ΠΡΟΓΡΑΜΜΑ ΜΕΤΑΠΤΥΧΙΑΚΩΝ ΣΠΟΥΔΩΝ

«ΚΑΡΔΙΟΜΕΤΑΒΟΛΙΚΗ ΙΑΤΡΙΚΗ»

ΕΘΝΙΚΟ ΚΑΙ ΚΑΠΟΔΙΣΤΡΙΑΚΟ ΠΑΝΕΠΙΣΤΗΜΙΟ ΑΘΗΝΩΝ

ΙΑΤΡΙΚΗ ΣΧΟΛΗ

ΔΙΠΛΩΜΑΤΙΚΗ ΕΡΓΑΣΙΑ

ΘΕΜΑ: «Systematic review of the microRNAs association with metabolic dysfunction associated steatotic liver disease (MASLD)»

«Συστηματική ανασκόπηση της συσχέτισης των microRNAs με τη λιπώδη νόσο του ήπατος σχετιζόμενη με μεταβολική δυσλειτουργία»

ΑΝΤΩΝΙΟΥ ΕΛΕΝΗ

Α. Μ.: 7450752200001

ΕΠΙΒΛΕΠΩΝ ΚΑΘΗΓΗΤΗΣ: ΟΙΚΟΝΟΜΟΥ ΕΥΑΓΓΕΛΟΣ

ΣΥΝΕΠΙΒΛΕΠΟΝΤΕΣ ΚΑΘΗΓΗΤΕΣ: ΛΑΜΠΑΔΙΑΡΗ ΒΑΙΑ

ΚΑΣΣΗ ΕΥΑΝΘΙΑ

ΑΘΗΝΑ

(ΙΟΥΛΙΟΣ 2024)

ΠΕΡΙΛΗΨΗ

Εισαγωγή: Η μη αλκοολική λιπώδης νόσος του ήπατος (NAFLD) είναι μια νοσηρότητα που χαρακτηρίζεται από υπερβολική συσσώρευση λιπιδίων στο ήπαρ. Θεωρείται ένας τύπος νοσηρότητας του μεταβολικού συνδρόμου και αυξάνει τον κίνδυνο καρδιαγγειακής νόσου. Ο κίνδυνος καρδιαγγειακής νόσου είναι αυξημένος στη NAFLD, αλλά δεν σχετίζεται γραμμικά με τη σοβαρότητα της NAFLD. Η NAFLD και η στεφανιαία νόσος (CAD) έχουν κοινούς παθοφυσιολογικούς μηχανισμούς. Η δυσλιπιδαιμία, η οποία χαρακτηρίζει τη NAFLD, οδηγεί σε στεφανιαία σύνδρομα και ισχαιμική καρδιακή νόσο. Τα microRNAs αποτελούνται από 20-22 νουκλεοτίδια. Είναι μικρά μονόκλωνα μη κωδικοποιητικάRNAs που ρυθμίζουν τη μετα-μεταγραφική έκφραση των RNAs-στόχων τους. Τα MiRNAs προάγουν τη διάσπαση ή εμποδίζουν την έκφραση των συμπληρωματικών RNAs-στόχων τους. Τα MiRNAs ελέγχουν μια μεγάλη ποικιλία μεταβολικών μονοπατιών και μπορούν να βρεθούν σε πολλούς διαφορετικούς ιστούς. Η συγκέντρωση MiRNAs στον ορό, μεταβάλλεται αντιστρόφως ανάλογα σε σχέση με τη συγκέντρωση MiRNAs στο ήπαρ.

Μέθοδοι: Οι μηχανές βάσεων δεδομένων που χρησιμοποιήθηκαν κατά τη διάρκεια του Μαΐου 2024 ήταν η PubMed και η βιβλιοθήκη Cochrane. Οι λέξεις-κλειδιά αναζήτησης που επιλέχθηκαν ήταν : ("nonalcoholic fatty liver disease" OR "non alcoholic fatty liver disease" OR "NAFLD" OR "metabolic dysfunction fatty liver disease" OR "MAFLD" OR "fatty liver" OR "fatty liver disease" OR "steatosis" OR "liver steatosis" OR "hepatic steatosis" OR "steatohepatitis" OR "NASH") AND ("miRNA" OR "microRNA" OR "MicroRNA" OR "MicroRNA" OR "small noncoding RNA " OR "small- noncoding RNA" OR "MICRO RNA" OR "MICRORNA" OR "small temporal RNA"

OR "MIR" OR "MIRNA" OR "small regulator RNA" OR "small-regulator RNA" OR "micro ribonucleic acids" OR "micro-ribonucleic acids" OR "smallRNA" OR "noncoding RNA" OR "non-codingRNA" OR "sncRNA"). Η παρούσα ανασκόπηση ακολούθησε τις κατευθυντήριες γραμμές του Prisma 2020.

Από 1925 άρθρα επιλέχθηκαν 44 για την παρούσα ανασκόπηση.

Αποτελέσματα: Το Mir122 θεωρείται ότι αποτελεί το 70% των ηπατικών miRNA. Τα επίπεδα του mir122 στον ορό σχετίζονται με τη σοβαρότητα της στεάτωσης. Η ήπια στεάτωση χαρακτηρίζεται από χαμηλότερα επίπεδα mir122 από τη μέτρια στεάτωση, ενώ η μέτρια στεάτωση έχει ακόμη χαμηλότερα επίπεδα mir122 στον ορό από τη σοβαρή στεάτωση. Αν και η ίνωση θεωρείται πιο σοβαρή παθολογία από τη στεάτωση, το mir122 είναι χαμηλότερο στην ίνωση και ιδιαίτερα στη σοβαρή ίνωση. Το mir122, ως βιοδείκτης, παρέχει επίσης προγνωστικές πληροφορίες για τη NAFLD. Για 1 μονάδα μείωσης των επιπέδων του mir122 είναι 40% πιθανότερο για τον ασθενή να αναπτύξει NASH συγκριτικά με τη NAFLD (τα επίπεδα του mir122 μετρώνται σε σχέση με τα επίπεδα του mir23a). Το mir34αυξάνει την απόπτωση αυξάνοντας την ακετυλίωση του p53 και επάγοντας προαποπτωτικά γονίδια. Είναι επίσης αυξημένο στον ιστό του ηπατοκυτταρικού καρκινώματος (HCC). Το Mir34a είναι υψηλότερο στη NAFLD και περαιτέρω αυξημένο σε ασθενείς με στεάτωση. Τα πρώιμα στάδια ίνωσης χαρακτηρίζονται από υψηλότερα επίπεδα σε σύγκριση με τη NAFLD, ενώ η σοβαρή ίνωση χαρακτηρίζεται από χαμηλότερα επίπεδα mir34a στον ορό σε σύγκριση με τους ελέγχους. Το mir29a προστατεύει το ήπαρ από τη λιποτοξικότητα με επιγενετικές τροποποιήσεις και μεταβολές στην ομοιόσταση των μιτοχονδρίων. Το mir193-5p μπορεί να θεωρηθεί ως προοδευτικός βιοδείκτης της NAFLD. Επίσης, είναι πιο ευαίσθητος και ακριβής βιοδείκτης σε σύγκριση με το mir122 και το πάνελ

