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Review Article

Deciphering the Non-invasive Tests in NAFLD: A Systematic Review and Meta-analyses

Sanul Shahi Salim, DNB, Shamim Hyder, DNB, Jamila Hameed *, MD

Department of Radiology, Karuna Medical College, Vilayodi, Kerala, 678103, India.

*Corresponding Author: Jamila Hameed, MD; *hameedjamila78@gmail.com*

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Abstract

Background: Non-alcoholic fatty liver disease (NAFLD) is a significant health issue worldwide now referred to as metabolic dysfunction-associated steatotic liver disease (MASLD), varying from mild fat accumulation in the liver to non-alcoholic steatohepatitis (NASH). ***Aim and objective:*** The primary aim of this study was to answer the question:" How does the combination of non-invasive tests help in enhancing the accuracy for advancing fibrosis in NAFLD patients and how this could assist in clinical practice and management?". ***Methods:*** The systematic review and meta-analyses included studies published from 2016 to 2024 assessing non-invasive tests (NITs) in the diagnosis of advanced fibrosis in NAFLD patients across Scopus, PubMed and Embase. Fifteen studies were finally selected with seven studies included in the meta-analyses. ***Results:*** The pooled estimates for mean AUC, sensitivity and specificity for various NITs were 0.82, 0.53 and 0.83 respectively. Magnetic resonance elastography (MRE) and enhanced liver fibrosis (ELF) tests showed high accuracy in the diagnosis of advanced fibrosis. ***Conclusion:*** A combination of non-invasive tests will aid in enhancing the accuracy of fibrosis detection and progression in NAFLD improving patient outcomes. Emerging methods like the use of AI and ML, microRNA signatures, optical coherence tomography, and future directions including liquid biopsy and molecular imaging techniques show promising results in the diagnostic performance and accuracy of NAFLD.

Keywords: ***Non-alcoholic fatty liver disease, Non-invasive tests, Area under curve, Liver fibrosis, Systematic review, Meta-analyses.***

Introduction

Non-alcoholic fatty liver disease (NAFLD) is a major global health concern, affecting ~25% of adults worldwide, especially in Western countries (Younossi ZM et al, 2016). The disease, also now officially called metabolic dysfunction associated steatotic liver disease (MASLD), is part of a spectrum from simple steatosis to non-alcoholic steatohepatitis (NASH) that can advance to advanced fibrosis and cirrhosis associated with increased morbidity and mortality. Regardless of NAFLD-related causes, accurate staging of liver fibrosis provides an effective opportunity to evaluate disease prognosis and develop treatment approaches. Traditionally, liver biopsy has served as the gold standard for fibrosis staging, but given its invasive nature, risks, and sampling variability, there has been a search for reliable non-invasive alternatives (Heyens LJ et al, 2021). Today, non-alcoholic fatty liver disease (NAFLD) is a formidable global health threat, with approximately one-fourth of all adults affected, especially in Western countries. A systematic review and meta-analysis of studies, including patients with NAFLD, that assessed the performance of NITs in diagnosing advanced fibrosis were performed. A combination of NITs along with liver biopsy can enhance the accuracy (Mathew JF et al, 2024) The understanding of the disease progression and timely intervention are essential in improving the patient outcomes. As such, the prevalence of NAFLD is increasing. The accurate diagnosis of fibrosis and its management pushed us to conduct the systematic review and meta-analyses.

Methodology

This systematic review and meta-analyses followed the Preferred Reporting Item for Systematic Review and Meta-Analyses (PRISMA) guidelines (**Figure 1**) (Page MJ et al, 2021).

Literature search

A comprehensive literature search was done to find out studies published between 2016 to 2024 on the various NITs used in the diagnosis of liver fibrosis in NAFLD. Electronic database search was done in PubMed, Scopus, and Embase using the keywords "Non-invasive tests", "Liver fibrosis" and "NAFLD".

Inclusion Criteria

- Adults diagnosed with NAFLD (non-alcoholic fatty liver disease) with more than 5% fat in their liver but no other liver conditions like hepatitis B, hepatitis C, or autoimmune diseases.
- Studies that focus on non-invasive tests for checking liver fibrosis, such as:
  i. Enhanced Liver Fibrosis (ELF) test
  ii. Fibrosis-4 Index (FIB-4)
  iii. NAFLD Fibrosis Score (NFS)
  iv. Vibration Controlled Transient Elastography (VCTE)
  v. Other blood tests, like acoustic radiation force impulses (ARFI), aspartate aminotransferase to platelet ratio index (APRI) or AST/ALT ratio

- Studies that use methods like liver biopsy or imaging tests such as liver stiffness measurement (LSM) and vibration controlled transient elastography (VCTE) to accurately diagnose liver fibrosis stages.

Exclusion Criteria

- Studies like case reports, conference abstracts, and animal studies.
- Studies involving people with other chronic liver conditions or those who drink excessive amounts of alcohol (more than 10 grams per day for men, or 20 grams per day for women).

Data Extraction

The article's eligibility based on criteria search was completed by two authors (S.S and S.H) and the full text of the studies was analyzed using Microsoft Excel 2016. The two authors assessed the articles' methodology and quality by using the New Castle Ottawa assessment scale (Luchini C et al, 2017). Finally, a total of 15 studies met the quality of assessment. The data shows different studies from different parts of the world. The first author's name with year of publication, country of study, study design, sample size and sample characteristics were tabulated (**Table 1**). Seven studies out of 15 studies were included in the meta-analyses. Two studies were considered for APRI(Siddiqui MS et al, 2019; Kaswala DH et al, 2016), three for FIB-4(Siddiqui MS et al, 2019; Kaswala DH et al, 2016;Staufer K et al 2019), three for NFS(Siddiqui MS et al, 2019; Kaswala DH et al, 2016;Staufer K et al 2019), two for AST/ALT ratio(Siddiqui MS et al, 2019; Kaswala DH et al, 2016), three studies for ELF score(Staufer K et al 2019; Kaswala DH et al, 2016; Vali Y et al, 2020), two studies for FibroMeter(Staufer K et al 2019; Kaswala DH et al, 2016), two studies for MRE(Cui J et al, 2016; Loomba R et al, 2016) were considered. Forest graphs were plotted.

