BMJ Open

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

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2022-063959
Article Type:	Protocol
Date Submitted by the Author:	19-May-2022
Complete List of Authors:	Mettananda, Chamila; University of Kelaniya, Department of Pharmacology Egodage, Thimira; University of Kelaniya Faculty of Medicine, Department of Pharmacology, Clinical Trials Unit Dantanarayana, Channaka; University of Kelaniya Faculty of Medicine, Department of Pharmacology, Clinical Trials Unit Fernando, Rumal; Noth Colombo Teaching Hospital Ranaweera, Lakmali; Noth Colombo Teaching Hospital Luke, Nathasha; University of Kelaniya, Department of Pharmacology Ranawaka, Chamila; Noth Colombo Teaching Hospital Kottahachchi, Dulani; University of Kelaniya Faculty of Medicine, Department of Physiology Pathmeswaran, Arunasalam; University of Kelaniya Faculty of Medicine, Public Health de Silva, Hithanadura; University of Kelaniya Faculty of Medicine, Medicine Dassanayake, Anuradha; University of Kelaniya Faculty of Medicine, Pharmacology
Keywords:	Hepatology < INTERNAL MEDICINE, Other metabolic, e.g. iron, porphyria < DIABETES & ENDOCRINOLOGY, Gastroenterology < INTERNAL MEDICINE

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

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

Chamila Mettananda

Department of Pharmacology, Faculty of Medicine, University of Kelaniya, Ragama, Sri Lanka

chamila@kln.ac.lk

Full name, department, institution, city and country of all co-authors

- Chamila Mettananda, Department of Pharmacology, University of Kelaniya, Ragama, Sri Lanka
- Thimira Egodage, Department of Pharmacology, University of Kelaniya, Ragama, Sri Lanka
- Channaka Dantanarayana, Department of Pharmacology, University of Kelaniya, Ragama, Sri Lanka
- Rumal Fernando, North Colombo Teaching Hospital, Ragama, Sri Lanka
- Lakmali Ranaweera, North Colombo Teaching Hospital, Ragama, Sri Lanka
- Nathasha Luke, Department of Pharmacology, University of Kelaniya, Ragama, Sri Lanka
- Chamila Ranawaka, North Colombo Teaching Hospital, Ragama, Sri Lanka
- Dulani Kottahachchi, Department of Physiology, University of Kelaniya, Ragama, Sri Lanka
- Arunasalam Pathmeswaran, Department of Public Health, University of Kelaniya, Ragama, Sri Lanka
- Janaka de Silva, Department of Medicine, University of Kelaniya, Ragama, Sri Lanka
- Anuradha Dassanayake, Department of Pharmacology, University of Kelaniya, Ragama, Sri Lanka

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

1986

Abstract:

Introduction;

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

Methods and analysis;

This cross-sectional study will be conducted among all consenting adults with T2DM with NAFLD at the Colombo North Teaching Hospital, Ragama, Sri Lanka. All patients will have the FIB-4 score calculated. Those with FIB-4 ≥1.3 will undergo VCTE (with FibroScan® by Echosens). Risk associations for progression to advanced liver fibrosis/cirrhosis will be identified by comparing patients with significant fibrosis (LSM ≥8 kPa) and without significant fibrosis (FIB-4<1.3). A model to predict significant liver fibrosis will be developed using logistic regression.

Ethics and dissemination. Ethical approval has been obtained from the Ethics Committee of the Faculty of Medicine, University of Kelaniya (P/66/07/2021). Results of the study will be disseminated as scientific publications in reputed journals.

Keywords: Nonalcoholic Fatty liver disease, NAFLD, Diabetes mellitus, Prediction, significant liver fibrosis, cross-sectional study, vibration controlled transient elastography

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, F 3 is advanced fibrosis and F 4 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 ≥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

NAFLD is the hepatic manifestation of insulin resistance, which is the hallmark of type 2 diabetes mellitus (T2DM) (9). It is three times more prevalent among diabetics than in the general population (10). Globally, 55.5% of patients with T2DM and 62% of Asians with T2DM have NAFLD (11) (12). On the other hand, NAFLD is considered a risk factor for T2DM (13, 14), and NAFLD patients have a two-fold increased risk of developing T2DM compared to those without NAFLD (15). Diabetes is independently associated with the degree of steatosis, NAFLD progression to NASH, advanced fibrosis, and the development of HCC (16-19). The progression of NAFLD to advanced fibrosis is two times higher among diabetics 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)(20). Therefore, both the American Diabetes Association(2020) (21) and the European Association for the Study of the Liver(2016) (22) recommend screening of all T2DM patients for NAFLD to prevent liver-related complications of NAFLD. There are two proposed algorithms for screening diabetic patients for NAFLD, and both propose annual screening of diabetics with NAFLD but using two different screening tools; one using the Fibrosis-4 score (FIB-4) (23, 24) and the other using the NAFLD fibrosis score (NFS)(25, 26). The proposed algorithm involves two steps. The first is an annual screening of diabetics with NAFLD and the second step is liver stiffness measurement (LSM) using transient elastography (25) for those with FIB-4 ≥1.3 (24) or NFS between -1.455 to 0.676 (25). 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 (24, 27) (28).

Diabetes has become an epidemic in low-middle-income countries including those in Asia. Of the world's diabetic population, 60% are Asian (29). 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 (30, 31). Asians are at higher risk of developing T2DM compared with people of European ancestry with genetic predisposition through PNPLA3 SNPs and polymorphisms in apolipoprotein (29). However, VCTE is expensive and is not freely available for all diabetics in low-middle income countries.

Therefore, we aimed to identify clinical predictors of NASH (i.e.: LSM ≥8 kPa) (28)) in type 2 diabetics who are at risk of progression to advanced liver fibrosis and cirrhosis, and develop a model to identify patients at high risk of progression to advanced fibrosis. This we hope will help to guide clinicians in primary and secondary care in resource-poor settings to prioritize referrals for VCTE and to specialized liver centres.