ALT/AST/FIB4. Τα επίπεδα mir192 των ασθενών με NAFLD είναι αυξημένα στην πρώιμη ίνωση. Η ηπατική στεάτωση ευνοείται από το mir132 σε ασθενείς με NAFLD. Η συγκέντρωση του mir9 στον ορό είναι υψηλότερη σε μέτρια έως σοβαρή NAFLD σε σύγκριση με την ήπια NAFLD και ακόμη υψηλότερα στη στεάτωση. Η μετάσταση στο HCC και η αντίσταση με τη μεσολάβηση της αυτοφαγίας καταστέλλεται από το mir30a, το οποίο έχει προστατευτικό ρόλο στην εξέλιξη του καρκίνου και τη μεταστατική συμπεριφορά. Οι ασθενείς με χαμηλότερα επίπεδα mir194 στον ορό διατρέχουν μεγαλύτερο κίνδυνο ανάπτυξης HCC. Τα mir194 και mir214 εμπλέκονται στην ανάπτυξη της εξωκυτταρικής ουσίας και μπορούν να χρησιμοποιηθούν για τη διάγνωση της NAFLD. Οι ασθενείς με NAFLD έχουν υψηλότερα επίπεδα του mir103 το οποίο εμπλέκεται στην αντίσταση στην ινσουλίνη. Το mir155 συμμετέχει στην καρκινογένεση και πολλαπλασιάζει την αντι-απόπτωση και τη μετάσταση. Το LNCrab11bas1 μπορεί να καταστείλει το mir426-5p και να αποτρέψει τον πολλαπλασιασμό, τη μετάσταση και την εισβολή του HCC αυξάνοντας τα επίπεδα του mRNA bab11b. Στη βιοψία ήπατος το mir21 χαρακτηρίζει τους ασθενείς με NASH, ενώ τα mir122 και mir192 μπορούν να βρεθούν τόσο στη NAFLD όσο και στη NASH. Το mir122-5p σχετίζεται με δυσλιπιδαιμία, ενώ τα επίπεδα του mir34-5p σχετίζονται με υψηλότερα επίπεδα CRP και φλεγμονώδη απόκριση. Το Mir122 που εκφράζεται στον ηπατικό ιστό έχει 10 φορές χαμηλότερα επίπεδα στη NASH σε σύγκριση με τη στεατοηπατίτιδα. Το HCC σχετίζεται με το mir182. Το mir301 είναι ένα βασικό miRNA που σχετίζεται με την εξέλιξη του HCC.

Συμπεράσματα: Αρκετές μελέτες έχουν αξιολογήσει το ρόλο των microRNAs στη NAFLD. Τα πιο μελετημένα microRNAs είναι τα mir122 και mir34. Αυξημένα επίπεδα mir34a και mir122 παρατηρούνται σε ασθενείς με NAFLD. Ειδικότερα, η ρύθμιση του mir122 στον ορό σχετίζεται με τη θνησιμότητα της NAFLD και σχετίζεται με τη σοβαρότητα της στεάτωσης. Το mir122

ελέγχει τον μεταβολισμό των λιπιδίων, ενώ το mir34a σχετίζεται με την αντίσταση στην ινσουλίνη. Παρόλο που τα microRNAs δεν έχουν εισαχθεί στην καθημερινή κλινική πρακτική, πάνελ microRNAs και βιοδεικτών χρησιμοποιούνται για την αξιολόγηση των ασθενών σε κίνδυνο NASH και επίσης δύναται να χρησιμοποιηθούν για την πρόβλεψη του ποσοστού του ηπατικού λίπους. Το NIS2+ είναι ένα διαγνωστικό τεστ που αποτελείται από δύο βιοδείκτες ορού (mir34a και YKL40) και είναι κατάλληλο για υποπληθυσμούς ανδρών και γυναικών και για άτομα με διαβήτη. Το NIS2+ πέτυχε ευαισθησία και ειδικότητα 85% για τον αποκλεισμό της NASH. Επίσης, υπάρχει μια ποσοτική μέθοδος πρόβλεψης του ηπατικού λίπους, με σφάλμα 4-5%, μέσω της ποσοτικοποίησης 23 miRNAs του ορού. Περαιτέρω μελέτες πρέπει να καταλήξουν στον ρόλο των microRNAs στην παθοφυσιολογία, την εξέλιξη, τη διάγνωση και τη διαχείριση του φάσματος της NAFLD.

Περιεχόμενα

1 Contents

Abstract

INTRODUCTION : Non-Alcoholic Fatty Liver Disease (NAFLD) is a board spectrum morbidity that is characterized by excessive lipids accumulation in the liver. It is considered to be a type of metabolic syndrome morbidity and elevates the risk of cardiovascular disease. Cardiovascular disease risk is elevated in NAFLD but it is not linear related to NAFLD severity. NAFLD and Coronary Artery Disease (CAD) have common pathophysiological mechanisms. Dyslipidemia, which characterizes NAFLD, leads to coronary syndromes and ischemic heart disease. MicroRNAs consist of 20-22 nucleotides. They are short single stranded non-codingRNAs which regulate the post-transcriptional expression of their target RNAs. MiRNAs promote the cleavage or block the expression of their complementary target RNAs. MiRNAs control a great variety of metabolic pathways and they can be found in many different tissues. Serum MiRNAs concentration is reversibly related to liver MiRNAs concentration.

MATERIAL AND METHODS: Database engines used during May 2024 were PubMed and Cochrane library. Search key words selected were : (“nonalcoholic fatty liver disease” OR “non-alcoholic fatty liver disease” OR “NAFLD” OR “metabolic dysfunction fatty liver disease” OR ”MAFLD” OR “fatty liver” OR “fatty liver disease” OR “steatosis” OR “liver steatosis” OR “hepatic steatosis” OR “steatohepatitis” OR “NASH”) AND (“miRNA” OR “microRNA” OR “MicroRNA” OR “small noncoding RNA “ OR “small-noncoding RNA” OR “MICRO RNA” OR “MICRORNA” OR “small temporal RNA” OR “MIR” OR “MIRNA” OR “small regulator RNA” OR “small-regulator RNA” OR “micro ribonucleic acids” OR “micro-ribonucleic acids” OR “smallRNA” OR “noncoding RNA” OR “non-codingRNA” OR “sncRNA”). This review followed Prisma 2020 guidelines.

From 1925 articles 44 were selected for this review.