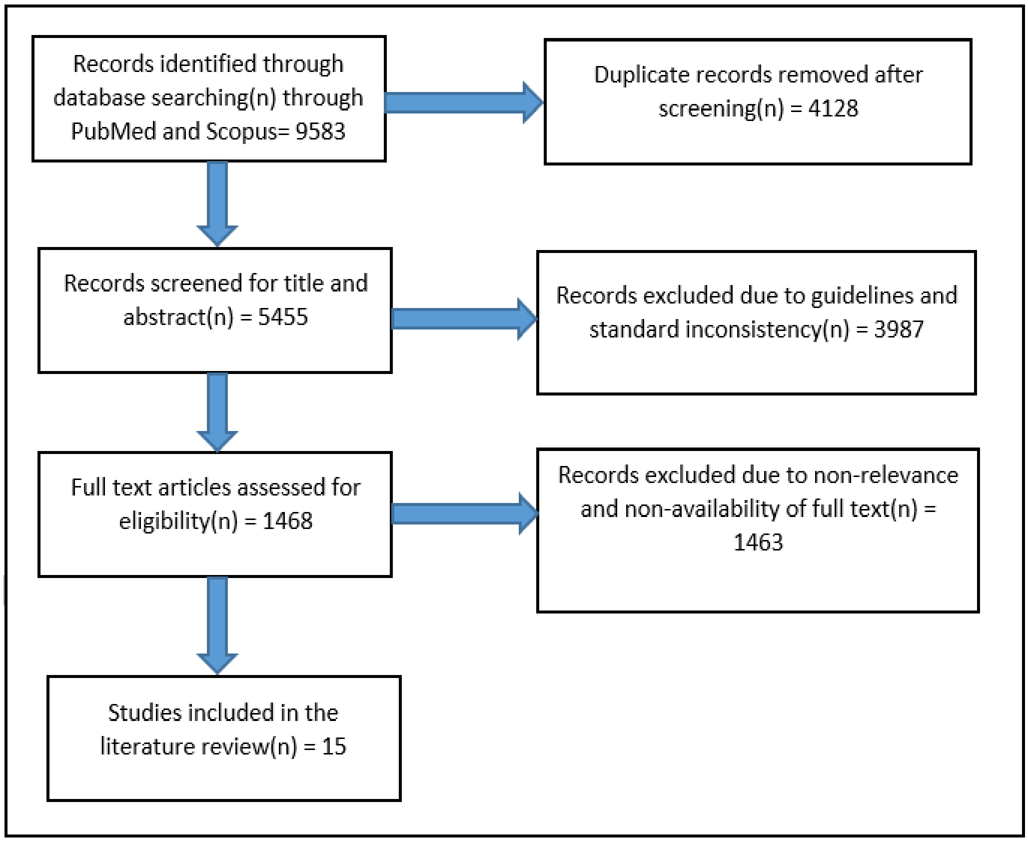

Figure 1: Flowchart for selection of studies

Results

Screening flow

According to the search flow strategy, a total of 9583 articles were retrieved from different electronic databases like PubMed, Embase and Scopus of which 4128 duplicate articles were removed. During the abstract and title screening process, 3987 articles were removed out of 5455 articles. Out of the rest of the 1468 articles 1458 articles were excluded during full-text screening phase. Finally, 15 articles were selected for the systematic review of which six articles were considered for meta-analysis.

The forest graphs were plotted for mean AUC, sensitivity and specificity for various NITs and the pooled estimates were 0.82[95% CL: 0.74-0.89], 0.53[95% CL: 0.45-0.61] and 0.83[95% CL:0.65-0.94] respectively and heterogeneity I2= 98.79%, 99.89% and 99.90% respectively (**Figure 2 a. b and c**). The data regarding the mean AUC, sensitivity and specificity (%) for various NITs were tabulated (**Table 2**).

These results indicate that MRE (particularly 3D-MRE) and the ELF score are among the most effective non-invasive tests for diagnosing advanced fibrosis in NAFLD, followed closely by the FIB-4 Index and NFS. The findings support the use of these non-invasive models as reliable alternatives to liver biopsy.

Funnel and Egger's test

The funnel plot showed asymmetry attributed to the chronological and geographical variations in the data (**Figure 3**). The Egger's test p-values for mean AUC, sensitivity and specificity were $p = 0.027$, $p = 0.006$, and $p = 0.981$ respectively.

The meta regression bubble graph was plotted (**Figure 4 c**).

Table 1: Study Characteristics

S No	First Author's Name	Country of Study	Study Design	Sample Size	Sample Size Characteristics
1	Jeffrey Cui et al (2016)	United States	Prospective Study	102	Mean age: 51.3 years, 58.8% women, Mean BMI: 31.7 kg/m², biopsy-proven NAFLD patients.
2	Dharmesh H. Kaswala et al (2016)	United States	Review Article	APRI=358, FIB4=292, NFS=733, ALT/ALT = 358	NAFLD prevalence (30% in adults, 10% in children), associated with obesity, diabetes, and metabolic syndrome.
3	Jeffrey Cui et al (2016)	United States	Prospective Cohort Study	125	Mean age: 48.9 years, 54.4% female, Mean BMI: 31.8 kg/m², 71 patients obese (BMI ≥ 30).
4	Rohit Loomba (2016)	United States	Prospective Study	100	Mean age: 50.2 years, 56% women, Mean BMI: 32.1 kg/m², biopsy-proven NAFLD patients.
5	Cheah MCC et al (2017)	Singapore	Review Article	NFS=4099, FIB4=541, BARD=827, ELF=196, MRE=325, ARFI=541	Not specified
6	Yoneda M et al (2018)	Japan	Review Article	VCTE=2735, ARFI=723	Not specified
7	M. Shadab Siddiqui et al (2019)	United States	Retrospective Analysis	1904	Mean age: 48.9 years, Mean BMI: 34.4 kg/m², 39% with diabetes, 62% with hyperlipidaemia, 58% with hypertension, 58% with definite NASH
8	Katharina Staufer et al (2019)	Austria	Prospective, Biopsy-Controlled	186	Mean age: 52 years, 57% male, 30% with diabetes, 50% with NASH, 38% with significant fibrosis (F ≥ 2), 26% with advanced fibrosis (F ≥ 3)
9	Yuanzi Liang et al (2020)	China	Systematic Review and Meta-Analysis	910	Included 12 studies, with a range of ages and BMI; focused on patients with NAFLD and varying stages of liver fibrosis.
10	Yasaman Vali et al (2020)	Netherlands	Systematic Review and Meta-Analysis	4,452	Mean age: 54 years, 60% male, 25% with diabetes, 40% with obesity, varying stages of fibrosis (F0-F4).
11	Zobair M. Younossi et al (2021)	United States	Retrospective Cross-Sectional Study	829	Mean age: 53.1 years, 43.8% men, 35.5% with type 2 diabetes, mean FIB-4 score: 1.34.
12	Ferenc Emil Mózes et al (2021)	Multiple Countries	Individual Patient Data Meta-Analysis	5735	Median age: 54 years, 45% women, 33% with type 2 diabetes, 30% with advanced fibrosis (F3-F4).
13	Boursier J et al (2022)	France	Multicentre Cohort Study	1,057	Median age was 55 years, 62% of the patients were male, 37% had diabetes, 44% were under antihypertensive treatment, and 27% were under lipid-lowering treatment.
14	Sanyal AJ et al (2023)	USA, France, China	Review Article	APRI, NFS=625, VCTE=1268, ELF & LSM=1668, FIB4=41354	Not specified
15	Wang J-L et al (2024)	China	Review Article	2D-MRE=100, 3D-MRE=100, ELF=371, MRE=104	Not specified