Methods and analysis

Study design and setting

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

Study objectives

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

Study population and eligibility criteria

All adult patients 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 who have ultrasonographic evidence of NAFLD will be the study population.

Inclusion criteria

- Patients with confirmed T2DM
- Patients who are aged over 18 years
- Patients who have ultrasonic (US) evidence of fatty liver in a US scan done within the previous 3 months of recruitment

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 ≥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

Hospital and the consultant in charge of the Diabetic and Endocrine clinics to carry out the study.

Figure 3

Study procedure

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

Statistical analysis

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

Data management and monitoring

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

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.

Funding: This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors

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

Acknowledgement : Jayani Manchanayake and Dileepa Ediriweera for helping in developing the protocol.

References

1. Ekstedt M, Hagström H, Nasr P, Fredrikson M, Stål P, Kechagias S, et al. Fibrosis stage is the strongest predictor for disease-specific mortality in NAFLD after up to 33 years of follow-up. Hepatology. 2015;61(5):1547-54.
2. Younossi ZM, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global epidemiology of nonalcoholic fatty liver disease-Meta-analytic assessment of prevalence, incidence, and outcomes. Hepatology. 2016;64(1):73-84.
3. Amarapurkar DN, Hashimoto E, Lesmana LA, Sollano JD, Chen PJ, Goh KL. How common is non-alcoholic fatty liver disease in the Asia-Pacific region and are there local differences? Journal of gastroenterology and hepatology. 2007;22(6):788-93.
4. Diehl AM, Day C. Cause, Pathogenesis, and Treatment of Nonalcoholic Steatohepatitis. N Engl J Med. 2017;377(21):2063-72.
5. Castera L, Forns X, Alberti A. Non-invasive evaluation of liver fibrosis using transient elastography. J Hepatol. 2008;48(5):835-47.
6. Brunt EM, Janney CG, Di Bisceglie AM, Neuschwander-Tetri BA, Bacon BR. Nonalcoholic steatohepatitis: a proposal for grading and staging the histological lesions. Am J Gastroenterol. 1999;94(9):2467-74.
7. Diehl AM, Day C. Cause, Pathogenesis, and Treatment of Nonalcoholic Steatohepatitis. New England Journal of Medicine. 2017;377(21):2063-72.
8. Eslam M, Sanyal AJ, George J. MAFLD: A Consensus-Driven Proposed Nomenclature for Metabolic Associated Fatty Liver Disease. Gastroenterology. 2020;158(7):1999-2014.e1.
9. Alkhouri N, Poordad F, Lawitz E. Management of nonalcoholic fatty liver disease: Lessons learned from type 2 diabetes. Hepatology Communications. 2018;2(7):778-85.
10. Saponaro C, Gaggini M, Gastaldelli A. Nonalcoholic fatty liver disease and type 2 diabetes: common pathophysiologic mechanisms. Curr Diab Rep. 2015;15(6):607.
11. Younossi ZM, Golabi P, de Avila L, Paik JM, Srishord M, Fukui N, et al. The global epidemiology of NAFLD and NASH in patients with type 2 diabetes: A systematic review and meta-analysis. J Hepatol. 2019;71(4):793-801.

12. Herath HMM, Kodikara I, Weerarathna TP, Liyanage G. Prevalence and associations of non-alcoholic fatty liver disease (NAFLD) in Sri Lankan patients with type 2 diabetes: A single center study. Diabetes Metab Syndr. 2019;13(1):246-50.
13. Adams LA, Waters OR, Knuiman MW, Elliott RR, Olynyk JK. NAFLD as a risk factor for the development of diabetes and the metabolic syndrome: an eleven-year follow-up study. Am J Gastroenterol. 2009;104(4):861-7.
14. Shibata M, Kihara Y, Taguchi M, Tashiro M, Otsuki M. Nonalcoholic Fatty Liver Disease Is a Risk Factor for Type 2 Diabetes in Middle-Aged Japanese Men. Diabetes Care. 2007;30(11):2940-4.
15. Kasturiratne A, Weerasinghe S, Dassanayake AS, Rajindrajith S, de Silva AP, Kato N, et al. Influence of non-alcoholic fatty liver disease on the development of diabetes mellitus. Journal of gastroenterology and hepatology. 2013;28(1):142-7.
16. Hossain N, Afendy A, Stepanova M, Nader F, Srishord M, Rafiq N, et al. Independent predictors of fibrosis in patients with nonalcoholic fatty liver disease. Clin Gastroenterol Hepatol. 2009;7(11):1224-9, 9.e1-2.
17. Vernon G, Baranova A, Younossi ZM. Systematic review: the epidemiology and natural history of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in adults. Aliment Pharmacol Ther. 2011;34(3):274-85.
18. Loomba R, Abraham M, Unalp A, Wilson L, Lavine J, Doo E, et al. Association between diabetes, family history of diabetes, and risk of nonalcoholic steatohepatitis and fibrosis. Hepatology. 2012;56(3):943-51.
19. Fracanzani AL, Valenti L, Bugianesi E, Andreoletti M, Colli A, Vanni E, et al. Risk of severe liver disease in nonalcoholic fatty liver disease with normal aminotransferase levels: a role for insulin resistance and diabetes. Hepatology. 2008;48(3):792-8.
20. Singh S, Allen AM, Wang Z, Prokop LJ, Murad MH, Loomba R. Fibrosis progression in nonalcoholic fatty liver vs nonalcoholic steatohepatitis: a systematic review and meta-analysis of paired-biopsy studies. Clin Gastroenterol Hepatol. 2015;13(4):643-54.e1-9; quiz e39-40.
21. Association AD. 4. Comprehensive Medical Evaluation and Assessment of Comorbidities: Standards of Medical Care in Diabetes—2020. Diabetes Care. 2019;43(Supplement_1):S37-S47.
22. EASL-EASD-EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease. J Hepatol. 2016;64(6):1388-402.
23. Shah AG, Lydecker A, Murray K, Tetri BN, Contos MJ, Sanyal AJ. Comparison of noninvasive markers of fibrosis in patients with nonalcoholic fatty liver disease. Clin Gastroenterol Hepatol. 2009;7(10):1104-12.
24. Vieira Barbosa J, Lai M. Nonalcoholic Fatty Liver Disease Screening in Type 2 Diabetes Mellitus Patients in the Primary Care Setting. Hepatology Communications. 2021;5(2):158-67.
25. Castera L, Vilgrain V, Angulo P. Noninvasive evaluation of NAFLD. Nature Reviews Gastroenterology & Hepatology. 2013;10(11):666-75.
26. Angulo P, Hui JM, Marchesini G, Bugianesi E, George J, Farrell GC, et al. The NAFLD fibrosis score: a noninvasive system that identifies liver fibrosis in patients with NAFLD. Hepatology. 2007;45(4):846-54.
27. Petroni ML, Brodosi L, Bugianesi E, Marchesini G. Management of non-alcoholic fatty liver disease. BMJ (Clinical research ed). 2021;372:m4747.
28. Li Q, Huang C, Xu W, Hu Q, Chen L. Accuracy of FibroScan in analysis of liver fibrosis in patients with concomitant chronic Hepatitis B and nonalcoholic fatty liver disease. Medicine. 2020;99(23).