RESULTS: Mir122 considers to be 70% of hepatic miRNA. Serum mir122 levels are related with the severity of steatosis. Mild steatosis is characterized by lower mir122 levels than moderate steatosis, while moderate steatosis has even lower mir122 serum levels than severe steatosis. Although fibrosis is considered to be a more severe pathology than steatosis, mir122 is lower in fibrosis and especially in severe fibrosis. Mir122, as a biomarker, it also confers prognostic information for NAFLD. For 1 unit decrease of mir122 levels it is 40% more likely for the patient to develop NASH comparatively to NAFLD (mir122 levels are measured relatively to mir23a levels). Mir34increases apoptosis by increasing p53 acetylation and inducting proapoptotic genes. It is also elevated in Hepatocellular Carcinoma (HCC) tissue. Mir34a is higher in NAFLD and further increased in patient with steatosis. Early fibrosis stages are characterized by higher levels of compared to NAFLD, while severe fibrosis is characterized by lower mir34a serum levels compared to controls. Mir29a protects liver from lipotoxicity with epigenetic modulations and alterations in homeostasis of mitochondria. Mir193-5p can be considered as a progressive NAFLD biomarker. Also, it is a more sensitive and accurate biomarker compared to mir122 and ALT/AST/FIB4 panel. NAFLD patient's mir192 levels are elevated in early fibrosis. Hepatic steatosis is favored by mir132 in NAFLD patients. Mir9 serum concentration is higher in moderate to severe NAFLD compared to mild NAFLD and even higher in steatosis. Metastasis in HCC and autophagy mediated resistance is repressed by mir30a, which has a protective role in the cancer progression and metastatic behavior. Patients with lower mir194 serum levels are in greater risk of developing HCC. Mir194 and mir214 are involved in the extracellular matrix development and can be used to diagnose NAFLD though all its stages. NAFLD patients have higher levels of mir103

which is involved in insulin resistance. Mir155 participates in tumorigenesis and proliferates anti-apoptosis and metastasis. LNCrab11bas1 can repress mir426-5p and prevent HCC proliferation, metastasis and invasion by elevating bab11b mRNA levels. In liver biopsy mir21 characterizes NASH patients, while mir122 and mir192 can be found in both NAFLD and NASH. Mir122-5p is related with dyslipidemia while mir34-5p levels are related with higher CRP levels and inflammatory response with proinflammatory cytokines. Mir122 expressed in the liver tissue has 10-fold lower levels in NASH compared to steatohepatitis. HCC is related with mir182. Mir301 is a key miRNA associated with HCC progression.

CONCLUSION : Several studies have evaluated the role of microRNAs in NAFLD. The most studied microRNAs are mir122 and mir34. Elevated mir34a and mir122 levels are observed in NAFLD patients. Especially, upregulation of serum mir122 is associated with NAFLD mortality and is related with steatosis severity. Mir122 controls lipid metabolism while mir34a is related to insulin resistance. Although microRNAs have not been introduced in everyday clinical practice, panels of microRNAs and biomarkers are used to evaluate patients in risk of NASH and also predict hepatic fat percentage. NIS2+ is a screening NAFLD test that consist of two serum biomarkers (mir34a and YKL40) and it is appropriate for male and female subpopulations and for individuals with diabetes. NIS2+ achieved sensitivity of 85% for ruling out at risk NASH and specificity of 85% to rule in at-risk NASH. Also, there is a quantitate method of predicting hepatic fat, with an 4-5% error, by quantifying 23 serum miRNAs. Further studies need to conclude on the role of microRNAs in the pathophysiology, progression, diagnosis and management of NAFLD spectrum. **KEYWORDS**: nonalcoholic fatty liver disease, metabolic dysfunction fatty liver disease, steatosis, steatohepatitis, miRNA, non-codingRNA