Table 2: Overall mean AUC, sensitivity and specificity (%) values for various NITs across studies

NITs	Mean AUC (%)	Sensitivity (%)	Specificity (%)
APRI	75.5	30	93
FIB-4	78.67	16.67	28.16
NFS	76	51	96
AST/ALT Ratio	71	21	90
ELF Score	83	77.66	86.66
Fibro Meter	89.5	81	84
MRE	96.9	96.1	92.2

Table 3: Important findings of various studies

S No	First Author's Name	Key Findings
1	Jeffrey Cui et al (2016)	An AUROC of 0.957 was showed by 2D-MRE in prediction of advanced fibrosis outperforming FIB-4 that showed an AUROC of 0.861. A sensitivity and specificity of 0.922 and 0.904(cutoff: 3.64 kPa) was reported for 2D-MRE. 2D-MRE in short ruled with higher accuracy in diagnosis of advanced fibrosis in NAFLD patients
2	Dharmesh H. Kaswala et al (2016)	Western countries reported a prevalence of 20-25% in western countries showing advanced fibrosis as a significant predictor of mortality. Liver biopsy was stated as the gold standard in the assessment of fibrosis, however it had its own limitations.
3	Jeffrey Cui et al (2016)	MRE outperformed ARFI (AUROC 0.799 vs 0.664) in the diagnosis of any fibrosis. MRE showed higher accuracy in patients with obesity in comparison to ARFI (AUROC: 0.850 vs 0.603). It showed high AUROCs as well for advanced fibrosis stages (F2: 0.885, F3: 0.934, F4: 0.882)
4	Rohit Loomba (2016)	3D-MRE showed better resulted in advanced fibrosis diagnosis in comparison to 2D-MrRE at 40 Hz (AUROC: 0.981 vs 0.921). 3D-MRE at a threshold of 2.43 kPa for 40Hz exhibited a sensitivity and specificity of 1 and 0.94 respectively. Both 2D and 3D MRE showed high accuracy in the diagnosis of advanced fibrosis
5	Cheah MCC et al (2017)	The effectiveness of NITs such as NFS and VCTE in liver fibrosis assessment was highlighted. VCTE showed a higher AUROC (0.93) in comparison to NFS (AUROC: 0.85), indicating that it is a more reliable method for diagnosis.
6	Yoneda M et al (2018)	VCTE and MRE were reported as effective methods in assessment of liver fibrosis. MRE showed a high AUROC of 0.90 for advanced fibrosis detection making it a better alternative to liver biopsy. VCTE on the other hand showed an AUROC of 0.85 and 0.92 respectively in the detection of significant and advanced fibrosis.
7	M. Shadab Siddiqui et al (2019)	FIB-4 and NFS showed the best results in advanced fibrosis detection than other techniques (C-statistics: 0.80 for FIB-4, 0.78 for NFS). C-statistics for detection of progression to advanced fibrosis was: APRI(0.82), FIB-4(0.81) and NFS(0.80)
8	Katharina Staufer et al (2019)	In comparison to FIB-4 and NFS, ELF score, FibroMeter V2G/V3G and LSM demonstrated better diagnostic accuracy for fibrosis staging. AUROC for ELF score: 0.85(F≥2), 0.90 (F≥3), 0.90 (F≥3 & NASH). LSM per protocol showed an AUROC of 0.87(F≥2), 0.95(F≥3) and 0.91(F≥3 & NASH)
9	Yuanzi Liang et al (2020)	MRE was reported to be a reliable method for staging of liver fibrosis in patients with NAFLD. Its AUsROC was 0.89, 0.93, and 0.95 for respective stages. The pooled sensitivity and specificity for MRE in diagnosis of liver fibrosis stages were high:F≥1(0.77,0.90), F≥2(0.87,0.86), F≥3(0.89,0.84) and F≥4(0.94, 0.75)
10	Yasaman Vali et al (2020)	ELF test's effectiveness in ruling out advanced fibrosis at cut-off of 7.7 with a high sensitivity of 0.93 was demonstrated. At thresholds as high as 10.51 specificity increased to 0.93, however sensitivity dropped to 0.51.
11	Zobair M. Younossi et al (2021)	Advanced fibrosis was effectively diagnosed in NAFLD patients with AUROC of 0.81(biopsy) and 0.79(TE). A high PPV value was indicated for the condition with an ELF and FIB-4 score ≥9.8 and ≥2.9 respectively. A high NPV value on the other hand was indicated by ELF and FIB-4 scores ≥7.2 and ≥0.74 respectively for ruling out advanced fibrosis
12	Ferenc Emil Mózes et al (2021)	The need for liver biopsies can be decreased from 33% to 19% using the sequential combination of FIB-4 and LSM-VCTE. AUROCs for LSM-VCTE, FIB-4 and NFS for advanced fibrosis were reported as 0.85, 0.76 and 0.73 respectively.
13	Boursier J et al (2022)	FIB-4 and VCTE demonstrated good accuracy with Harrell's C indexes of 0.817 and 0.878 respectively. The risk of LREs was effectively stratified with the step-wise FIB4-VCTE algorithm reporting a 12.4 times increased risk of LREs in patients with FIB4≥1.30 and VCTE≥12.0kPa
14	Sanyal AJ et al (2023)	VCTE showed higher AUROC in comparison to ELF test (0.92 vs 0.85). FIB4 showed an AUROC of 0.84 while NFS showed an AUROC of 0.81 for the diagnosis of advanced fibrosis.
15	Wang J-L et al (2024)	Various non-invasive biomarkers like 20-carboxy arachidonic acid (20-COOH AA) and 13, 14-dihydro-15-keto prostaglandin D2 (dhk PGD2) showed diagnostic accuracies of 0.95 and 0.93 respectively.

