TITLE: Association Between Severity of Liver Fibrosis and cardiovascular Disease Risk Among Diabetic and Prediabetic Bangladeshi People: A population Based Multicenter Cross-Sectional Study

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Introduction:

Patients with type 2 diabetes (T2D) have a twofold to fourfold higher risk of fatal cardiovascular events than the general population. Cardiovascular disease (CVD) affects approximately 32.2% of all patients with T2D globally and is the principal cause of death in patients with T2D. CVD propensity is elevated in patients with T2D due to a complex combination of various risk factors.The traditional risk factors of CVD are hypertension, obesity and abdominal obesity, reduced physical activity, smoking, duration of diabetes, atherogenic dyslipidemia, high glycated hemoglobin level, dysglycemia, and insulin resistance.[1] Recently, several studies have demonstrated that liver fibrosis is independently associated with poor cardiovascular outcomes, cerebrovascular outcomes, and mortality in non-alcoholic fatty liver disease (NAFLD) as well as in the general population.[2]

Metabolic-associated fatty liver disease (MAFLD) is a novel terminology that was proposed recently by international experts instead of NAFLD in patients with overweight/obesity, type 2 diabetes, or evidence of metabolic dysregulation, in addition to hepatic steatosis.[3]A cross-sectional study reported that approximately 23.8% and 15.4% of

US diabetic adults had, respectively, significant liver fibrosis and advanced liver fibrosis . The vulnerability of liver fibrosis in diabetic patients can attribute to insulin abnormalities. Insulin plays an essential role in the normal functioning of the liver. Insulin mediates the glucose intake of the liver, and insulin resistance can lead to hepatic lipid accumulation and abnormal glucose regulation, which may eventually result in liver fibrosis.[4]Recently, increasing evidence has linked NAFLD to an increased risk of atherosclerotic cardiovascular disease (ASCVD) and heart failure (HF) development. Although some observational studies have shown inconsistent results, with NAFLD reported to have no relation to acute myocardial infarction or stroke after adjustment for cardiovascular disease (CVD) factors, there is a growing body of evidence linking NAFLD to increased ASCVD risk and severity.[5]

To diagnose and prevent the progression of NAFLD and ASCVD, early identification of individuals with NAFLD is crucial. Liver biopsy is the most accurate method for evaluation and diagnosis, but it is invasive and uneconomical for large population samples.

Ultrasound is a common noninvasive test but has limitations in detecting mild steatosis (affected by different operators) and accurately quantifying severity. To address these limitations, aggregate scores based on routine clinical and biochemical data such as the fibrosis 4 index(FIB-4), have been proposed as noninvasive markers for early detection and management of NAFLD.[5] FIB-4 index is calculated by using age, platelet count (PLT) and aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels. Hence, this index can be used for the early detection of liver fibrosis among patients with T2D. The FIB-4 index is measured according the formula that follows: Age(years)* AST(Unit/Liter)/ (PLT (109/L)*√ALT(Unit/Liter)).[3]

The aim of this study is to investigate the potential link between FIB-4 index and cardiovascular risk in patients with NAFLD or MAFLD. which may help to identify the predictive value of FIB-4 of CVD and its poor prognosis in the diabetic population.

Rationale: Liver fibrosis is a progressive condition that is associated with both NAFLD and MAFLD. Recent evidence indicates that liver fibrosis severity is related to an increased risk of fatal and non-fatal CVD events.[6] Several studies have demonstrated that the prevalence of clinically manifest cardiovascular disease (CVD) significantly increased among patients with NAFLD. Worryingly, NAFLD was also associated with a higher prevalence of high risk and vulnerable coronary artery plaques, independently of traditional CVD risk factors and the extent and severity of coronary atherosclerosis.[7] Although the cross-sectional association between NAFLD and increased CVD prevalence is strong and consistent, it remains uncertain whether the presence of NAFLD predicts incident CVD events or whether the more severe forms of NAFLD are associated with an even higher risk of future CVD events. However, few studies have investigated the association between severity of fibrosis and CVD risk among patients with T2D in bangladesh. Thus, in this study, we will find out the magnitude of the association between NAFLD and the risk of

CVD risk . We will also investigate whether the severity of NAFLD is associated with a higher risk of CVD events. Clarification of these issues may have important clinical implications for management of diabetic and prediabetic patients with NAFLD and cardiovascular disease.