Abbreviations

ABCG1 ATP Binding Cassette Subfamily G Member 1

ACC Acetyl-CoA carboxylase

ACC Acetyl-CoA carboxylase

AKT AKT Serine/Threonine Kinase

ALT: Alanine Aminotransferase

AMPK Adenosine monophosphate-activated protein kinase

ARHGAP1 Rho GTPase-activating protein 1

AST: Aspartate Aminotransferase

AUC Area Under the Curve

AUC: Area Under the Curve

BMI: Body Mass Index

CAD: Coronary Artery Disease

CAMP Cyclic adenosine monophosphate

CCL20 C-C Motif Chemokine Ligand 20

CHREBP Carbohydrate-responsive element-binding protein

CK18-Asp396 caspase-cleaved cytokeratin 18

CNM1 Cyclin and CBS Domain Divalent Metal Cation Transport Mediator 1

COL1A1 Collagen Type I Alpha 1 Chain

COX2 Cyclooxygenase-2

CRP C-reactive protein

CRP: C-reactive Protein

DGRC8 DiGeorge syndrome critical region 8

EVS: Extracellular Vehicles

FABP Fatty Acid Binding Protein

FABP1 Fatty Acid-Binding Protein 1

FASN Fatty Acid Synthase

FFA: Free Fatty Acids

FIB4: Fibrosis Index 4

FOA2 Forkhead box transcription factor 2

GA Growth Arrest

GCKR Glucokinase Regulatory Protein

GLUT4 Glucose transporter type 4

GP8 Glutathione peroxidase 8

GSDMD Gasdermin D

HBP1a Histone-specific transcription factor a

HCC: Hepatocellular Carcinoma

HCV: Hepatitis C Virus

HDL: High Density Lipoprotein

hist2h2be Histone H2B type 2-E

HMG-CoA-reductase

HMGCR 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase

HSC Hematopoietic stem cell

HSPD1 Heat Shock Protein Family D (Hsp60) Member 1

IGF2 Insulin-Like Growth Factor 2

IL6 Interleukin 6

IRS1 Insulin Receptor Substrate 1

ITGB1 Integrin Subunit Beta 1

JACK Janus kinase

KLF3 Krüppel-like Factor 3

LDL: Low Density Lipoprotein

LDLR Low Density Lipoprotein Receptor

LKB1 Liver kinase B1

LncRNA: Long non-coding Ribonucleic Acid

LPL Lipoprotein lipase

LXa: Liver X receptor a

MAFLD Metabolic Dysfunction Associated Fatty Liver Disease

MAFLD: Metabolic dysfunction associated fatty liver disease

MiRNA: Micro Ribonucleic acid

MMP-8 Matrix Metallopeptidase 8

MRI: Magnetic Resonance Imaging

MTHFR Methylenetetrahydrofolate reductase

MTOR Mammalian target of rapamycin

MYD88 MYD88 Innate Immune Signal Transduction Adaptor

NAD Nicotinamide adenine dinucleotide

NAFLD: Non-Alcoholic Fatty liver Disease

NASH: Non-alcoholic steatohepatitis

NEAT 35

NFKBP65 nuclear factor NF-kappa-B p65 subunit

PAI-1 Plasminogen Activator Inhibitor 1

PCSK7 Proprotein Convertase 7

PGC-1b peroxisome proliferator-activated receptor-γ coactivator 1β

PGSK-3B Glycogen synthase kinase 3beta

PI3K Phosphatidylinositol-4,5-Bisphosphate 3-Kinase

PNPLA3 Patatin Like Phospholipase Domain Containing 3

PNPLA3 Patatin-like phospholipase domain-containing protein 3

PPARa Proliferator-activated receptor a

PPARG Peroxisome Proliferator Activated Receptor Gamma

PTEN Phosphatase and Tensin Homolog

RAB11B RAB11B, Member RAS Oncogene Family

RAP2A Ras-related protein Rap-2a

RNA: Ribonucleic Acid

RUBX1 Runt-related transcription factor 1

SCD1 Stearoyl-CoA desaturase 1

SIRT1 Silent information regulator Sirtulin 1

SLC10A1 Sodium/bile acid and sulphated solute cotransporter 1

SLC2A4 Solute Carrier Family 2 Member 4

SLC7A1 Solute Carrier Family 7 Member 1

SNHG Small Nucleolar RNA Host Gene

SRBP1c Sterol Regulatory Element-Binding Transcription Factor 1

SREBP1 Sterol Regulatory Element-Binding Transcription Factor 1

STAT3 Signal Transducer And Activator Of Transcription 3

STAT3 Signal transducer and activator of transcription 3

TGF4 Tumor growth factor 4

TIMP-2 TIMP Metallopeptidase Inhibitor 2

TLR4 Toll Like Receptor 4

TM6FS2 Transmembrane 6 superfamily member 2

TM6SF2 Transmembrane 6 Superfamily Member 2

TNF Tumor Necrosis Factor

UTR: Untranslated Region

WNT Wingless-Type

YKL40 Chitinase-3-like protein 1

1 Introduction

Non-Alcoholic Fatty Liver Disease (NAFLD) is a board spectrum morbidity that is characterized by excessive lipids accumulation in the liver (1). The NALFD classification is based on Histological findings. Biopsy is the golden standard examination that validates NAFLD diagnosis. A common characteristic of NAFLD is triglycerides aggregation in the liver tissue, this aggregation triggers liver tissue damage and inflammation.

NAFLD and Coronary Artery Disease (CAD) have common pathophysiological mechanisms. Dyslipidemia, which characterizes NAFLD, leads to coronary syndromes and ischemic heart disease. Especially in cirrhotic patients with NAFLD, CAD is present in 21.6% while in cirrhotic patients of other etiologies (viral or excessive alcohol consumption), CAD is present in 5% (2). Moreover, when CAD and NAFLD coexist, more complex CAD is observed. Usually more than 3 coronary arteries are involved and plaques are more vulnerable and unstable (3).

NAFLD diagnosis and follow up biomarkers are limited and not highly accurate, while imaging techniques have their limitations when examined alone for NAFLD staging NAFLD .

MicroRNAs are promising biomarkers. MicroRNAs consist of 20-22 nucleotides. They are short single stranded non-codingRNAs which regulate the post-transcriptional expression of their target RNAs. MiRNAs control a great variety of metabolic pathways and they can be found in many different tissues. MiRNAs are used as therapeutic targets in many diseases such as different cancer subtypes and neurodegenerating pathologies. Especially mir122 is a necessary Hepatitis

C Virus (HCV) co-factor enhancing the virus replication and thus it can be used as a therapeutic agent in HCV infections (4). MiRNAs expressed in the liver, blood, serum and visceral adipose tissue are the ones taken into consideration, especially the ones involved in the pathogenesis of NAFLD.

NAFLD and NASH are asymptomatic in the early stages of disease and liver enzymes are usually into the normal range even though the liver is steatotic. We study MiRNAs that are involved into NAFLD pathogenesis and which miRNAs can be used as early biomarkers for NAFLD diagnosis and prognosis. We also investigate how miRNAs can be used to distinguish between the different pathologies of the broad NAFLD's spectrum and evaluate their potential as diagnostic biomarkers.

2 In General

2.1 NAFLD

2.1.1 NAFLD Pathophysiology

Non-Alcoholic Fatty Liver Disease (NAFLD) is a board spectrum morbidity that is characterized by excessive lipids accumulation in the liver. Lipid accumulation >5%, without inflammation, can be considered as simple steatosis. If inflammation coexists with the lipid accumulation, then the clinical syndrome is known as steatohepatitis. When steatohepatitis remains and progresses, the persistence inflammation causes liver cell death and tissue scarring which leads to cirrhosis. A cirrhotic liver can be a great substrate for Hepatocytic cell cancer to occur, this cancer subtype is the 4th worldwide leading death cause (1). A common characteristic of NAFLD is triglycerides aggregation in the liver tissue, this aggregation triggers liver tissue damage and inflammation.

2.1.2 NAFLD Diagnosis

The NALFD classification is based on Histological findings. Biopsy is the golden standard examination that validates NAFLD diagnosis. Although biopsy is the best diagnostic method, it is costly and has many complications. For those reasons, it is needed to introduce new diagnostic tools. Fibro scan is a great diagnostic tool for low steatosis levels but is limited by patient's body mass index. Ultrasound sensitivity range as a diagnostic tool is between 60-94% and its specificity is estimated between 66-95%. Ultrasound can categorize NAFLD severity. Mild NAFLD is known for diffuse hyperechoic echo texture. Moderate NAFLD presents with increased echogenity of the liver compared to kidneys echogenity but at the same time the visual images of intrahepatic

vessels are clear. Severe NAFLD is characterized by vascular blurring and increased echogenity of the liver accompanied with poor penetration of the posterior segment of the liver and vessel images. Metabolic Dysfunction Associated Fatty Liver Disease (MAFLD) activity score stage 1 by biopsy is usually related with mild steatosis confirmed by ultrasound and stage 3 activity level of MAFLD is related with severe steatosis by ultrasound (5). Ultrasound is not considered to be a reliable diagnostic method when more than 30% of hepatocytes are steatotic. Computed tomography is a semi quantitative method with high accuracy for moderate to severe steatosis but not for mild steatosis. MRI is a great option for diagnosing steatosis (liver fat content can be estimated by proton density) but it is not easy accessibly and is costly. MRI is not also suitable for patients with metallic prosthesis and electronic devices. Apart from imaging tools, biomarkers in blood can be used to detect liver damage, however Liver enzyme levels can be normal in NAFLD (6–8). Overall, potential of biomarkers for NAFLD diagnosis and follow up is limited with low levels of accuracy. Imaging techniques have their limitations especially in regard to NAFLD staging. NAFLD and NASH are asymptomatic in the early stages of disease and liver enzymes are usually into the normal range even though the liver is steatotic.

2.1.3 NAFLD and CAD

NAFLD and Coronary Artery Disease (CAD) have common pathophysiological mechanisms. Dyslipidemia, which characterizes NAFLD, leads to coronary syndromes and ischemic heart disease. Dyslipidemia and especially the heterogeneity of cholesterol molecules observed in NAFLD are causing lipotoxicity and elevated oxidative stress. Vascular inflammation, due to oxidative stress, leads to endothelial dysfunction and damage, which causes plaque rupture.

