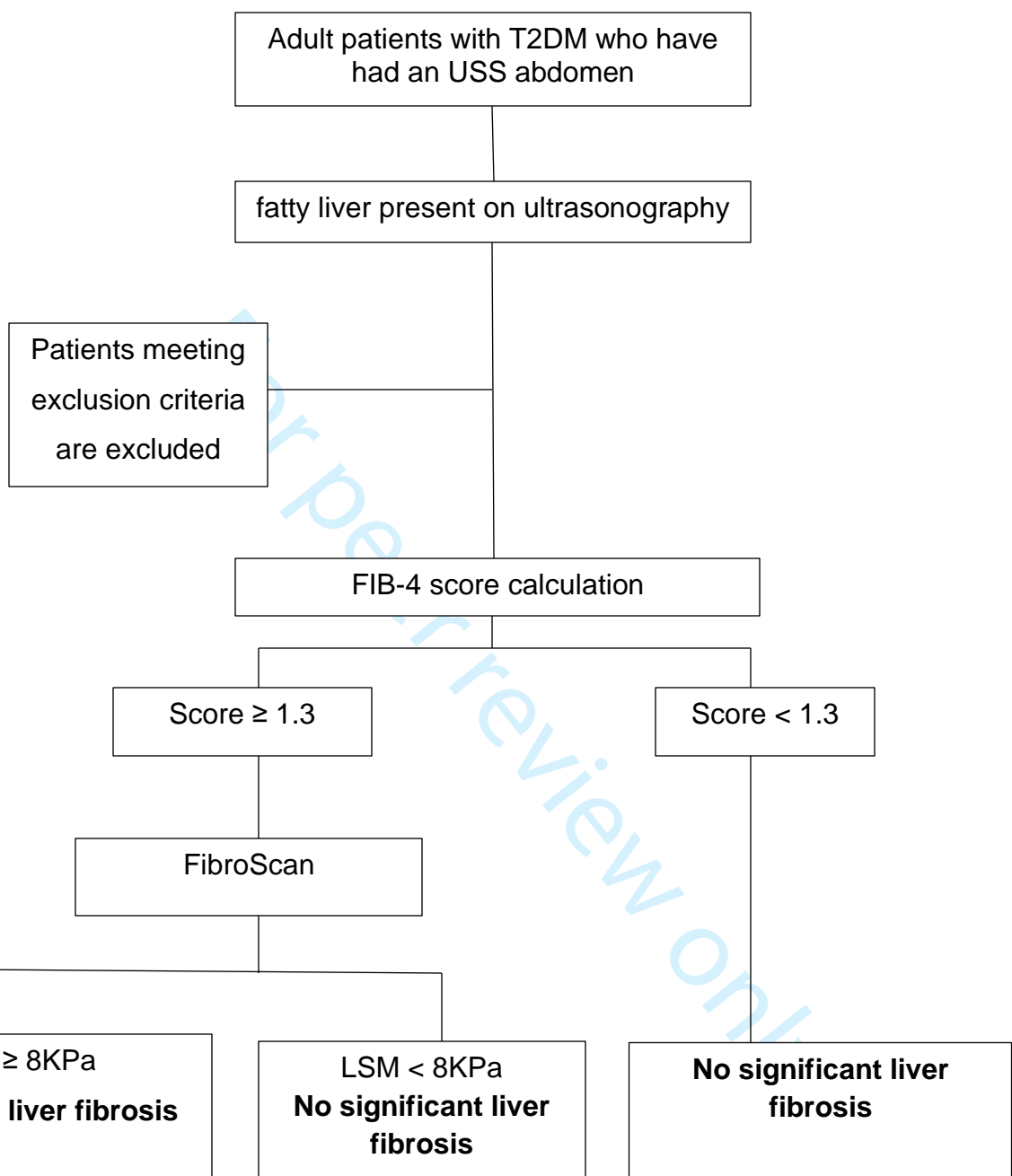


Figure 3 Study design and participant flow through the study

STROBE Statement—Checklist of items that should be included in reports of *cross-sectional studies*