29. Chan JCN, Malik V, Jia W, Kadowaki T, Yajnik CS, Yoon K-H, et al. Diabetes in Asia: Epidemiology, Risk Factors, and Pathophysiology. Jama. 2009;301(20):2129-40.
30. Dassanayake AS. Nonalcoholic Fatty Liver Disease: Identifying the Disease Burden in Sri Lanka. Euroasian J Hepatogastroenterol. 2018;8(1):69-72.
31. Niriella MA, Pathmeswaran A, De Silva ST, Kasturiratna A, Perera R, Subasinghe CE, et al. Incidence and risk factors for non-alcoholic fatty liver disease: A 7-year follow-up study among urban, adult Sri Lankans. Liver Int. 2017;37(11):1715-22.
32. Riley RD, Ensor J, Snell KIE, Harrell FE, Jr., Martin GP, Reitsma JB, et al. Calculating the sample size required for developing a clinical prediction model. BMJ (Clinical research ed). 2020;368:m441.
33. Joie Ensor [aut c, Emma C. Martin [aut], Richard D. Riley [aut]. pmsampsize: Calculates the Minimum Sample Size Required for Developing a Multivariable Prediction Model 2022 [1.1.2:[Available from: https://cran.r-project.org/web/packages/pmsampsize/index.html.
34. Harrison SA, Oliver D, Arnold HL, Gogia S, Neuschwander-Tetri BA. Development and validation of a simple NAFLD clinical scoring system for identifying patients without advanced disease. Gut. 2008;57(10):1441-7.
35. Sumida Y, Yoneda M, Hyogo H, Itoh Y, Ono M, Fujii H, et al. Validation of the FIB4 index in a Japanese nonalcoholic fatty liver disease population. BMC Gastroenterology. 2012;12(1):2.
36. Gupta A, Anoop S, Ansari IA, Prakash S, Misra A. High prevalence of hepatic steatosis and hepatic fibrosis in patients with type 2 diabetes mellitus. Clinical Nutrition ESPEN. 2021;46:519-26.
37. Tuong TTK, Tran DK, Phu PQT, Hong TND, Dinh TC, Chu DT. Non-Alcoholic Fatty Liver Disease in Patients with Type 2 Diabetes: Evaluation of Hepatic Fibrosis and Steatosis Using Fibroscan. Diagnostics (Basel). 2020;10(3):159.
38. Wong VW-S, Chan W-K, Chitturi S, Chawla Y, Dan YY, Duseja A, et al. Asia–Pacific Working Party on Non-alcoholic Fatty Liver Disease guidelines 2017—Part 1: Definition, risk factors and assessment. Journal of gastroenterology and hepatology. 2018;33(1):70-85.