Rupture of atheromatous plaque results in thrombosis. NAFLD is characterized by a procoagulant status and impaired fibrinolysis due to elevated PAI-1 levels. Liver is the main determinant of PAI-1 levels. Liver dysfunction increase PAI-1 levels and hence thrombotic potential. Especially in cirrhotic patients with NAFLD, CAD is present in 21.6% while in cirrhotic patients of other etiologies (viral or excessive alcohol consumption), CAD is present in 5% (2). Moreover, when CAD and NAFLD coexist, more complex CAD is observed. Usually more than 3 coronary arteries are involved and plaques are more vulnerable and unstable (3).

BMI is usually elevated in both CAD and NAFLD. However, during the statistical analysis, when adjusting for BMI, in NAFLD patients the risk of CAD is independent of obesity. Diabetic patients develop CAD two times more often than the non-diabetic ones. This is explained by insulin resistance, while insulin dysfunction can enhance atherosclerosis progression and vulnerable plaque formation. PNPLA3, TM6FS2 and GCKR genes are related with NAFLD. PNPLA3 and TM6FS2 have protective action against CAD while GCKR is positively related with CAD (3,9).

In general, NAFLD is characterized by a hyperdynamic hemodynamics status due to elevated peripheral resistance, causing cardiac output to increase. As a result, diastolic and later systolic dysfunction occurs. Also, QT prolongation is observed in patients with both NAFLD and CAD (2,3,9–15)

NAFLD and cardiovascular disease are both pathologies related to metabolic syndrome. NAFLD is caused by lipid accumulation in the liver and cardiovascular disease is caused by coronary arteries atheromatosis.

2.2 MiRNAs

2.2.1 Function

MicroRNAs are promising biomarkers. MicroRNAs consist of 20-22 nucleotides. They are short single stranded non-codingRNAs which regulate the post-transcriptional expression of their target RNAs. Their role is to bind to the 3' untranslated region of their target messenger RNA and repress its expression. RNApolymerase 2 assists with the transcription of mRNA genes. The outcome of this transcription is monosistronic, bisostronic and polysystronic miRNA precursors. MiRNAs have complementary target RNAs and block those target RNAs translation or promotes their cleavage (16). Mirna processing takes part in the nucleus starting with the transcription of the miRNA gene. The product of primiRNA contains 1000 nucleotides. After transcription endonuclease DROSHA and its cofactor DGRC8 are producing a mature 70 nucleotide with an accurate 3' end. The premiRNA is then exposed to the cytosol. Cytosolic dicer 1 cleaves the premiRNA and generate the 5' premiRNA's end. The final miRNA product consists of 19-23 nucleotides mature miRNA strand. The mature final miRNA can now translocate and silence it's targets gene expression (17). MiRNAs control a great variety of metabolic pathways and they can be found in many different tissues (18–20). In this review miRNAs expressed in the liver, blood serum and visceral adipose tissue are the ones taken into great consideration, especially the ones involved in the pathogenesis of NAFLD. MicroRNAs can be found as free forms in the serum or binding to exosomes. Exosomes are considered as a category of extracellular vehicles. Extracellular vehicles are small membrane band particles, usually have a diameter smaller than 150nm. They can be extracted by tissues and can be released by all cell types into blood, urine

and other body fluids. They originate from endocytic pathways assisting in cell membrane internalization which also leads to internalization of receptors, fluids and macromolecules. EVS can contain nucleic acids, proteins and lipids from the cell they originate from. MiRNAs are binding to Argonaut proteins and HDL. Especially human Ago2 participates in the catalytic pathway of mRNA degeneration. Mir122 is circulating as a free serum form binding to Ago2 and other EV's with ribonucleoprotein vehicles. Protein associated fraction are always been detected with mir122 leading to the conclusion that mir122 is always accompanied by a protein carrier (21–23).

2.3 MiRNAs and NAFLD

Serum MiRNAs concentration is reversibly related to liver MiRNAs concentration. This is observed because miRNAs related to hepatocytes' damage are accumulated into the hepatocytes leading to their inflammation, fibrosis and eventually apoptosis. During Hepatocytes' apoptosis, miRNAs stored into the cells are released. Especially mir122 is elevated in serum after apoptosis of hepatocytes. Apart from mir122, also mir192 is associated with liver biochemistry and elevated liver enzymes after hepatocytes membrane rupture during apoptosis (24).

3 Methods

3.1 Search Strategy

A systematic literature search was conducted. Database engines used during May 2024, were PubMed and Cochrane library. Search key words selected were : (“nonalcoholic fatty liver disease” OR “non-alcoholic fatty liver disease” OR “NAFLD” OR “metabolic dysfunction fatty liver disease” OR ”MAFLD” OR “fatty liver” OR “fatty liver disease” OR “steatosis” OR “liver steatosis” OR “hepatic steatosis” OR “steatohepatitis” OR “NASH”)AND(“miRNA” OR “microRNA” OR “MicroRNA” OR “small noncoding RNA “ OR “small-noncoding RNA” OR “MICRO RNA” OR “MICRORNA” OR “small temporal RNA” OR “MIR” or “MIRNA” OR “small regulator RNA” OR “small-regulator RNA” OR “micro ribonucleic acids” OR “micro-ribonucleic acids” OR “smallRNA” OR “noncoding RNA” OR “non-codingRNA” OR “sncRNA”). This review followed Prisma 2020 guidelines.

3.2 Selection Process

From this systematic review were excluded: Reviews, systematic reviews, case reports, conference abstracts, thesis, editorials, duplicate studies, animal-based data, not full papers, case reports, letters to editor, articles that involved specific populations such as only children or only women studies and articles without sufficient efficacy endpoints or without clear methods. After scanning articles titles and abstracts, an eligibility full text screening was conducted in order to select the most relevant original articles. Due to result’s heterogeneity, a narrative aspect of

review was followed. All articles selected were peer reviewed and published in English language. From the 1925 articles screened, 44 finally include in this systematic review ***(Figure 1)***. Records were identified from 2 databases (PubMed and Cochrane) and the number of total registers was 1925. Before screening, 19 duplicate records were excluded. Also, 1.591 other articles were removed. From those 1.591 articles, 287 articles were reviews, while 6 were systematic reviews. The population studied in 34 related articles was pediatric and 256 studies concerned women only population, 983 were animal studies and finally 25 articles weren't written in English language so they were excluded. All articles were retrieved, 11 articles weren't included due to insufficient efficacy endpoints and unclear methods.

3.3 Data Collection Process

Information was selected and categorized by miRNA type and origin (serum Mirna, liver Mirna, visceral adipose tissue Mirna) and by NAFLD's spectrum of disease (steatosis , fibrosis and HCC). From 44 articles only 14 concerned about serum miRNAs expressed in NAFLD, 18 concerned about serum miRNAs expressed in steatosis, 8 discussed about serum miRNAs in fibrosis, 10 mentioned about serum miRNAs in HCC, 2 mentioned about tumoral miRNA expression in HCC, 4 articles mentioned about liver miRNAs associated with steatosis, 2 mentioned liver expression of miRNAs associated with HCC, 2 mentioned about visceral adipose tissue miRNAs associated with steatosis and 2 concerned about ascetic fluid miRNAs in HCC ***(Table 1)***.

