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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

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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 Fibrosis-4 (FIB-4) score and exclusion of significant fibrosis with vibration-controlled transient elastography (VCTE) have 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 prioritise referral of patients with diabetes and NAFLD for VCTE.

Methods and analysis This cross-sectional study is conducted among all consenting adults with type 2 diabetes mellitus 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 (liver stiffness measure (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 reputable journals.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ Individual data of patients are used. Advanced fibrosis is confirmed with vibration-controlled transient elastography (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.
⇒ This is limited to a Sri Lankan cohort and therefore is not generalisable to all other nations.
⇒ This cohort is of a community of very low seroprevalence of hepatitis B and C. However, we have not studied hepatitis B and C serology in individual patients.

is a spectrum ranging from simple steatosis, non-alcoholic 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–F1 have no or minimal fibrosis, F2 is significant fibrosis with risk of progression to advanced fibrosis, 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 2.5 and 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 (F4).[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
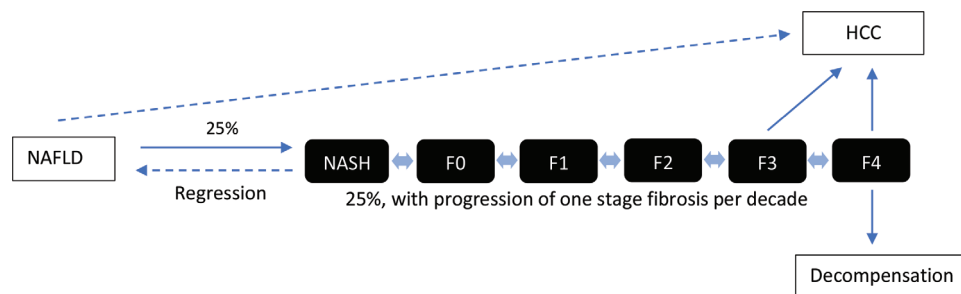
INTRODUCTION

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

Figure 1 Natural history of non-alcoholic fatty liver disease (NAFLD). HCC, hepatocellular carcinoma; NASH, non-alcoholic steatohepatitis.

and lead to cirrhosis. However, the majority of patients with NAFLD 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-income and low-income countries.[9]

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 patients with NAFLD have a twofold increased risk of developing T2DM compared with 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 patients with T2DM 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 patients with T2DM 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 LSM using transient elastography[26] for those with FIB-4 ≥1.3[25] or NFS between −1.455 and 0.676.[26] The patients at high risk of advanced fibrosis/cirrhosis with an LSM ≥8 kPa are advised referral to specialised liver centres for further assessment and management.[25 28–31]

Diabetes has become an epidemic in low/middle-income countries including those in Asia. Of the world's population with diabetes, 60% are Asians.[32] Diabetes is on the rise among Asians due to urbanisation, 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 (ie, 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 prioritise referrals for VCTE and to specialised 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
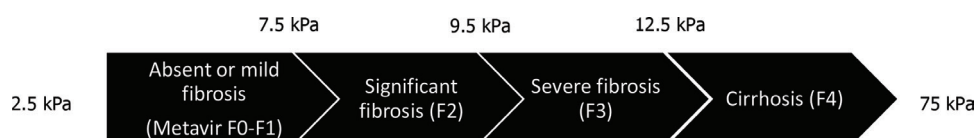

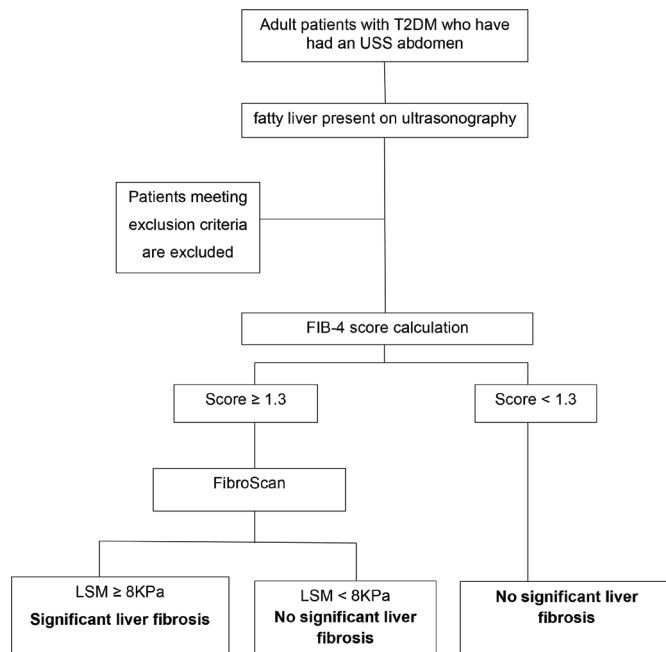**Figure 2** Liver stiffness cut-offs in chronic liver diseases.

Figure 3 Study design and participant flow through the study FIB-4 score. FIB-4, Fibrosis-4; LSM, liver stiffness measure; T2DM, type 2 diabetes mellitus; USS, ultra sound scan.

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, for example, tamoxifen, methotrexate, etc.

Sample size

The sample size was calculated for developing a clinical prediction model[35][36] assuming an R^2 value of 0.2 for the logistic model, 10 parameters in the model, and 0.05 acceptable difference in apparent and adjusted R^2, 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, that is, LSM ≥8 kPa). This is likely to provide six events per predictor parameter.

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.

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 Controlled Attenuation Parameter (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 V.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 receiver operating characteristic 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 becomes 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 who 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 Asians 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 are 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]

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