	Item No	Recommendation	Page No
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract	1,2
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any prespecified hypotheses	6
Methods			
Study design	4	Present key elements of study design early in the paper	6
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	6,7
Participants	6	(a) Give the eligibility criteria, and the sources and methods of selection of participants	6.
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	8
Data sources/measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	7,8
Bias	9	Describe any efforts to address potential sources of bias	7
Study size	10	Explain how the study size was arrived at	7
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	8
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	8
		(b) Describe any methods used to examine subgroups and interactions	8
		(c) Explain how missing data were addressed	
		(d) If applicable, describe analytical methods taking account of sampling strategy	
		(e) Describe any sensitivity analyses	8
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	7,8
		(b) Give reasons for non-participation at each stage	NA
		(c) Consider use of a flow diagram	8
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	NA
		(b) Indicate number of participants with missing data for each variable of interest	NA
Outcome data	15*	Report numbers of outcome events or summary measures	NA
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	NA

		(b) Report category boundaries when continuous variables were categorized	
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	NA
Discussion			
Key results	18	Summarise key results with reference to study objectives	NA
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	3, 10
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	NA
Generalisability	21	Discuss the generalisability (external validity) of the study results	NA
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	10

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

Identification of patients with type 2 diabetes with non-alcoholic fatty liver disease who are at increased risk of progressing to advanced fibrosis: A cross-sectional study

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2022-063959.R1
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Primary Subject Heading:	Gastroenterology and hepatology
Secondary Subject Heading:	Gastroenterology and hepatology, Diabetes and endocrinology
Keywords:	General diabetes < DIABETES & ENDOCRINOLOGY, General endocrinology < DIABETES & ENDOCRINOLOGY, Hepatology < INTERNAL MEDICINE, Gastroenterology < INTERNAL MEDICINE

SCHOLARONE™
Manuscripts

Title Page

Title of the article - Identification of patients with type 2 diabetes with non-alcoholic fatty liver disease who are at increased risk of progressing to advanced fibrosis: A cross-sectional study

Full name, postal address and e-mail of the corresponding author –

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Word count, excluding title page, abstract, references, figures and tables

2095

1
2 35 **Abstract:**
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4 36
5 37 **Introduction;**
6
7 38 Identification of advanced hepatic fibrosis in non-alcoholic fatty liver disease (NAFLD) is
8
9 39 important as this may progress to cirrhosis and hepatocellular carcinoma. The risk of
10
11 40 hepatic fibrosis is especially high among patients with diabetes with NAFLD. Annual
12
13 41 screening of patients with diabetes for fatty liver and calculation of FIB-4 score and
14
15 42 exclusion of significant fibrosis with vibration controlled transient elastography (VCTE) has
16
17 43 been recommended. However, VCTE is expensive and may not be freely available in
18
19 44 resource-limited settings. We aim to identify predictors of significant liver fibrosis who are at
20
21 45 increased risk of progression to advanced liver fibrosis and to develop a prediction model to
22
23 46 prioritize referral of patients with diabetes and NAFLD for VCTE.
24

25 47
26 48 **Methods and analysis;**

27 49 This cross-sectional study is conducted among all consenting adults with T2DM with
28
29 50 NAFLD at the Colombo North Teaching Hospital, Ragama, Sri Lanka. All patients get the
30
31 51 FIB-4 score calculated. Those with FIB-4 ≥ 1.3 undergo VCTE (with FibroScan® by
32
33 52 Echosens). Risk associations for progression to advanced liver fibrosis/cirrhosis will be
34
35 53 identified by comparing patients with significant fibrosis (LSM ≥ 8 kPa) and without
36
37 54 significant fibrosis (LSM < 8 kPa). A model to predict significant liver fibrosis will be
38
39 55 developed using logistic regression.
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41 56
42 57 **Ethics and dissemination.** Ethical approval has been obtained from the Ethics Committee
43
44 58 of the Faculty of Medicine, University of Kelaniya (P/66/07/2021). Results of the study will
45
46 59 be disseminated as scientific publications in reputed journals.
47