Objectives of the study:

General objective:

To assess the prevalence of Non-alcoholic fatty liver disease and their FIB-4 index, and to find out their association with cardiovascular Disease Risk (ASCVD risk) among urban diabetic and Prediabetic Bangladeshi population.

Specific objectives:

1. To determine the prevalence and severity of NAFLD among urban diabetic and prediabetic population.
2. To estimate FIB-4 index and ASCVD risk in diabetic and prediabetic subjects.
3. To evaluate fibrosis risk stratifications according to FIB-4 index and ASCVD risk according to ASCVD risk calculator.
4. To evaluate association of NAFLD with BMI, waist circumference, HbA1c, hypertension, dyslipidemia and ASCVD risk.

Methodology:

Study Design:

This will be a cross-section, observational study.

Place of study:

This study will be conducted at Dhaka Medical College Hospital, Dr. Sirajul Islam Medical College and CIMEC Health center, Dhanmondi, Dhaka, Bangladesh.

Study period:

This study will be conducted from February 2024 to July 2024.

Study population:

Non-pregnant adults having T2DM and prediabetes without any evidence of cardiovascular diasease, aged ≥18 years to ≤65 years who are willing to give consent to participate in the study.

Inclusion criteria:

- Non-pregnant adults having T2DM and Prediabetes, aged ≥18 years to ≤65 years
- eGFR ≥30 ml/min/1.73m2
- Only consented subjects having all the required data available
- No previous history of CVD

Exclusion criteria:

- Having Type 1 diabetes
- History of CVD before diagnosis T2DM
- Age ≤18 years or > 65 years
- Adult women with pregnancy
- eGFR < 30 ml/min/1.73m2
- History of significant alcohol consumption (defined as ingestion of >21 standard drinks per week in men and >14 standard drinks per week in women over a 2-year period preceding baseline liver histology)
- Patients with coinfection with hepatitis B and C virus or human immunodeficiency virus or any cause associated with chronic liver disease, advanced liver disease, hepatic congestion, cardiac failure or on hepatotoxic drugs
- Coexisting significant medical illness(e.g. terminal stage of congestive heart failure or renal failure, and uncontrolled infection or malignant disease)
- Unwilling to give consent and with incomplete data were excluded from the study

Study sample size:

Emerging evidence based on several large population-based studies has demonstrated an exponential increase in MAFLD burden in the Asia–Pacifc region over the past three

decades.[8] A recent systematic review and meta-analysis of MAFLD prevalence from an Asian context suggested that the prevalence of MAFLD in this region is 29.62%.[9]

For 95% confidence level and 5% margin of error-

Sample size is $n = \{\frac{(1.96)2\times0.29\times0.71}{(0.05)2}\}$ = 316

In Indian subcontinent, prevalence of NAFLD is recorded to be 16–32% in urban population.[10]

So considering prevalence 32%, our sample size is ,

n={((1.96)2×0.32×0.68)/((0.05)2)} = 334.3

Considering these two prevalences, our proposed study sample size is 400.

Sampling technique:

Study sample will be collected purposively among the type 2 diabetic and prediabetic patients.

Data collection instrument:

After taking consent, a pre-formed printed data record form will be used to collect data through face-to-face interview and from investigations reports that are done routinely.

Data record procedure:

Data of each participant will be recorded in single visit. Demographic data comprising age and sex were obtained for each subject. All relevant laboratory test results data for the study were obtained from Case record. Data will be collected of serum aminotransferase (ALT), and aspartate aminotransferase (AST) activity, serum creatinine,blood HbA1c and platelet counts. Serum ALT, AST, and creatinine will be included in standard biochemistry profile and will measured through a biochemical analyzer.

In this study ,The patients were stratified into three groups based on the probability of advanced liver fibrosis using conventional cutoffs of FIB-4: FIB-4 < 1.30 a (low probability),FIB-4 1.30–2.67 (intermediate probability), and FIB-4 > 2.67 (high probability).

The predicted cardiovascular risk at the time of T2D diagnosis was calculated using the 10-year atherosclerotic CVD (ASCVD) risk prediction model of the 2013 American College of Cardiology/American Heart Association guidelines.11The 10-year ASCVD risk classified into low risk (<5%), medium risk (5% to 9%), or high risk (≥10%).