From 44 articles, 3 article contained information about Japanese individuals, 2 article concerned Egyptian individuals, 2 articles studied Chinese individuals and the rest 37 articles

studied Caucasian individuals. A broad spectrum of BMI, from lean to overweight and obese was observed in individuals from both sexes.

4 Results

4.1 Serum miRNAs

From 44 articles, 13 concerned about serum miRNAs expressed in the board spectrum of NAFLD pathology ***(Table 2)***. Elevated mir34a and mir122 levels are observed in NAFLD patients (6). Elevated serum mir122 is associated with NAFLD mortality and is related with steatosis severity (6). Mir122 controls lipid metabolism while mir34a is related to insulin resistance (25). Mir193-5p is a more accurate biomarker than mir122 for NAFLD staging (1).

4.1.1 NAFLD

4.1.1.1 Serum Mir122 in NAFLD

Mir122 controls lipid metabolism by controlling the activity of enzymes involved in lipid deposition metabolic pathway such as acetyl-coA-carboxylase 2, fatty acid synthase, sterol regulatory binding proteins, HMG-CoA reductase. Especially HMG-CoA-reductase is considered to be a leading enzyme in triglycerides hydrolysis. Mir122 inhibition leads to increasing LPLGLUT4 FABP activity. Increased GLUT4 concentration and activity, leads to increasing transport of glucose into the cells which causes the insulin receptor to desensitize. Insulin receptor desensitizing promotes lower expression of IRS1, SREBP1, FAS, FOA2 under the signaling of insulin molecule, while on the other hand, it increases the level of IL6, TNFA, CCL2, CCL3, CCR4 chemokines. Cholesterol induces lysosomal dysfunction by increasing exosomal released mir122-

5p from the hepatocytes. Exosomes released from cells are filled with cholesterol (exosomes with high cholesterol levels). Cholesterol causes M1 polarization and macrophage induced inflammation. Also, mir122-5p elevates inos-1 expression which is a macrophage marker (21,22).

Mir122 inhibition, inhibits TLR4/MYD88/NFKBP65 signaling pathway which contributes to lipid accumulation. AlsoIL6, IL8, TNF-α, cox2 were inhibited after mir122 inhibition. As a result, we can assume that mir122 inhibition reduces lipid accumulation and reduces the inflammatory state whose are the two main pathophysiological marks leading to NAFLD. Hepatocytes release mir122 to blood circulation after their apoptosis due to liver inflammation (23,24). Low circulating serum levels of mir122 is an independent factor of increased mortality in NAFLD patients (26).

Molecules that mimic mir122 reverse liver steatosis, fibrosis and insulin resistance, pathophysiological processes that characterize NAFLD. In contrast molecules that inhibit mir122 promote fibrosis progression in a dose dependent pattern, the more the liver organoid is exposed to mir122 inhibitors the more fibrotic tissue is accumulated. After mir122 inhibition, MMP-8, MMP-9 and their inhibitor TIMP-2 were overly expressed as well as lipoprotein lipase and SLC2A4GLUT. On the other side LDLD receptor was under expressed with mir122 inhibition (24).

4.1.1.2 Serum Mir34 in NAFLD

Elevated mir34a levels are related with biopsies histological findings parameters in patients with NAFLD and resistance in insulin's action. Mir34a is also associated with genetic factors, such as PNPLA3 and EI6K variant in TM6SF2, who are both related with lipid and lipoprotein metabolism pathways in the liver. Higher levels of mir34a and mir192 are observed in NAFLD patient's serum (27,28).

4.1.1.3 Serum Mir193-5p in NAFLD

Mir193-5p can be considered as a progressive NAFLD biomarker. As mir193-5p levels elevate, NAFLD severity aggravates. Also it is a more sensitive and accurate biomarker when compared to mir122 and ALT/AST/FIB4 panel (1).

4.1.1.4 Serum microRNA206 in NAFLD

Lipogenesis in hepatocytes is induced by LXa. Mir206 suppress LXa by targeting the 3' UTR region. Inhibiting LXa gene translation and function is leading to decreased lipogenesis. The cascade promoted by LXa enhances the expression of genes related to lipogenesis such as SRBP1c, FFS, CHREBP and ACC. As a result, a higher cholesterol and fatty acid uptake is observed when LXa gene is activated (6).

4.1.1.5 Other serum mIRNAs in NAFLD

Sirtulin 1 and AMPK are downregulated by mir141. Mir141 decreases hepC2 cell survival (7). Overexpression of Mir125a-5p, mir142-3p and mir409-3p downregulates PCSK7 mRNA and especially mir125a-5p represses PCSK7 cleavage activity on Human transferring receptor 1 (28). Mir199-5p is related to deregulated fat b oxidation of mitochondria. Mir2916-3p deactivates adenosine 5' monophosphate AMP protein kinase and by this pathway promotes hepatic lipogenesis. Mir2125p is suppressed by free fatty acids and lipid accumulation is promoted. Mir1225p interacts with lncRNA NEAT which suppress mir1225p concentration (29).

Mir30a-5p targets PPARa and suppresses its expression. PPARa is associated with cellular growth and differentiation. At the same time Mir30a-3p upregulates ACC, Pgsk-3B and FASN

(proteins related to lipid metabolism) and affects Camp signaling pathway. When mir30a-3p is overexpressed, it leads to lipid accumulation in the liver causing steatosis and elevated triglycerides levels contributing to NAFLD pathology (30). Mir146 and mir214 are upregulated in NAFLD patients and participate in inflammation pathology and also affect HSC activity by interacting with STAT3/RUNX1 transcriptional factors. Mir 149 alters the MTHFR expression (methylation from homocysteine to methionine) and its role is important in NAFLD development. Mir98-5p inhibits PGC-1b activity and reduces lipogenesis in the liver. Mir325p also lowers lipogenesis but has a different target, KLF3 (31). NAFLD patients have higher levels of mir103 which is involved in insulin resistance, adipogenesis by triglycerides accumulation and downregulates caveolin1 (participates in insulin receptor regulation) (20). Focal necrosis found on liver biopsy was associated with DICER and DRG8 levels. DICER and DRG8 levels were elevated when mir7-1 was expressed. Steatohepatitis and ballooning degeneration were inhibited by mir125b (17).

Mir4488 in lean individuals with NAFLD can be a diagnostic biomarker. Mir4488 controls pathways such as choline metabolism in cancer, TNF, p53 and also affects hub genes such as ARHGAP1 and SLC10A1. However, in a long-term follow up, non-lean and lean patients have the same morbidity (32).

4.1.2 Steatosis

The role of serum miRNAs expressed in steatosis has been examined in 18 articles ***(Table 3)***.

4.1.2.1 Serum mir122 in steatosis

MiRNA mir122 considers to be 70% of hepatic origin miRNA. By repressing SIRT1 and inactivating LKB1 AMPK pathway, mir122 disables intrahepatic accumulation of fat. Serum mir122 levels are severity related (33), mild steatosis is characterized by lower mir122 levels than moderate steatosis and moderate steatosis has even lower mir122 serum levels than severe steatosis (5,34).