48 60
49 61 **Keywords:** Nonalcoholic Fatty liver disease, NAFLD, Diabetes mellitus, Prediction,
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51 62 significant liver fibrosis, cross-sectional study, vibration controlled transient elastography
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1
2 67 **Article Summary**
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4 68 **Strengths**
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- 6 69 • Individual data of patients are used
7
8 70 • Advanced fibrosis is confirmed with VCTE (with FibroScan® by Echosens)
9
10 71 • Risk factors for advanced fibrosis out of over 100 variables will be selected
11
12 72 • All possible risk factors will be studied without prejudice and the risk factors with the
13 73 highest predictive values will be used in the prognostic model
14
15 74

16 75 **Limitations**
17

- 18 76 • This is limited to a Sri Lankan cohort and therefore is not generalizable to all other
19 77 nations
20
21 78 • This cohort is of a community of very low seroprevalence of Hep B and C. However,
22 79 we have not studied Hepatitis B and C serology in individual patients.
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81 Main text

82

83 Introduction

84

85 Non-alcoholic fatty liver disease (NAFLD) is the most common liver disease in the world
86 with a global prevalence of 25%, with the highest rates in the Middle East and South
87 America and the lowest in Africa (1-3). NAFLD is a spectrum ranging from simple
88 steatosis, nonalcoholic steatohepatitis (NASH) to cirrhosis (Figure 1)(4). NASH is strongly
89 associated with liver fibrosis and is defined histologically on a scale ranging from F0 to F4.
90 Stages F0 to F1 have no or minimal fibrosis, F2 is significant fibrosis with risk of
91 progression to advanced fibrosis, F3 is advanced fibrosis and F4 cirrhosis (Figure 2) (5).
92 Fibrosis of the liver can be measured non-invasively with vibration-controlled transient
93 elastography (VCTE) with FibroScan® by Echosens which measures liver stiffness. A liver
94 stiffness measure (LSM) between 2.5 - 7 kPa is suggestive of absent or mild fibrosis, ≥ 7.1
95 kPa significant fibrosis ($\geq F2$), ≥ 9.5 kPa advanced fibrosis ($\geq F3$) and ≥ 12.5 kPa, cirrhosis
96 (F=4)(5, 6).

97

98 NAFLD is a dynamic condition that can regress to simple steatosis with liver-directed
99 therapy, smoulder at a relatively constant level of activity, or cause progressive fibrosis and
100 lead to cirrhosis. However, the majority of NAFLD patients have a benign course and only
101 10% develop progressive fibrosis leading to cirrhosis and hepatocellular carcinoma (HCC)
102 (7, 8). There is no widely accepted pharmacological treatment for established cirrhosis and
103 the only curative treatment is liver transplantation. However, progressive NASH and its
104 complications such as cirrhosis could be delayed or prevented if people with increased risk
105 of progressive fibrosis are detected early in the course of the disease (at F2-3 stage or
106 LSM ≤ 8 kPa) and liver-directed therapies are initiated. This is the key to reducing the liver-
107 related burden of NAFLD in both high- and low-income countries (9).

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110 *Figure 1*

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Figure 2

NAFLD is the hepatic manifestation of insulin resistance, which is the hallmark of type 2 diabetes mellitus (T2DM) (10). It is three times more prevalent among patients with diabetes than in the general population (11). Globally, 55.5% of patients with T2DM and 62% of Asians with T2DM have NAFLD (12) (13). On the other hand, NAFLD is considered a risk factor for T2DM (14, 15), and NAFLD patients have a two-fold increased risk of developing T2DM compared to those without NAFLD (16). Diabetes is independently associated with the degree of steatosis, NAFLD progression to NASH, advanced fibrosis, and the development of HCC (17-20). The progression of NAFLD to advanced fibrosis is two times higher among patients with diabetes than in the general population. Around 20% of T2DM patients with NAFLD progress to advanced liver fibrosis over a mean of 5.9 years (9)(21). Therefore, both the American Diabetes Association(2020) (22) and the European Association for the Study of the Liver(2016) (23) recommend screening of all T2DM patients for NAFLD to prevent liver-related complications of NAFLD. There are two proposed algorithms for screening patients with diabetes for NAFLD, and both propose annual screening of patients with diabetes with NAFLD but using two different screening tools; one using the Fibrosis-4 score (FIB-4) (24, 25) and the other using the NAFLD fibrosis score (NFS)(26, 27). The proposed algorithm involves two steps. The first is an annual screening of patients with diabetes with NAFLD and the second step is liver stiffness measurement (LSM) using transient elastography (26) for those with FIB-4 ≥ 1.3 (25) or NFS between -1.455 to 0.676 (26). The patients at high risk of advanced fibrosis/cirrhosis with an LSM ≥ 8 kPa are advised referral to specialized liver centres for further assessment and management (25, 28) (29-31).