Figures -

Figure 4 Natural history of nonalcoholic fatty liver disease

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

Figure 5 Liver stiffness cut-offs in chronic liver diseases

kPa - kilo Pascal

Figure 6 Study design and participant flow through the study

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

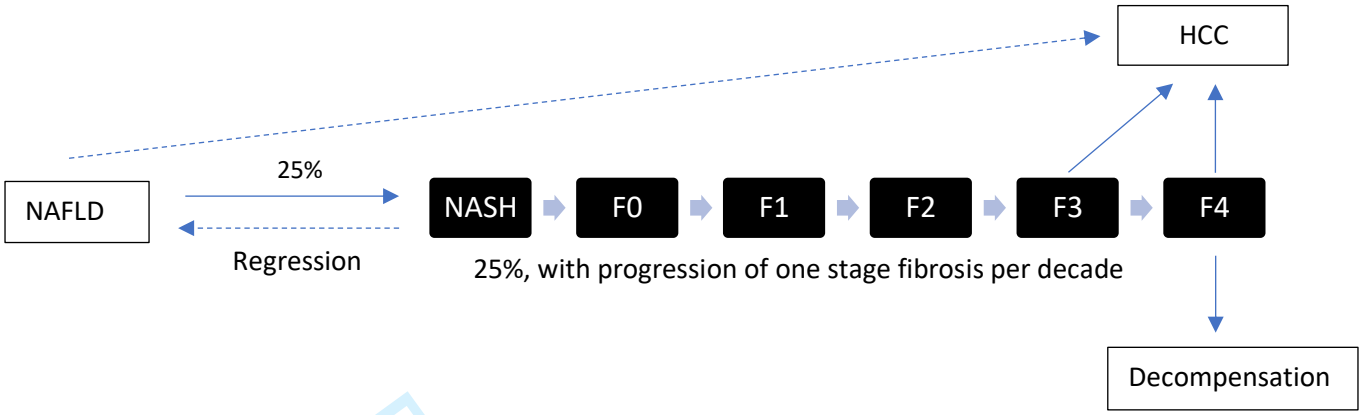

Figure 1 Natural history of nonalcoholic fatty liver disease

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

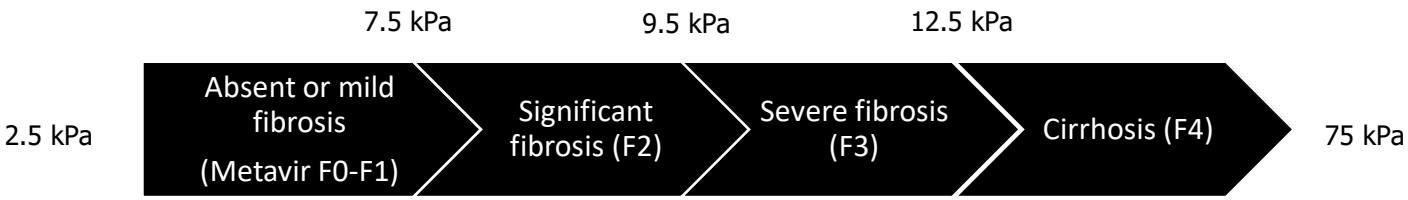

Figure 2 Liver stiffness cut-offs in chronic liver diseases

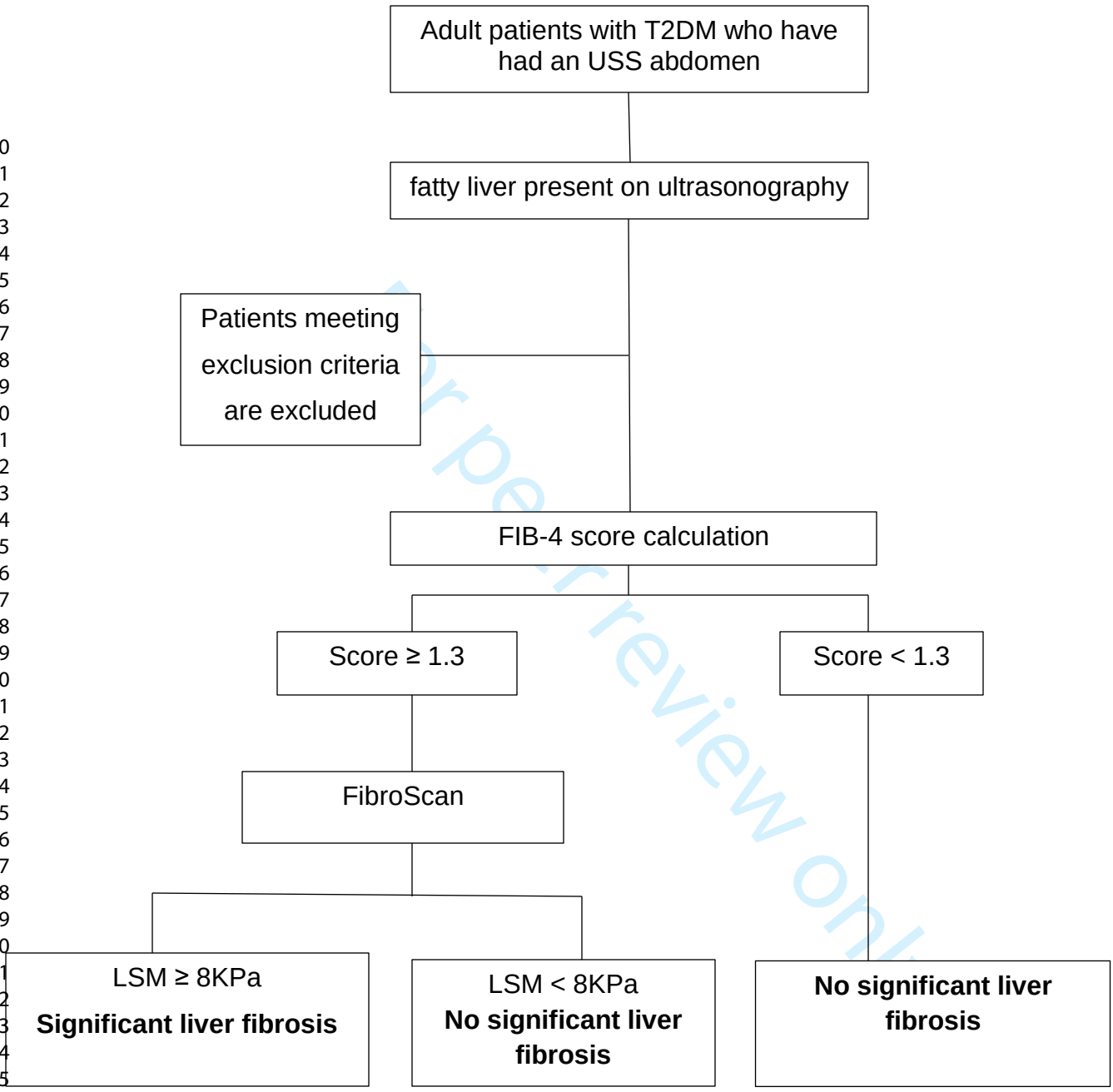

Figure 3 Study design and participant flow through the study

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:	*BMJ Open*
Manuscript ID	bmjopen-2022-063959.R1
Article Type:	Protocol
Date Submitted by the Author:	11-Nov-2022
Complete List of Authors:	Mettananda, Chamila; University of Kelaniya, Department of Pharmacology Egodage, Thimira; University of Kelaniya Faculty of Medicine, Department of Pharmacology, Clinical Trials Unit Dantanarayana, Channaka; University of Kelaniya Faculty of Medicine, Department of Pharmacology, Clinical Trials Unit Fernando, Rumal; Noth Colombo Teaching Hospital Ranaweera, Lakmali; Noth Colombo Teaching Hospital Luke, Nathasha; University of Kelaniya, Department of Pharmacology Ranawaka, Chamila; Noth Colombo Teaching Hospital Kottahachchi, Dulani; University of Kelaniya Faculty of Medicine, Department of Physiology Pathmeswaran, Arunasalam; University of Kelaniya Faculty of Medicine, Public Health de Silva, Hithanadura; University of Kelaniya Faculty of Medicine, Medicine Dassanayake, Anuradha; University of Kelaniya Faculty of Medicine, Pharmacology
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