Patients with improved histopathological features (steatosis, ballooning stage, lobular inflammation in HCC patients) have lower mir122 levels. Mir122 levels are related with severity of NAFLD. Apart from diagnosing NAFLD, mir122 as a biomarker is also capable of predicting NAFLD prognosis. For 1 unit decrease of mir122 levels (expressed relative to mir23a) it is 40% more likely for the patient to develop NASH comparatively to NAFLD (2,25). Serum mir122 are lower in mild than moderate and severe steatosis. Despite this severity correlation, mir122 is lower in severe fibrosis. Mir122 is negatively correlated with HDL. Micro122 and liver enzymes are released from the hepatic cells during hepatic cell injury and membrane impairment (14). Apart from mir122, also mir192 is related with a state of liver injury. Both these miRNAs are more associated with steatosis than fibrosis (24,25). Mir122 serum levels in NASH patients is 31-fold higher than in healthy controls (24).

4.1.2.2 Serum mir34a in steatosis

Mir34a is higher in NAFLD, further increased in patient with steatosis, and if combined with other biomarkers such as patient's BMI, serum CRP and serum cholesterol levels, they can diagnose NAFLD with an accuracy of AUC 0.98 (27).

PPARa, which is related to hepatic steatosis mechanisms and oxidative stress pathology, is inhibited by mir34a and also mir34a is modulated by circrna0046367. If circrna0046367 levels are into normal range, then mir34a action on PPARa is inhibited and steatosis is prevented. Also, when circ0046367 level decreases, mir34 levels increase and affect WNT/b-catenin pathway. Circ0046367 levels are decreased in steatosis. WNT/b-catenin pathway represses adipogenesis, with epigenetic mechanisms, and at the same time promotes tolerance in glucose and contributes to increased insulin sensitivity. In overall there is a protective effect against NAFLD. There is a negative correlation between mir34a and WNT1 (negative feedback loop). Circrna0046367 normalization protects from steatosis and at the same time, in a steatotic liver circ0046367 loses its protective function (35).

4.1.2.3 Serum mir29a in steatosis

When circrnaHIPK3 is upregulated, mir29a is downregulated and affects WNT/b-catenin pathway, which affects lipid metabolism and regulation. Mir29a protects liver from lipotoxicity with epigenetic modulations and alterations in homeostasis of mitochondria. Hepatic injury caused due to cholestasis and liver damage are decreased with elevation of mir29a. A positive

feedback loop is observed is observed between mir29a and WNT1 (36,37). Steatosis is characterized by a mismatch in lipid supply, which is increased and lipid consumption which is decreased causing lipid accumulation in the liver. Intrahepatic lipid content is associated with mir29b. Excessive lipid accumulation leads to NAFLD. Despite the fact that lipotoxicity and lipid accumulation leads to steatosis and liver cell dysfunction though time, aminotransferase levels are normal in many patients with steatotic livers. Also, mir29b is related with Diabetes type 2 (38).

4.1.2.4 Other serum miRNAs in steatosis

Mir193a-5p, 378d, 38e levels are elevated in liver steatosis (39). Mir185 and mir29 have a protective role against steatosis by repressing Fatty acid synthase, HMGCR and SPEEP1C2, which participate in lipid clearance from the liver (36). NASH is associated with mir126 which is downregulated in diabetes type 2. Iris1 is related with lever damage severity in NAFLD and it's a mir126 target (40). Hepatocyte steatosis is ameliorated by mir3941, mir4517 and mir4672. FABP1 is the target of those miRNAs, causing hepatic injury and steatosis. Hepatic steatosis is favored by mir132 in NAFLD patients, while mir103 targets FASN and SCD1, inhibiting lipogenesis and reducing NAFLD (30).

Mir9 serum concentration is higher in moderate to severe NAFLD compared to mild NAFLD and even higher in steatosis. Onecut and SIRT1 in insulin secreting cells are negatively regulated by mir9 while SIRT1 is the main mir9 target in steatosis, assuming NAFLD and diabetes pathologies are correlated. Also, SIRT1 is repressed when mir50-30p in elevated. AMPK is activated by deacetylation caused by LKB1 which is activated by SIRT1. Activated AMPK alter

NAD/NADH ratio and further increase SIRT1 activation. When SIRT1 is upregulated, it leads to AMPK activation. Insulin sensitivity is improved by SIRT1. As a result, hepatic steatosis is suppressed (40,41). CCTAT1 participates in lipogenesis while mir613 is inhibiting its action and at the same time CXAT1 inhibits mir613. Dicer1, DORSHA and DCG8 are elevated in NASH. Dicer1 cleaves the cytosolic passenger miRNA strand (42). Mir615 can reverse lipotoxicity by reducing triglyceride levels in NASH by reducing MTOR and SREBP1 on protein level and miRNA transcriptional level (42). After liver transplantation, liver graft is also susceptible to NAFLD and steatosis. Mir33a in the serum of a patient after liver transplantation is associated with dyslipidemia and resistance in insulin, due to this fact it is considered to be a useful predictive steatosis and inflammation biomarker after liver transplantation (43).

4.1.3 Fibrosis

From 44 articles, 8 discussed about serum miRNAs in liver fibrosis ***(Table 4)***. Mir214 was downregulated while progressing to cirrhosis from fibrosis. Mir197 and mir99 are elevated when pericellular fibrosis occurs in NASH (44). In early stages of fibrosis, mir34a and mir192 are overexpressed, while advanced fibrosis is characterized by elevated mir193a-5p (45,46).

4.1.3.1 Serum mir122 in fibrosis

Once liver injury has led to inflammation and liver tissue is exposed for a long time to proinflammatory and inflammatory factors (IL6, TNFA and other inflammatory cytokines), fibrosis occurs. Those cytokines promote fibrosis by increasing the level of collagen 3. Although fibrosis

is considered to be a more severe pathology than steatosis, mir122 is lower in fibrosis and especially in severe fibrosis (5,20).

4.1.3.2 Serum mir34a in fibrosis

However, mir34a in early fibrosis is characterized of higher levels when compared to NAFLD, while in severe fibrosis it is distinguished for lower serum levels compared to controls. There is also a correlation with other morbidities related to NAFLD, such as hypertension and higher fasting plasma glucose levels (which is related to diabetes gradual progression) (36,45).

4.1.3.3 Other serum miRNAs in fibrosis

Higher levels of mir193-5p are associated with NAFLD severity as graded by biopsy by activity grade and fibrosis stage (39). Patients with NAFLD have elevated serum mir192 levels, especially in early fibrosis (sensitivity of 63.3% and specificity of 93.3%) (45). Fibrosis in NAFLD is associated with macrophage infiltration. GasdeminD is upregulated in this pathology and especially mir4517/GSDMD cascade which alters immunity associated with macrophages. This cascade alteration leads to fibrosis by a free fatty acid induced macrophage injury (47). Fibrosis related pathways are associated with mir214, which can differentiate fibrosis and cirrhosis. Mir214 was downregulated while progressing to cirrhosis from fibrosis (48). Mir590-5p represses CCL20, which has a profibrogenic role in liver fibrosis (49).