Diabetes has become an epidemic in low-middle-income countries including those in Asia. Of the world's population with diabetes, 60% are Asian (32). Diabetes is on the rise among Asians due to urbanization, changing to Western lifestyles and dietary habits in addition to a relatively high genetic predisposition (33, 34). Asians are at higher risk of developing T2DM compared with people of European ancestry with genetic predisposition through PNPLA3 SNPs and polymorphisms in apolipoprotein (32). However, VCTE is expensive and is not freely available for all patients with diabetes in low-middle income countries.

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4 146 Therefore, we aimed to identify clinical predictors of significant liver fibrosis with risk of
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6 147 progression to advanced liver fibrosis (i.e.: LSM \geq 8 kPa) (29)) in patients with type 2
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8 148 diabetes who are at risk of progression to advanced liver fibrosis and cirrhosis, and develop
9
10 149 a model to identify patients at high risk of progression to advanced fibrosis. This we hope
11
12 150 will help to guide clinicians in primary and secondary care in resource-poor settings to
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14 151 prioritize referrals for VCTE and to specialized liver centres.
15

16 153 **Methods and analysis**

17 154 **Study design and setting**

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19 155 This study is an ongoing cross-sectional study to identify patients with diabetes with NAFLD
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21 156 who are at risk of progression to advanced liver fibrosis and/or cirrhosis. We compare the
22
23 157 risk associations of patients with advanced liver fibrosis diagnosed by an LSM \geq 8 kPa by
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25 158 transient elastography and patients with no or minimal liver fibrosis diagnosed by FIB-4
26
27 159 score $<$ 1.3. The study is being conducted at the Colombo North Teaching Hospital,
28
29 160 Ragama, Sri Lanka.
30

31 162 **Study objectives**

32
33 163 To identify patients with diabetes with NAFLD who are at increased risk of progression to
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35 164 advanced liver fibrosis/cirrhosis and to develop a prediction model for the same. We further
36
37 165 plan to validate the FIB-4 score among Sri Lankans.
38

39 166 40 167 **Study population and eligibility criteria**

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42 168 All adult patients with T2DM attending Medical/Diabetes Clinics of three Consultants at
43
44 169 three private sector hospitals of the Gampaha District of Sri Lanka over 12 consecutive
45
46 170 months who have ultrasonographic evidence of NAFLD will be the study population.
47

48 171 49 172 **Inclusion criteria**

- 50 173
- 51 174 • Patients with confirmed T2DM
 - 52 175 • Patients who are aged over 18 years
 - 53 176 • Patients who have ultrasonic (US) evidence of fatty liver in a US scan done within the
54 previous 3 months of recruitment
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Exclusion criteria

- 179 • Patients without consent
- 180 • Patients with established cirrhosis on US scan
- 181 • Males consuming alcohol > 14 units/week and females consuming alcohol > 7
- 182 units/week
- 183 • Evidence of Hepatitis B or C infection
- 184 • Patients with diagnosed liver diseases of known aetiology other than NAFLD
- 185 • Patients on medications known to cause fatty liver or liver fibrosis e.g., tamoxifen,
- 186 methotrexate etc.

187

Sample size

189 The sample size was calculated for developing a clinical prediction model (35, 36)
190 assuming an R-squared value of 0.2 for the logistic model, 10 parameters in the model, and
191 0.05 acceptable difference in apparent & adjusted R-squared, 0.05 margin of error in the
192 estimation of intercept and an anticipated prevalence of advanced liver fibrosis of 15%
193 among T2DM with NAFLD (37).