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 –

Chamila Mettananda

Department of Pharmacology, Faculty of Medicine, University of Kelaniya, Ragama, Sri Lanka

chamila@kln.ac.lk

Full name, department, institution, city and country of all co-authors

- Chamila Mettananda, Department of Pharmacology, University of Kelaniya, Ragama, Sri Lanka
- Thimira Egodage, Department of Pharmacology, University of Kelaniya, Ragama, Sri Lanka
- Channaka Dantanarayana, Department of Pharmacology, University of Kelaniya, Ragama, Sri Lanka
- Rumal Fernando, North Colombo Teaching Hospital, Ragama, Sri Lanka
- Lakmali Ranaweera, North Colombo Teaching Hospital, Ragama, Sri Lanka
- Nathasha Luke, Department of Pharmacology, University of Kelaniya, Ragama, Sri Lanka
- Chamila Ranawaka, North Colombo Teaching Hospital, Ragama, Sri Lanka
- Dulani Kottahachchi, Department of Physiology, University of Kelaniya, Ragama, Sri Lanka
- Arunasalam Pathmeswaran, Department of Public Health, University of Kelaniya, Ragama, Sri Lanka
- Janaka de Silva, Department of Medicine, University of Kelaniya, Ragama, Sri Lanka
- Anuradha Dassanayake, Department of Pharmacology, University of Kelaniya, Ragama, Sri Lanka

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

2095

Abstract:

Introduction;

Identification of advanced hepatic fibrosis in non-alcoholic fatty liver disease (NAFLD) is important as this may progress to cirrhosis and hepatocellular carcinoma. The risk of hepatic fibrosis is especially high among patients with diabetes with NAFLD. Annual screening of patients with diabetes for fatty liver and calculation of FIB-4 score and exclusion of significant fibrosis with vibration controlled transient elastography (VCTE) has been recommended. However, VCTE is expensive and may not be freely available in resource-limited settings. We aim to identify predictors of significant liver fibrosis who are at increased risk of progression to advanced liver fibrosis and to develop a prediction model to prioritize referral of patients with diabetes and NAFLD for VCTE.

Methods and analysis;

This cross-sectional study is conducted among all consenting adults with T2DM with NAFLD at the Colombo North Teaching Hospital, Ragama, Sri Lanka. All patients get the FIB-4 score calculated. Those with FIB-4 ≥1.3 undergo VCTE (with FibroScan® by Echosens). Risk associations for progression to advanced liver fibrosis/cirrhosis will be identified by comparing patients with significant fibrosis (LSM ≥8 kPa) and without significant fibrosis (LSM <8 kPa). A model to predict significant liver fibrosis will be developed using logistic regression.

Ethics and dissemination. Ethical approval has been obtained from the Ethics Committee of the Faculty of Medicine, University of Kelaniya (P/66/07/2021). Results of the study will be disseminated as scientific publications in reputed journals.

Keywords: Nonalcoholic Fatty liver disease, NAFLD, Diabetes mellitus, Prediction, significant liver fibrosis, cross-sectional study, vibration controlled transient elastography

Article Summary

Strengths

- Individual data of patients are used
- Advanced fibrosis is confirmed with VCTE (with FibroScan® by Echosens)
- Risk factors for advanced fibrosis out of over 100 variables will be selected
- All possible risk factors will be studied without prejudice and the risk factors with the highest predictive values will be used in the prognostic model

Limitations

- This is limited to a Sri Lankan cohort and therefore is not generalizable to all other nations
- This cohort is of a community of very low seroprevalence of Hep B and C. However, we have not studied Hepatitis B and C serology in individual patients.

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 progression to advanced fibrosis, F 3 is advanced fibrosis and F 4 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 2.5 - 7 kPa is suggestive of absent or mild fibrosis, ≥ 7.1 kPa significant fibrosis (≥F2), ≥ 9.5 kPa advanced fibrosis (≥ F3) and≥12.5 kPa, cirrhosis (F=4)(5, 6).

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) (7, 8). 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 ≤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 (9).

Figure 1

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.

Therefore, we aimed to identify clinical predictors of significant liver fibrosis with risk of progression to advanced liver fibrosis (i.e.: LSM ≥8 kPa) (29)) in patients with type 2 diabetes who are at risk of progression to advanced liver fibrosis and cirrhosis, and develop a model to identify patients at high risk of progression to advanced fibrosis. This we hope will help to guide clinicians in primary and secondary care in resource-poor settings to prioritize referrals for VCTE and to specialized liver centres.

Methods and analysis

Study design and setting

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

Study objectives

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

Study population and eligibility criteria

All adult patients 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 who have ultrasonographic evidence of NAFLD will be the study population.

Inclusion criteria

- Patients with confirmed T2DM
- Patients who are aged over 18 years
- Patients who have ultrasonic (US) evidence of fatty liver in a US scan done within the previous 3 months of recruitment

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 (35, 36) 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 (37).

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

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 Hospital and the consultant in charge of the Diabetic and Endocrine clinics to carry out the study.

Figure 3

Study procedure

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

Statistical analysis

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

Data management and monitoring

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

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 at the North Colombo Teaching Hospital which is the only state hospital with a fibro scanner in Sri Lanka. 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 patients with diabetes 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 patients with diabetes 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 (24), BRAD (38), and NFS (27), none of these has been developed specifically for patients with diabetes and has been validated in Asian except among Japanese (39).

Apart from a few studies from North India (40) and Vietnam (41), there are no reports of VCTE data in patients with diabetes 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 (42). 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 (25, 26).

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.