Table 4: Strengths and weaknesses of each study

S No	First Author's Name	Merits	Gaps
1	Jeffrey Cui et al (2016)	Robust evidence was provided to support the use of 2D-MRE	Limited generalizability.
2	Dharmesh H. Kaswala et al (2016)	The clinical implications were well discussed for advanced fibrosis	Original research data was lacking,
3	Jeffrey Cui et al (2016)	Comprehensive comparison of MRE and ARFI was carried out	Further research needs to be conducted to evaluate the long-term outcomes and cost-effectiveness of MRE vs ARFI,
4	Rohit Loomba (2016)	The potential of 3D-MRE was highlighted in terms of its superiority as a non-invasive diagnostic tool. Clear diagnostic thresholds were provided for clinical applications	Small sample size
5	Cheah MCC et al (2017)	Detailed analysis of multiple non-invasive modalities provided a clear comparison of the diagnostic performance	Primary data was lacking

6	Yoneda M et al (2018)	Data regarding the invasive as well as non-invasive methods was provided along with detailed overview of VCTE and MRE	Recent advancements in non-invasive testing lacking
7	M. Shadab Siddiqui et al (2019)	Valuable insights into the diagnostic performance for various non-invasive models	Limited to a particular population reporting high prevalence of advanced fibrosis
8	Katharina Staufer et al (2019)	Optimized cut-offs were provided for ELF, FibroMeter and LSM	The generalizability might have been affected since the study was limited to data from two Austrian referral centres
9	Yuanzi Liang et al (2020)	Vivid analyses of MRE'S diagnostic accuracy	High heterogeneity reported across the studies
10	Yasaman Vali et al (2020)	Large sample size enhanced reliability, detailed analyses for ELF test's diagnostic accuracy in NAFLD	Differences in the study methodologies might affect the results
11	Zobair M. Younossi et al (2021)	Clear cut values for ELF and FIB4 scores were provided	Retrospective nature of the study might lead to bias
12	Ferenc Emil Mózes et al (2021)	Large sample size including robust data from multiple studies, the use of sequential testing to reduce liver biopsy was validated	There was lack of subgroup analysis like ethnic differences and differences in study quality and methodologies
13	Boursier J et al (2022)	Multicentre design of the study improved generalizability	The follow-up duration was limited
14	Sanyal AJ et al (2023)	Comprehensive overview of current NITs was provided with data specifically on the diagnostic performance	Lack of all recent advancements in non-invasive testing
15	Wang J-L et al (2024)	Specific data on the accuracy of different biomarkers as well as imaging techniques provided	Focused on existing literature and failed to provide new experimental findings

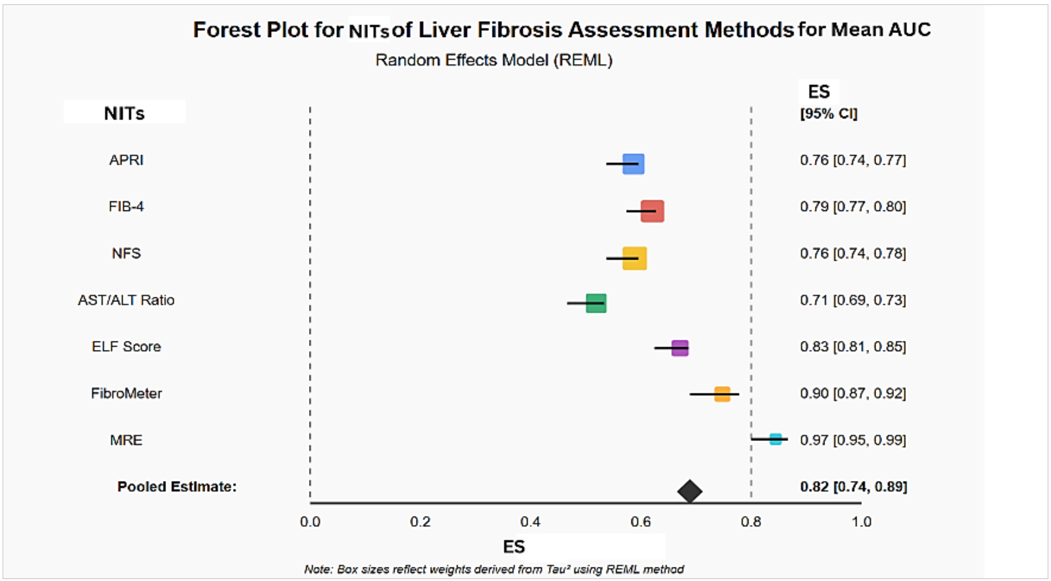

Figure 2 a): Forest plot of mean AUC for various NITs in liver fibrosis assessment in NAFLD patients

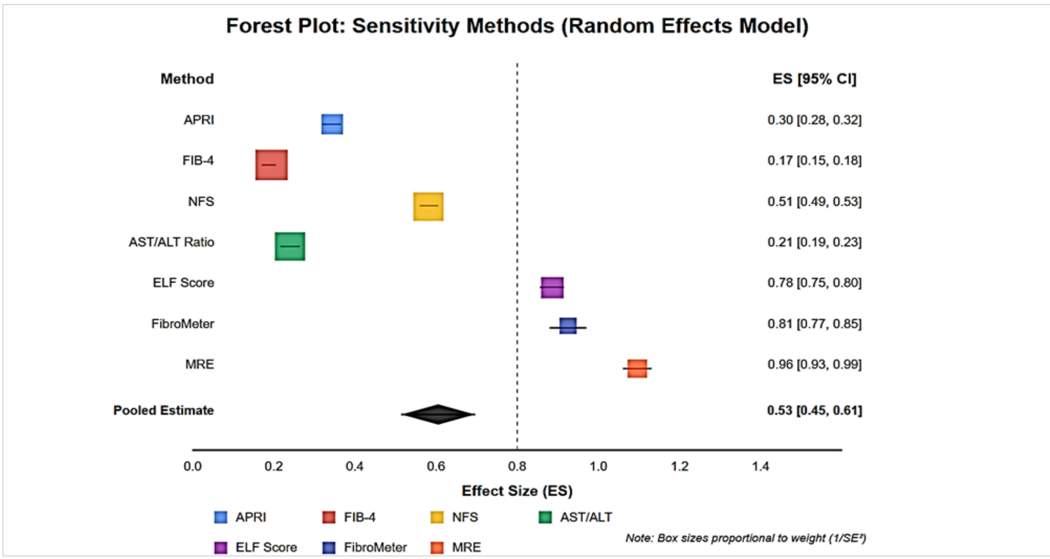

Figure 2 b): Forest plot of sensitivity for liver fibrosis diagnosis in NAFLD patients for various NITs