194

195 The calculated minimum sample size is 398 patients with T2DM with NAFLD (among whom
196 60 are expected to have significant fibrosis, i.e.: LSM \geq 8 kPa). This is likely to provide 6
197 events per Predictor Parameter (EPP).

198

Patient recruitment -

200 Consecutive adult patients (>18 years) with T2DM attending Medical/Diabetes Clinics of
201 three Consultants at three private sector hospitals of the Gampaha District of Sri Lanka
202 over 12 consecutive months starting from November 2021 are being screened and the
203 eligible patients are recruited to this study after being referred to the Gastroenterology and
204 Hepatology clinic of the Colombo North Teaching Hospital Ragama until the sample size is
205 achieved (Figure 3). All eligible patients are given a patient information sheet to read and
206 time to clarify doubts with investigators before consenting. The participants will be informed
207 about the study including the data collection procedure and that a subgroup of participants
208 will undergo a non-invasive VCTE of the liver free of charge. The patients will be made
209 aware of the ability to withdraw consent at any point without having to give reasons.

1
2 210 Informed written consent from all participants will be obtained before recruiting into the
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4 211 study. Permission has been obtained from the director of the North Colombo Teaching
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6 212 Hospital and the consultant in charge of the Diabetic and Endocrine clinics to carry out the
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8 213 study.

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12 216 Figure 3

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19 220 Study procedure

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21 221 Patients with diabetes mellitus and complying with inclusion and exclusion criteria will be
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23 222 interviewed and medical records will be assessed. Data will be collected using an
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25 223 interviewer-administered questionnaire. Information on demography, history of metabolic
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27 224 risk factors, medications, diet, and exercise will be recorded. Haematological and
28
29 225 biochemical investigations done within 3 months before recruitment will be extracted from
30
31 226 clinic records. Anthropometry: height, weight and waist circumference will be measured at
32
33 227 recruitment. FIB-4 score will be calculated for all patients using age, sex, and the most
34
35 228 recent AST, ALT and platelet count done within 3 months of recruitment to the study. All
36
37 229 patients with a FIB-4 score ≥ 1.3 will undergo VCTE of the liver free of charge by a single,
38
39 230 trained medical officer. LSM and CAP measures will be recorded. A subset of patients with
40
41 231 a FIB-4 score <1.3 will also undergo VCTE to confirm the validity of the FIB-4 score to
42
43 232 exclude significant liver fibrosis.

44 233

45 234 Statistical analysis

46
47 235 IBM SPSS 22.0 software will be used for data management and analyses. Multiple logistic
48
49 236 regression will be used to identify factors associated with significant and beyond liver
50
51 237 fibrosis. A model will be developed using the identified risk factors to predict “significant
52
53 238 liver fibrosis” according to their weighted scores (β -coefficient). Cut-off points and the
54
55 239 sensitivity and specificity of predictions will be determined using ROC curves. The
56
57 240 predictions of the new model will be compared with the predictions of the simple FIB-4
58
59 241 score.

60 242

243 Data management and monitoring

244 All completed questionnaires and VCTE reports will be stored securely. An electronic
245 screening log and database will be maintained as a password-protected file.

247 Ethical considerations

248 This is not an interventional study and is associated with no risks to the patients. Selected
249 patients will undergo non-invasive VCTE of the liver free of charge at the North Colombo
250 Teaching Hospital which is the only state hospital with a fibro scanner in Sri Lanka. There
251 are no risks associated with this scan. The results of the scan will only be divulged to the
252 treating physician of the patients for initiation of relevant treatment options. The findings of
253 the study could be beneficial to all patients with diabetes in the early diagnosis/prediction of
254 significant liver fibrosis in individuals. Participants will have the right to withdraw from the
255 study at any point without providing explanations. Ethical approval for the study has been
256 obtained from the Ethics Committee of the Faculty of Medicine, University of Kelaniya, Sri
257 Lanka (Ref. P/66/07/2021).