Funding: This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors

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

Acknowledgement : Jayani Manchanayake and Dileepa Ediriweera for helping in developing the protocol.

References

1. Ekstedt M, Hagström H, Nasr P, Fredrikson M, Stål P, Kechagias S, et al. Fibrosis stage is the strongest predictor for disease-specific mortality in NAFLD after up to 33 years of follow-up. Hepatology. 2015;61(5):1547-54.
2. Younossi ZM, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global epidemiology of nonalcoholic fatty liver disease-Meta-analytic assessment of prevalence, incidence, and outcomes. Hepatology. 2016;64(1):73-84.
3. Amarapurkar DN, Hashimoto E, Lesmana LA, Sollano JD, Chen PJ, Goh KL. How common is non-alcoholic fatty liver disease in the Asia-Pacific region and are there local differences? Journal of gastroenterology and hepatology. 2007;22(6):788-93.
4. Diehl AM, Day C. Cause, Pathogenesis, and Treatment of Nonalcoholic Steatohepatitis. N Engl J Med. 2017;377(21):2063-72.
5. Castera L, Forns X, Alberti A. Non-invasive evaluation of liver fibrosis using transient elastography. J Hepatol. 2008;48(5):835-47.
6. Castéra L, Vergniol J, Foucher J, Le Bail B, Chanteloup E, Haaser M, et al. Prospective comparison of transient elastography, Fibrotest, APRI, and liver biopsy for the assessment of fibrosis in chronic hepatitis C. Gastroenterology. 2005;128(2):343-50.
7. Brunt EM, Janney CG, Di Bisceglie AM, Neuschwander-Tetri BA, Bacon BR. Nonalcoholic steatohepatitis: a proposal for grading and staging the histological lesions. Am J Gastroenterol. 1999;94(9):2467-74.
8. Diehl AM, Day C. Cause, Pathogenesis, and Treatment of Nonalcoholic Steatohepatitis. New England Journal of Medicine. 2017;377(21):2063-72.
9. Eslam M, Sanyal AJ, George J. MAFLD: A Consensus-Driven Proposed Nomenclature for Metabolic Associated Fatty Liver Disease. Gastroenterology. 2020;158(7):1999-2014.e1.

10. Alkhouri N, Poordad F, Lawitz E. Management of nonalcoholic fatty liver disease: Lessons learned from type 2 diabetes. Hepatology Communications. 2018;2(7):778-85.
11. Saponaro C, Gaggini M, Gastaldelli A. Nonalcoholic fatty liver disease and type 2 diabetes: common pathophysiologic mechanisms. Curr Diab Rep. 2015;15(6):607.
12. Younossi ZM, Golabi P, de Avila L, Paik JM, Srishord M, Fukui N, et al. The global epidemiology of NAFLD and NASH in patients with type 2 diabetes: A systematic review and meta-analysis. J Hepatol. 2019;71(4):793-801.
13. Herath HMM, Kodikara I, Weerarathna TP, Liyanage G. Prevalence and associations of non-alcoholic fatty liver disease (NAFLD) in Sri Lankan patients with type 2 diabetes: A single center study. Diabetes Metab Syndr. 2019;13(1):246-50.
14. Adams LA, Waters OR, Knuiman MW, Elliott RR, Olynyk JK. NAFLD as a risk factor for the development of diabetes and the metabolic syndrome: an eleven-year follow-up study. Am J Gastroenterol. 2009;104(4):861-7.
15. Shibata M, Kihara Y, Taguchi M, Tashiro M, Otsuki M. Nonalcoholic Fatty Liver Disease Is a Risk Factor for Type 2 Diabetes in Middle-Aged Japanese Men. Diabetes Care. 2007;30(11):2940-4.
16. Kasturiratne A, Weerasinghe S, Dassanayake AS, Rajindrajith S, de Silva AP, Kato N, et al. Influence of non-alcoholic fatty liver disease on the development of diabetes mellitus. Journal of gastroenterology and hepatology. 2013;28(1):142-7.
17. Hossain N, Afendy A, Stepanova M, Nader F, Srishord M, Rafiq N, et al. Independent predictors of fibrosis in patients with nonalcoholic fatty liver disease. Clin Gastroenterol Hepatol. 2009;7(11):1224-9, 9.e1-2.
18. Vernon G, Baranova A, Younossi ZM. Systematic review: the epidemiology and natural history of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in adults. Aliment Pharmacol Ther. 2011;34(3):274-85.
19. Loomba R, Abraham M, Unalp A, Wilson L, Lavine J, Doo E, et al. Association between diabetes, family history of diabetes, and risk of nonalcoholic steatohepatitis and fibrosis. Hepatology. 2012;56(3):943-51.
20. Fracanzani AL, Valenti L, Bugianesi E, Andreoletti M, Colli A, Vanni E, et al. Risk of severe liver disease in nonalcoholic fatty liver disease with normal aminotransferase levels: a role for insulin resistance and diabetes. Hepatology. 2008;48(3):792-8.
21. Singh S, Allen AM, Wang Z, Prokop LJ, Murad MH, Loomba R. Fibrosis progression in nonalcoholic fatty liver vs nonalcoholic steatohepatitis: a systematic review and meta-analysis of paired-biopsy studies. Clin Gastroenterol Hepatol. 2015;13(4):643-54.e1-9; quiz e39-40.
22. Association AD. 4. Comprehensive Medical Evaluation and Assessment of Comorbidities: Standards of Medical Care in Diabetes—2020. Diabetes Care. 2019;43(Supplement_1):S37-S47.
23. EASL-EASD-EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease. J Hepatol. 2016;64(6):1388-402.
24. Shah AG, Lydecker A, Murray K, Tetri BN, Contos MJ, Sanyal AJ. Comparison of noninvasive markers of fibrosis in patients with nonalcoholic fatty liver disease. Clin Gastroenterol Hepatol. 2009;7(10):1104-12.
25. Vieira Barbosa J, Lai M. Nonalcoholic Fatty Liver Disease Screening in Type 2 Diabetes Mellitus Patients in the Primary Care Setting. Hepatology Communications. 2021;5(2):158-67.
26. Castera L, Vilgrain V, Angulo P. Noninvasive evaluation of NAFLD. Nature Reviews Gastroenterology & Hepatology. 2013;10(11):666-75.