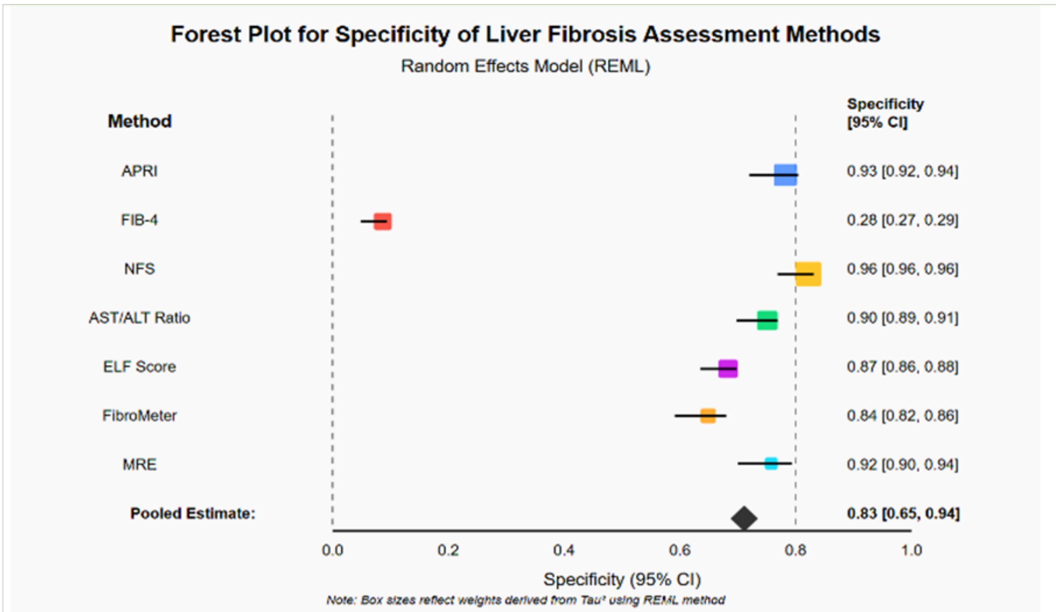

Figure 2 c): Forest plot of specificity for liver fibrosis diagnosis in NAFLD patients for various NITs