259 Termination of the study

260 The study will be terminated if:

- 261 • A new and cost-effective tool to predict significant liver fibrosis that changes current
262 guidelines become available.
- 263 • Significant violation of good clinical practice that compromises the ability to achieve
264 study objectives or compromises subject safety occurs.

266 Study status

267 The trial commenced in November 2021 according to the protocol version 2.0, 06 August
268 2021 and is currently open for recruitment. We have recruited 220 patients for the study so
269 far.

271 Patient and public involvement

272 Reports of the VCTE (LSM) done as part of the trial will be available to all participants who
273 request it and will be used in the standard management when required. All patients are
274 given a health education leaflet on Fatty Liver and secondary prevention. Results of the
275 VCTE are notified to the treating physician for necessary action. The results of the study

1
2 276 will be disseminated to study participants and other patients with diabetes and fatty liver
3
4 277 using patient education leaflets and lectures after completion of the study.

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7 279 **Discussion**

9 280 In this study, we aim to develop a practical, cost-effective model to predict patients with
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11 281 diabetes with NAFLD who are at increased risk of progressing to advanced liver fibrosis
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13 282 and/or cirrhosis to target those that require transient elastography. Furthermore, even
14
15 283 though there are non-invasive markers of liver fibrosis such as FIB-4 (24), BRAD (38), and
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17 284 NFS (27), none of these has been developed specifically for patients with diabetes and has
18
19 285 been validated in Asian except among Japanese (39).

20 286

21 287 Apart from a few studies from North India (40) and Vietnam (41), there are no reports of
22
23 288 VCTE data in patients with diabetes from Asia. There is no data on VCTE from Sri Lanka.
24
25 289 Furthermore, the FIB-4 score has not been validated among Sri Lankans. Through our
26
27 290 study, we hope to provide a low-cost, practical model to identify the patients with type 2
28
29 291 diabetes and NAFLD who are at increased risk of progression to advanced
30
31 292 fibrosis/cirrhosis.

32 293

33 294 **Limitations**

35 295 Liver biopsy is the gold standard for staging liver fibrosis but in our study, VCTE will be
36
37 296 used to diagnose significant liver fibrosis (42). However, liver biopsy is an invasive
38
39 297 procedure and current practice guidelines recommend VCTE as a surrogate to exclude
40
41 298 advanced fibrosis, with liver biopsy reserved for those with equivocal VCTE results (25, 26).

42 299

44 300 **Author Contributions:** CM conceived the study. CM,TE, CD, RF,NL, LR, CR, DK, AP
45
46 301 JdeS and AD contributed to the study design. CM drafted the manuscript and AD, and
47
48 302 JdeS significantly edited the manuscript. All authors assisted in developing the protocol and
49
50 303 have read, reviewed, edited, and approved the final manuscript.

51 304

52
53 305 **Funding:** This research received no specific grant from any funding agency in the public,
54
55 306 commercial or not-for-profit sectors

56 307

1
2 308 **Competing interests:** None declared.

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5 310 **Patient consent for publication:** Not required.

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9 312 **Data statement :** Technical appendix, statistical code, and dataset available from the
10
11 313 corresponding author on a valid request

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15 315 **Provenance and peer review:** Not commissioned

16
17 316

18 317 **Acknowledgement :** Jayani Manchanayake and Dileepa Ediriweera for helping in
19
20 318 developing the protocol.

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27 322 **References**

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8 447 Figures -

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10 448 Figure 4 Natural history of nonalcoholic fatty liver disease

11 449 NAFLD - nonalcoholic fatty liver disease, NASH - nonalcoholic steatohepatitis, HCC –
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13 450 hepatocellular carcinoma

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16 452 Figure 5 Liver stiffness cut-offs in chronic liver diseases

17 453 kPa - kilo Pascal

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21 455 Figure 6 Study design and participant flow through the study

22 456 FIB-4 score - fibrosis 4 score, LSM - liver stiffness measure, kPa - kilo Pascal