27. Angulo P, Hui JM, Marchesini G, Bugianesi E, George J, Farrell GC, et al. The NAFLD fibrosis score: a noninvasive system that identifies liver fibrosis in patients with NAFLD. Hepatology. 2007;45(4):846-54.
28. Petroni ML, Brodosi L, Bugianesi E, Marchesini G. Management of non-alcoholic fatty liver disease. BMJ (Clinical research ed). 2021;372:m4747.
29. Li Q, Huang C, Xu W, Hu Q, Chen L. Accuracy of FibroScan in analysis of liver fibrosis in patients with concomitant chronic Hepatitis B and nonalcoholic fatty liver disease. Medicine. 2020;99(23).
30. Cusi K, Isaacs S, Barb D, Basu R, Caprio S, Garvey WT, et al. American Association of Clinical Endocrinology Clinical Practice Guideline for the Diagnosis and Management of Nonalcoholic Fatty Liver Disease in Primary Care and Endocrinology Clinical Settings: Co-Sponsored by the American Association for the Study of Liver Diseases (AASLD). Endocrine Practice. 2022;28(5):528-62.
31. Mózes FE, Lee JA, Selvaraj EA, Jayaswal ANA, Trauner M, Boursier J, et al. Diagnostic accuracy of non-invasive tests for advanced fibrosis in patients with NAFLD: an individual patient data meta-analysis. Gut. 2022;71(5):1006-19.
32. Chan JCN, Malik V, Jia W, Kadowaki T, Yajnik CS, Yoon K-H, et al. Diabetes in Asia: Epidemiology, Risk Factors, and Pathophysiology. Jama. 2009;301(20):2129-40.
33. Dassanayake AS. Nonalcoholic Fatty Liver Disease: Identifying the Disease Burden in Sri Lanka. Euroasian J Hepatogastroenterol. 2018;8(1):69-72.
34. Niriella MA, Pathmeswaran A, De Silva ST, Kasturiratna A, Perera R, Subasinghe CE, et al. Incidence and risk factors for non-alcoholic fatty liver disease: A 7-year follow-up study among urban, adult Sri Lankans. Liver Int. 2017;37(11):1715-22.
35. Riley RD, Ensor J, Snell KIE, Harrell FE, Jr., Martin GP, Reitsma JB, et al. Calculating the sample size required for developing a clinical prediction model. BMJ (Clinical research ed). 2020;368:m441.
36. Joie Ensor [aut c, Emma C. Martin [aut], Richard D. Riley [aut]. pmsampsize: Calculates the Minimum Sample Size Required for Developing a Multivariable Prediction Model 2022 [1.1.2:[Available from: https://cran.r-project.org/web/packages/pmsampsize/index.html.
37. Lomonaco R, Godinez Leiva E, Bril F, Shrestha S, Mansour L, Budd J, et al. Advanced Liver Fibrosis Is Common in Patients With Type 2 Diabetes Followed in the Outpatient Setting: The Need for Systematic Screening. Diabetes care. 2021;44(2):399-406.
38. Harrison SA, Oliver D, Arnold HL, Gogia S, Neuschwander-Tetri BA. Development and validation of a simple NAFLD clinical scoring system for identifying patients without advanced disease. Gut. 2008;57(10):1441-7.
39. Sumida Y, Yoneda M, Hyogo H, Itoh Y, Ono M, Fujii H, et al. Validation of the FIB4 index in a Japanese nonalcoholic fatty liver disease population. BMC Gastroenterology. 2012;12(1):2.
40. Gupta A, Anoop S, Ansari IA, Prakash S, Misra A. High prevalence of hepatic steatosis and hepatic fibrosis in patients with type 2 diabetes mellitus. Clinical Nutrition ESPEN. 2021;46:519-26.
41. Tuong TTK, Tran DK, Phu PQT, Hong TND, Dinh TC, Chu DT. Non-Alcoholic Fatty Liver Disease in Patients with Type 2 Diabetes: Evaluation of Hepatic Fibrosis and Steatosis Using Fibroscan. Diagnostics (Basel). 2020;10(3):159.
42. Wong VW-S, Chan W-K, Chitturi S, Chawla Y, Dan YY, Duseja A, et al. Asia–Pacific Working Party on Non-alcoholic Fatty Liver Disease guidelines 2017—Part 1: Definition,

risk factors and assessment. Journal of gastroenterology and hepatology. 2018;33(1):70-85.