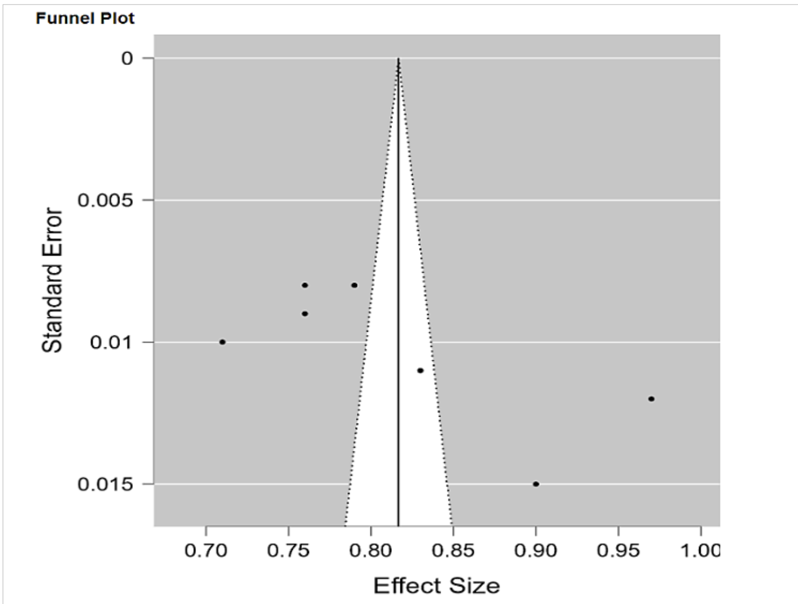

Figure 3: Funnel plot

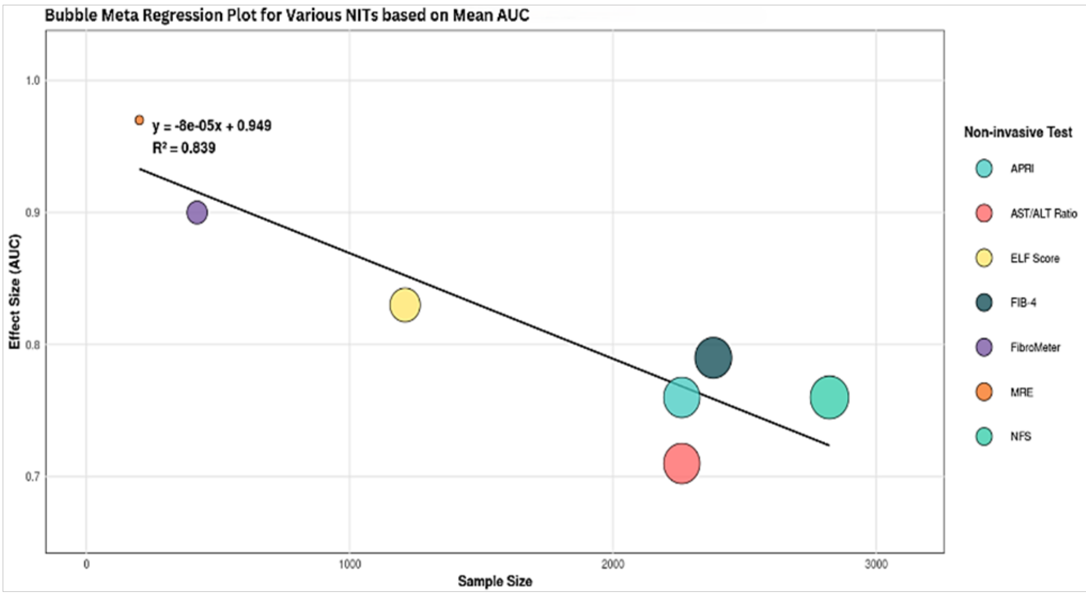

Figure 4 a): Bubble meta regression plot for various NITs based on mean AUC

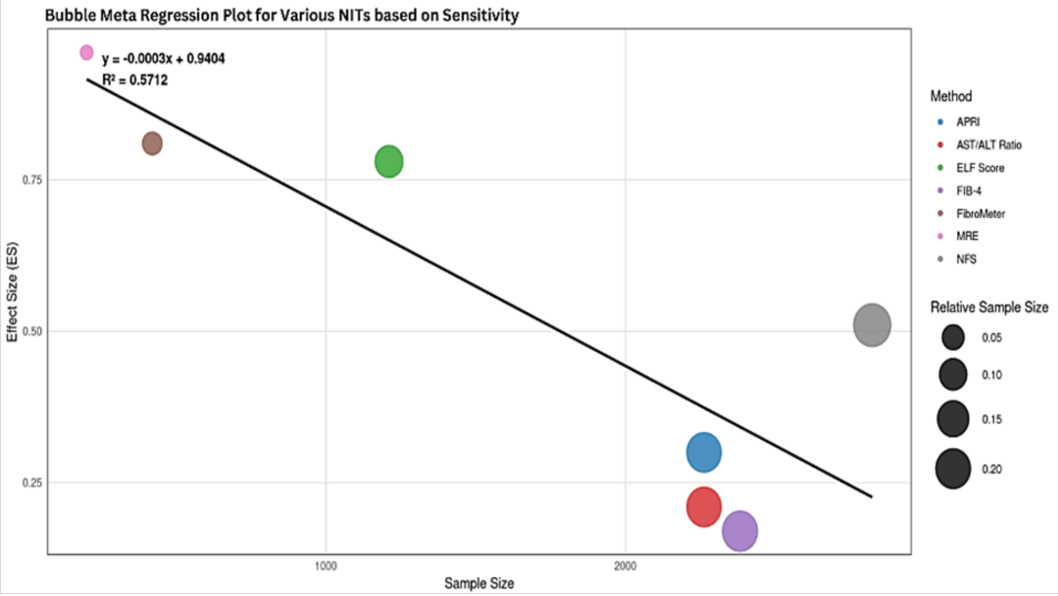

Figure 4 b): Bubble meta regression plot for sensitivity

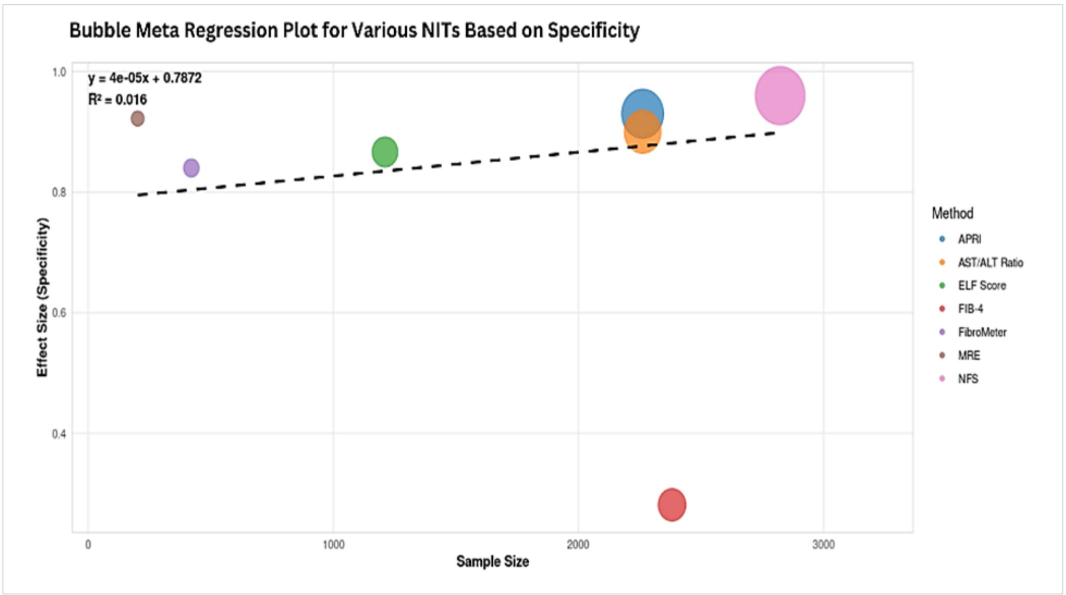

Figure 4 c) Bubble meta regression plot for specificity

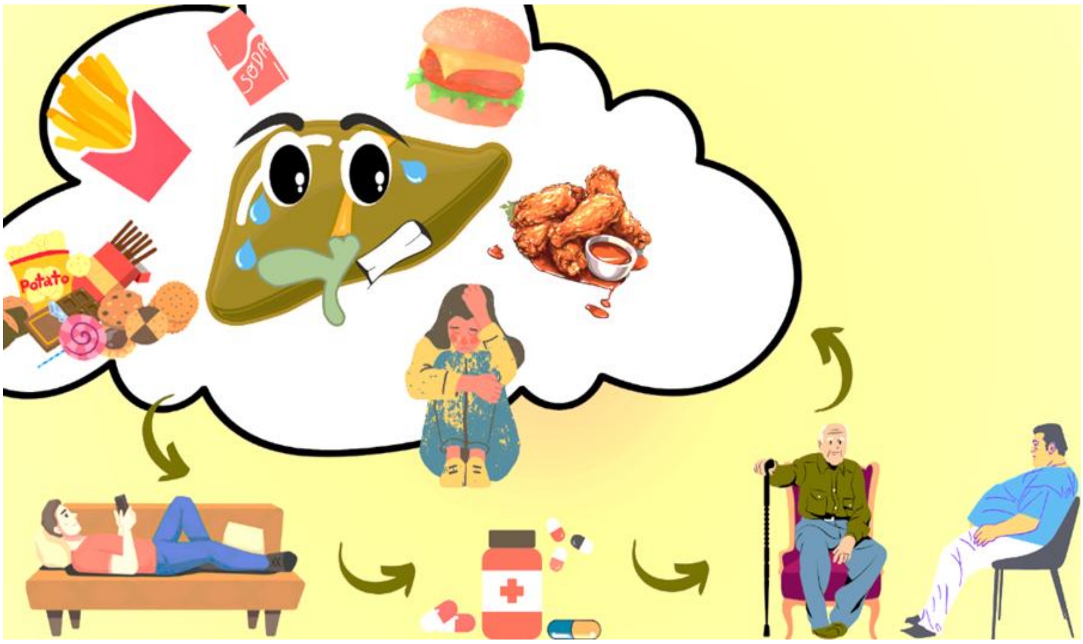

Figure 5: Etiological factors for NAFLD/MASLD

Statistical analysis

SPSS version 28 was used for data analysis. R Studio was used for plotting graphs.