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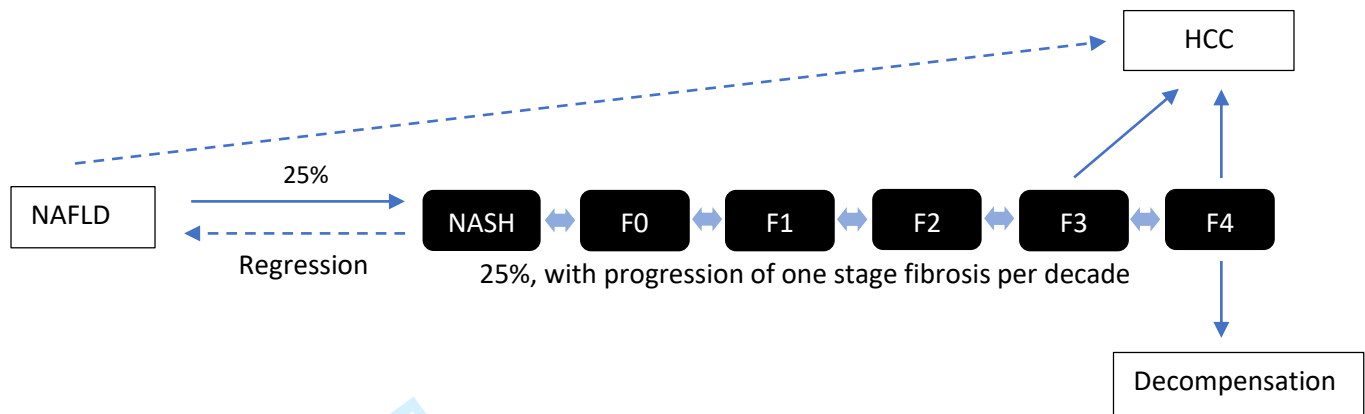


Figure 1 Natural history of nonalcoholic fatty liver disease

NAFLD - nonalcoholic fatty liver disease, NASH - nonalcoholic steatohepatitis, HCC – hepatocellular carcinoma

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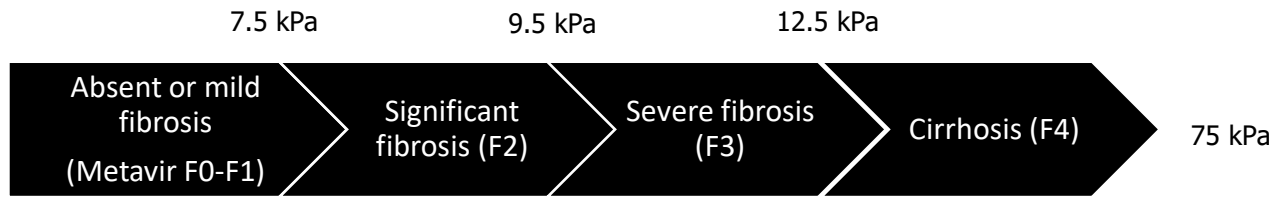