Statistical analysis:

Data will be analyzed using IBM SPSS for Windows version 20.0 (IBM SPSS Statistics for Windows, Version 20.0 IBM Corp. Armonk, NY, USA). Frequencies and percentages will be calculated for quantitative variables, while mean ± SD will be documented for qualitative variables. Categorical variables will be compared with each other using the chi-square test. Among the basic characteristics of the studied participants, the continuous variables will be compared with each other using the ANOVA test. Independent t- test and paired t- test will be used to analyze the association of clinical and biochemical parameters. Statistical significance will be set at $P<0.05$.

Literature Review

The present study will take cross-sectional data of both diabetic and prediabetic individuals from rural and urban areas of Bangladesh from February 2024 to July 2024 to explore the relationship between FIB-4 and ASCVD risk. A cross-sectional study conducted by Lihua Guan et al using the data from the National Health and Nutrition Examination Survey (NHANES) 1999–2008, the association between FIB-4, death, and CVD was analyzed in that study and the risk of all-cause mortality (HR 1.24; 95% CI,1.17–1.32) and CVD mortality (HR 1.17; 95% CI, 1.04–1.31) increased with each FIB-4 SD increase after adjusting for all covariates were found.4 FIB-4 is a highly-sensitive biomarker for evaluating advanced liver fibrosis. However, in recent years, studies have begun to focus on the predictive value of FIB-4 on the occurrence and prognosis of CVD.[12,13]

In another study by Park JH et al found that the histological severity of liver fibrosis can be an independent risk factor in predicting the incidence of CVD, indicating the potential role of FIB-4 in indirectly predicting CVD.[14] However, a multicenter study in Sweden revealed that the degree of liver fibrosis did not independently affect the risk of CVD.[15]

Though the definite cause behind the increased rate of CAD among patients with hepatic steatosis has not been determined as of yet, some speculations have been made. One of which is that NAFLD is commonly associated with T2D, a comorbidity also known as MAFLD, leading to insulin resistance and this increase the chances of developing CAD by triggering monocyte/macrophage adhesion to the vascular walls, and stimulating chemokine secretion by the smooth muscle cells of the vessels, and activating inflammation via macrophages, In line with the findings in patients with NAFLD, MAFLD can potentially influence the risk of CAD.[16] This is because of the overlap between NAFLD and MAFLD as well as the more metabolic derangements in patients with MAFLD, which

in turn further increases the risk of CAD.[17] In 2019, Song et al. carried out a study on patients with NAFLD without CAD and concluded that FIB-4 score as a non-invasive fibrosis marker is significantly associated with the coronary artery calcium score (CACS>100).[18] Also Lee, J. et al. showed the association between intermediate/high FIB-4 scores and the progression of coronary artery calcification (CAC) in patients with NAFLD.[19]

The largest population-based investigation carried out in an Asian population to explore the correlation between fatty liver index(FLI) and 10-year ASCVD risk by Jing Zhou et al demonstrated a significant association between FLI and 10-year ASCVD risk ($p < 0.001$). Adjusted for age, individuals with high FLI (≥60) had an odds ratio of 3.91 (95% CI 2.52–6.08) compared to those with low FLI (<30) and the findings consistently demonstrate that higher FLI levels are associated with an increased risk of ASCVD.

Our current study will conduct with a view establish FIB 4 score a reliable indicator of increased 10-year ASCVD risk in Bangladeshi diabetic and prediabetic patients. Subsequently it is important to pay close attention to cardiovascular diseases in patients with fatty liver disease. Individuals with elevated FIB 4 score should undergo thorough evaluation and regular monitoring for ASCVD to improve their prognoses.Finally our aim is to highlight the role of FIB4 in clinical practice for risk assessment and prevention strategies to reduce burden of non-communicable disease in Bangladesh.

Budget:

For data collection tools development, data processing, software, one laptop and related works, approximately 2,0,0000 BDT budget is needed.

Protocol approval:

Study protocol will be submitted for approval to IRB (Institutional Review Board) of DMCH.

Outcome measures of the study are:

BMI, waist circumference, systolic and diastolic blood pressure, fasting plasma glucose, HbA1c%, AST, ALT, Platelet count, eGFR, fasting lipid profile, FIB-4 index, ASCVD risk estimation.

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