Discussion

An author conducted a study on 2D-MRE, which showed an AUROC of 0.957 for predicting advanced fibrosis showing better outcomes than the FIB-4 clinical prediction rule, which had an AUROC of 0.861 confirming that MRE was a very accurate tool for diagnosing advanced fibrosis in people with NAFLD (Cui J et al, 2016). This was further elucidated upon in another study. The author reported that the AUROC was higher for MRE in comparison to FIB-4 and that it presented higher accuracy, however the study compared the combination of MRE and FIB-4 with MRE alone as well to report that the two-step strategy could be used in large population, its diagnostic accuracy was comparable to MRE alone and that it could be a better cost-effective method (Tamaki N et al, 2023).

Another author emphasized the important role of non-invasive tests for assessing advanced fibrosis and stated that advanced fibrosis is one of the strongest predictors of mortality. This study supported the application of non-invasive tests such as FIB-4 and NFS that are already commonplace in clinical contexts. The author's remarks accentuate the growing need for accurate diagnostic features with the increasing incidence of NAFLD. However, another study reported that FIB-4 and NFS were totally unreliable for liver fibrosis estimation in NAFLD patients (Hazzan R et al, 2024).

Jeffrey Cui examined MRE and ARFI and concluded that MRE was more accurate for diagnosing any level of fibrosis, especially in overweight patients. Moreover, it emphasized MRE's potential as a preferred non-invasive diagnostic tool for doctors managing NAFLD in clinical settings. However, another study concluded that ARFI showed higher diagnostic value when compared to other NITs such as FIB4, NFS, APRI and that it was a valuable tool in screening of advanced fibrosis in 136 NAFLD patients (Kang Y W et al, 2024).

In another study, an author discovered that 3D-MRE had a very high AUROC score of 0.981 when diagnosing advanced fibrosis. This score was noticeably higher than those for 2D-MRE and ARFI suggesting that 3D-MRE could be a more effective non-invasive diagnostic tool (Loomba R et al, 2016). These findings align with previous author's research, which also highlighted the benefits of MRE compared to traditional methods (Cui J et al, 2016). This was further elucidated upon in another study (Li M, 2021 et al).

Another author conducted a detailed review that underscored the effectiveness of non-invasive tests, particularly the NAFLD Fibrosis Score (NFS) and Vibration Controlled Transient Elastography (VCTE) (Cheah MC et al, 2017). The NFS demonstrated an area under the receiver operating characteristic curve (AUROC) of 0.85 when it came to predicting advanced fibrosis. In comparison, VCTE outperformed it with an impressive AUROC of 0.93, proving to be a valuable tool for diagnosing significant fibrosis. This was further supported by another study that reported that VCTE was superior with higher AUROC in comparison to NFS and had a lower misclassification rate (Tapper EB et al, 2016).

Another study also highlighted the importance of liver fibrosis in forecasting long-term outcomes for patients with NAFLD (Yoneda M et al, 2018). This review revealed that VCTE achieved an AUROC of 0.85 when it comes to identifying significant fibrosis (≥F2) and an impressive 0.92 for detecting advanced fibrosis (≥F3). Moreover, Magnetic Resonance Elastography (MRE) was acknowledged for its AUROC of 0.90 in identifying advanced fibrosis, which further supports the dependability of non-invasive methods in clinical settings. This was further supported by another who stated that NITs such as VCTE and other NITs showed good diagnostic accuracy for fibrosis assessment however met the minimum c riteria to show notable sensitivity and specificity rates (Selvaraj EA et al, 2021).

An author indicated that the Fibrosis-4 Index (FIB-4) and the NAFLD Fibrosis Score (NFS) were useful for screening advanced fibrosis in patients suffering from nonalcoholic fatty liver disease (NAFLD) (Siddiqui MS et al, 2019). The results exhibited C-statistics of 0.80 for FIB-4 and 0.78 for NFS, which showed very good diagnostic accuracy. In addition, the severity of fibrosis stage was significantly associated with changes in scores of APRI, FIB-4 and NFS, and C-statistics for proving progression to advanced fibrosis were 0.82 for APRI, 0.81 for FIB-4 and 0.80 for NFS. These results stressed upon the value of these models for non-invasive monitoring of disease progression. However, the reliance on a population with known advanced fibrosis may restrict the scope of the findings. However, the opposite was proposed by another author who reported that FIB4 and NFS proved to be suboptimal in the screening procedure due to high risk of overdiagnosis (Graupera I et al, 2022).

A study showed that the Enhanced Liver Fibrosis (ELF) score and Liver Stiffness Measurement (LSM) provided superior diagnostic accuracy for fibrosis staging compared to FIB-4 and NFS (Staufer K et al, 2019). The ELF score achieved an area under the receiver operating characteristic curve (AUROC) of 0.85 for significant fibrosis and 0.90 for advanced fibrosis. This aligned with Siddiqui's results, as both emphasized upon the importance of non-invasive tests in accurately assessing liver fibrosis. The author's findings further validated the clinical utility of the ELF score, particularly in a cohort with histologically confirmed NAFLD. This was further elucidated upon by yet another author who stated that the combination of ELF and LSM proved beneficial with increased specificity and PPC in comparison to ELF alone (Inadomi C et al, 2020).

Another author reported high pooled sensitivity and specificity for numerous stages of fibrosis by Magnetic Resonance Elastography with Area under the Receiver Operating Characteristic Curve (sROC) of 0.89 for F ≥1 and 0.94 for F ≥4 supporting the efficiency of FIB-4 and NFS (Liang Y, Li D, 2020). This was supported by another study that suggested that MRE was a quick and easy tool in the diagnosis of liver fibrosis even though dynamic contrast enhanced MRE could be used an alternative in its absence (Ren H et al, 2024).