Figure 2 Liver stiffness cut-offs in chronic liver diseases

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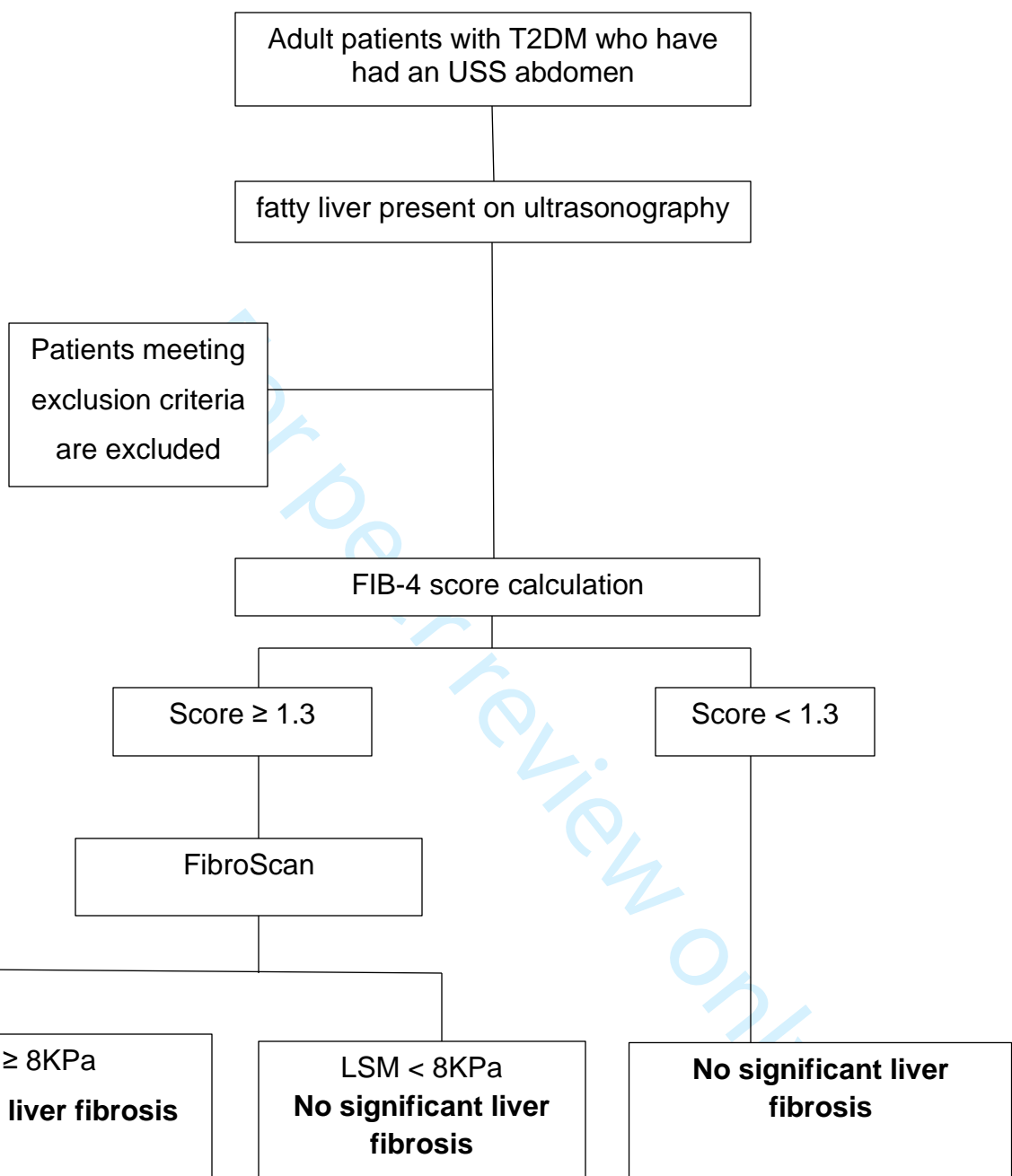


Figure 3 Study design and participant flow through the study

STROBE Statement—Checklist of items that should be included in reports of *cross-sectional studies*

	Item No	Recommendation	Page No
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract	1,2
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any prespecified hypotheses	6
Methods			
Study design	4	Present key elements of study design early in the paper	6
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	6,7
Participants	6	(a) Give the eligibility criteria, and the sources and methods of selection of participants	6.
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	8
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	7,8
Bias	9	Describe any efforts to address potential sources of bias	7
Study size	10	Explain how the study size was arrived at	7
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	8
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	8
		(b) Describe any methods used to examine subgroups and interactions	8
		(c) Explain how missing data were addressed	
		(d) If applicable, describe analytical methods taking account of sampling strategy	
		(e) Describe any sensitivity analyses	8
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	7,8
		(b) Give reasons for non-participation at each stage	NA
		(c) Consider use of a flow diagram	8
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	NA
		(b) Indicate number of participants with missing data for each variable of interest	NA
Outcome data	15*	Report numbers of outcome events or summary measures	NA
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	NA

		(b) Report category boundaries when continuous variables were categorized	
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	NA
Discussion			
Key results	18	Summarise key results with reference to study objectives	NA
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	3, 10
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	NA
Generalisability	21	Discuss the generalisability (external validity) of the study results	NA
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	10

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at www.strobe-statement.org.