Figures -

Figure 4 Natural history of nonalcoholic fatty liver disease

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

Figure 5 Liver stiffness cut-offs in chronic liver diseases

kPa - kilo Pascal

Figure 6 Study design and participant flow through the study

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

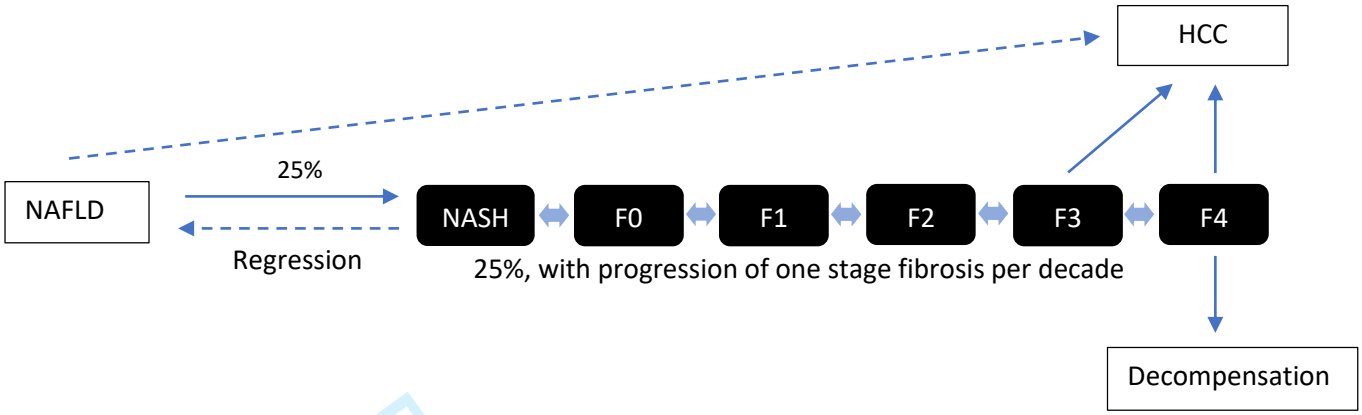

Figure 1 Natural history of nonalcoholic fatty liver disease

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

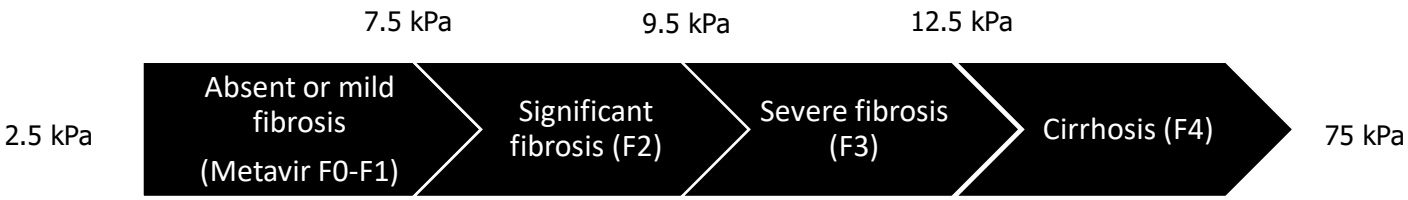

Figure 2 Liver stiffness cut-offs in chronic liver diseases

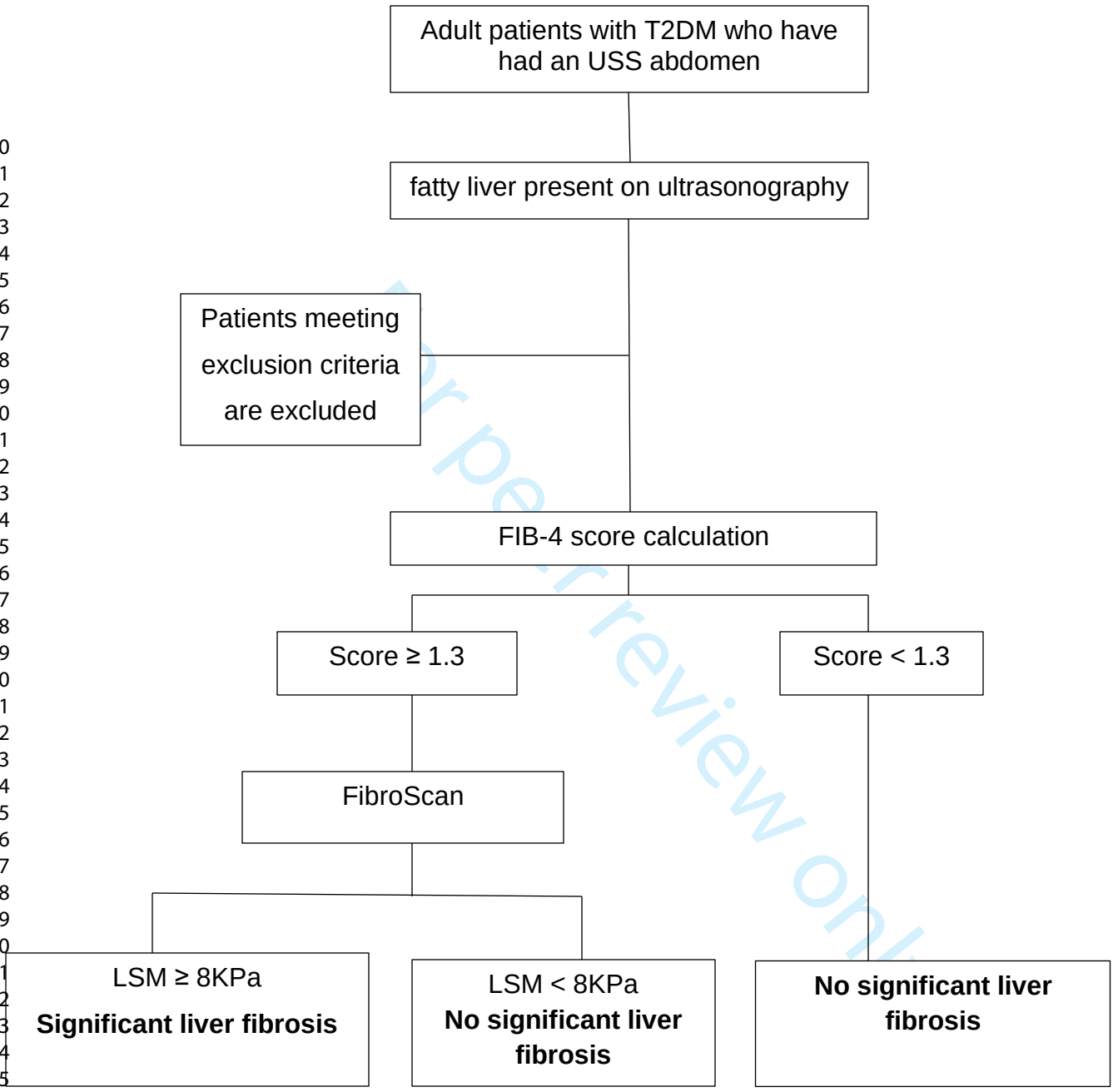

Figure 3 Study design and participant flow through the study

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.