Another author studied the effectiveness of the ELF test for diagnosing liver problems (Vali Y et al, 2020). The test showed a high sensitivity of 0.93 for ruling out advanced fibrosis when the cut-off point was set at 7.7 indicating that it was 93% effective at identifying people without the disease.

The test was not very specific, with a score of 0.34 meaning that it often showed positive results when the cut-off points were set higher. It's important to select the right cut-off numbers, especially when the disease is common.

An author confirmed that the ELF test was effective at identifying advanced liver fibrosis. The study showed that the ELF test had an AUROC of 0.81, meaning that it was quite accurate when compared to biopsy results (Younossi ZM et al, 2021). An ELF score of 9.8 or more indicated a 95% chance of having advanced fibrosis. This finding was similar to what another researcher who discovered about the ELF score's accuracy in diagnosis indicating that the test can be reliably used with different groups of people (Staufer K et al, 2019).

Yet another author shared important results on testing for serious liver disease, known as advanced fibrosis (Mozes FE et al, 2022). He reported AUROC for three methods: LSM-VCTE at 0.85, FIB-4 at 0.76, and NFS at 0.73 suggesting that using FIB-4 and LSM-VCTE together could help doctors avoid many liver biopsies, which are invasive. This idea aligned with findings by previous two authors (Siddiqui MS et al, 2019; Younossi ZM et al, 2021), who also emphasized the benefits of non-invasive tests in reducing the need for invasive procedures. This idea of combination of different modalities to enhance the performance of NITs was further discussed in another study (Lai).

Another study shed light on how effectively FIB-4 and VCTE can forecast liver-related events (LREs) in individuals with NAFLD. The results indicated that FIB-4 achieved a Harrell's C-index of 0.817, while VCTE had a score of 0.878, demonstrating their robust predictive capability (Boursier J et al, 2022). Patients with VCTE measurements greatly beyond 12.0 kPa faced a 12.4 times substantially greater risk. This research highlights the critical role of integrating non-invasive tests into clinical settings to enhance patient management.

Another author provided a thorough overview of the latest advancements in non-invasive assessments for liver fibrosis in NAFLD (Sanyal AJ et al, 2023). The review spotlighted the Enhanced Liver Fibrosis (ELF) test, which showed an AUROC of 0.85 for advanced fibrosis (≥F3), while VCTE scored even higher with an AUROC of 0.92 for the same stage. In addition to this, the FIB-4 index and NFS evaluations revealed that FIB-4 and NFS had AUROC values of 0.84 and 0.81 respectively in case of advanced fibrosis. The review suggested VCTE as a crucial tool for achieving superior accuracy in comparison to other methods. Similar findings echoed in another study (Chon YE et al, 2024).

Most recently, another study explored the current landscape and future directions for non-invasive diagnostic methods in NAFLD (Wang JL et al, 2024). The review brought attention to promising non-invasive biomarkers, such as 20-carboxy arachidonic acid (20-COOH AA) and 13,14-dihydro-15-keto prostaglandin D2 (dhk PGD2), which displayed diagnostic accuracies with AUROCs of 0.95 and 0.93, respectively, for diagnosing NAFLD. Imaging techniques like three-dimensional magnetic resonance elastography(3D-MRE) were reported to have an AUROC of 0.981 in diagnosis of significant fibrosis greater than or equal to F2. The study also reported FM-fibro LSM index to have an AUROC of 0.943 in case of advanced fibrosis greater than or equal to F3. It shed light on the need for conducting larger cohort studies to support these findings and improve diagnostic accuracy.

These studies highlighted the importance of non-invasive tests in assessing liver fibrosis in NAFLD for detecting advanced fibrosis. MRE (especially with using 3-dimensional MRE) and ELF score showed the best results followed by FIB-4 Index and NFS. Using these tests can be very helpful and can be used as a non-invasive alternative to liver biopsy, giving more certainty and improving patient management while avoiding unnecessary invasive procedures. Preference of test will be guided by clinical context, availability and particular patient characteristics. The etiological factors causing NAFLD such as junk fatty fried and sugary food, genetic factor, obesity, sedentary lifestyle, stress and certain medications (Satapathy SK et al, 2015) primarily were illustrated (**Figure 5**).

The important findings and merits and gaps were tabulated (**Table 3 and 4**).

Conclusion

The crucial role of non-invasive tests was highlighted in our systematic review and meta-analysis in improving the accuracy of advanced fibrosis diagnosis in NAFLD patients. Integrating tests like enhanced liver fibrosis score (ELF), fibrosis 4 index (FIB-4) and magnetic resonance elastography (MRE) can help further in enhancing the precision of diagnosis.

ELF and MRE were found to be the suitable alternative to liver biopsy. This is a gold standard but an invasive test. In addition to this FIB 4 and NAFLD Fibrosis score (NFS) also showed variation in specificity and sensitivity for significant fibrosis estimation.

The combination of non-invasive tests aids in not only comprehending the progression of disease but also helps in treatment optimization enhancing patient outcomes with increasing global prevalence of NAFLD. This will help in timely intervention and addressing of public health challenges and more future researches should be conducted to overcome the enigma of NAFLD.

Abbreviations

NAFLD: Non-alcoholic Fatty Liver Disease
MASH: Metabolic Dysfunction-associated Steatotic Liver Disease
NASH: Non-alcoholic Steatohepatitis
MRE: Magnetic Resonance Elastography
ELF: Enhanced Liver Fibrosis Test
FIB-4: Fibrosis-4 Index
NFS: NAFLD Fibrosis Score
VCTE: Vibration Controlled Transient Elastography
ARFI: Acoustic Radiation Force Impulses
APRI or AST/ALT ratio: Aspartate Aminotransferase to Platelet Ratio Index
LSM: Liver Stiffness Measurement

Declaration

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Conflicts of interests

The authors report no conflict of interest.

Ethical approval

Not required because the study was systematic review and meta analyses including the studies published between 2016 to 2024.

Consent to publication

Not applicable

Availability of supporting data

Not applicable as the study is a systematic review and meta-analyses.

Author contributions

Conceptualization and methodology, S.S. and S.H.; Formal analysis, S.S., S.H., J.H..; Visualization and writing – original draft S.S., S.H. and J.H.; Writing – review and editing, S.S., S.H. and J.H. All authors have read and agreed to the final version of the manuscript.

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