4.1.4 Hepatocellular Carcinoma

From 44 articles, 10 articles mentioned about serum miRNAs in HCC. HCC Is characterized by high mir21-5p and mir34a-5p levels, while usually HCC patients are characterized by lower mir130a-3p levels (50). Patients with lower mir194 serum levels are in greater risk of developing HCC. Mir194 and mir214 are involved in the extracellular matrix development and can be used to diagnose NAFLD though all its stages (52,53).

4.1.4.1 Serum miRNAs in HCC

Mir34 increases apoptosis by increasing p53 acetylation and inducting proapoptotic genes. It is also elevated in HCC tissue (27). Mir192 has lower serum levels in HCC compared to NASH and NAFLD (24,25). If taken in consideration, the amount of circulating mir122 combined with stage of fibrosis, history of HCC and FIB4 index, can foretell the mortality percentages of NAFLD patients (20,51). Liver failure and HCC are associated with mir191 but not NAFLD (45). Mir9 is suppressed by SNHG, which sponges to mir9-5p. This sponging is causing CNM1 to increase and promotes HCC. Also, mir9 targets CNM1 (cyclin mediator 1) (50). DNA methylation of HIST2H2BE induces HCC progression in NAFLD and NASH (48). Metastasis in HCC and autophagy mediated resistance is repressed by mir30a, which has a protective role in the cancer progression and metastatic behavior (30). Patients with lower mir194 serum levels are in greater risk of developing HCC. Mir194 and mir214 are involved in the extracellular matrix development and can be used to diagnose NAFLD though all its stages (52,53)

4.2 Tumoral MiRNAs

4.2.1 Hepatocellular Carcinoma

The role of MiRNAs extracted from Hepatocellular Carcinoma tissues has been examined in 2 articles. In Tumoral and peritumoral tissue mir21 levels are elevated. HCC is the last and most severe stage of NAFLD. NAFLD results in HCC due to excessive lipid accumulation. Lipid accumulation is enhanced by mir21. Mir21 downregulates HBP1a, which is a transcriptional activator of the tp53 gene. Also, in peritumoral and tumoral tissues mir21-5p, mir34a-5p and mir130a-3p overexpression is affecting p53 pathway. Mir130a-5p in samples of patients diagnosed with HCC is downregulated, while mir155-3p levels are elevated in HCC compared to NAFLD. Mir155 participates in tumorigenesis and proliferates anti-apoptosis and metastasis. WNT/b-catenin and p53 pathways are regulated by mir21-5p, 34a-5p and 130a-5p (35). HCC tissues express higher levels of mir4726-5p which regulates RAB11B antioncogene, meaning that mir4726-5p is repressing the antioncogenetic RAB11B action, leading to HCC. LNCRAB11BAS1 can repress mir426-5p and prevent HCC proliferation metastasis and invasion by elevating RAB11B mRNA levels (53).

4.3 Liver Tissue MiRNAs

4.3.1 Steatosis

Liver tissue miRNAs associated with steatosis was mentioned in 4 articles. In liver biopsy mir21 characterizes NASH patients, while mir122 and mir192 can be found in NAFLD and NASH. MicroRNA expression combined with scoring of CK18-Asp396 fragment level scoring can predict NASH with a higher accuracy. NAFLD biopsy parameters such as : degree of steatosis, lobular inflammation, ballooning and fibrosis are connected with mir21, mir122 and mir192. Mir 21 and mir122 were also related with lobular inflammation (23). In NASH patient's liver tissue were observed higher levels of mir34a-5p and lower levels of mir122-5p and mir29c-3p, compared to healthy individuals. NASH and NAFLD often coexist with cardiovascular diseases. Impaired metabolism of cholesterol and inflammation are common pathophysiological steps leading to both pathologies' evolvement. Mir122-5p is related with dyslipidemia while mir34-5p levels are related with higher CRP levels and inflammatory response with proinflammatory cytokines (54). Although mir34a-5p is more difficult to detect it is a sensitive biomarker (44). Mir122 expressed in the liver tissue has 10-fold lower levels in NASH compared to steatohepatitis (23).

When examining liver tissue, mir122 concentration is higher in simple steatosis when compared to control group and even higher in livers diagnosed with NAFLD by biopsy when compared to NASH livers (also diagnosed by biopsy). Mir122 regulates deposition of fat. Steatotic cells, are cells that accumulate more lipids and triglycerides than the control ones. Steatotic cells also express more mir122 than the control ones. If no mir122 is expressed, fat accumulation in enhanced (30).

4.3.2 Hepatocellular Carcinoma

4.3.2.1 mir193-5p and mir192 in HCC

Liver expression of miRNAs associated with HCC was mentioned in 2 articles. Mir 193-5p is decreased in the liver tissue (liver tissue miRNA) when liver damage is causing fibrotic remodeling and even HCC. RAP2A, IGF2, SLC7A1, COL1A1, GP8 are target genes of mir193-5p and they are associated to hepatic function. Especially GP8 function and expression is elevated when cells are transiting from epithelial to mesenchymal and is related to IL6/JACK/STAT3 cascade (48). Mir192 levels are lower in HCC compared to NAFLD and NASH, however this differentiation had a statistical significance only in severely obese patients (54).

4.4 Visceral Adipose Tissue miRNAs

4.4.1 Steatosis

From 44 articles, 2 articles related visceral adipose tissue miRNAs with steatosis. Visceral adipose tissue is a tissue with its own endocrine function. Visceral adipose tissue expresses its own miRNAs such as mir19 and mir99 that were positively related with NASH and especially with pericellular fibrosis that is observed in NASH. Those miRNAs are interacting with IL6 encoding genes and downregulate them (55). Mir132 that is expressed in visceral adipose tissue is related with lower liver enzymes levels, lower triglycerides serum concentration and lower blood pressure levels. When mir132 levels are low, heart failure and liver steatosis are less possible to

occur. Mir 132 participates in steatosis pathophysiology by regulating pTEN (phosphate and tensin homolog and sirtulin formation regulator 1) (56). In adipose tissue of obese NAFLD patients mir99a is under expressed and its levels are associated with inflammation and lipid content (IL6 and FFA levels). As a result, mir99a is lower in NAFLD and NASH. Apart from mir99b, mir19 is also related with pericellular fibrosis in NASH (55).

4.5 Ascetic Fluid miRNAs

4.5.1 Hepatocellular Carcinoma

From 44 articles, 2 concerned about ascetic fluid miRNAs in HCC. Mir182, mir301a, mir373 are elevated in serum exosomes of patients with NASH ascetic fluid, those exosomes originate also from cancer cells found in ascetic fluid. HCC is related with mir182, as mir182 elevated serum concentration levels are observed in intrahepatic steatosis and HCC. HCC is associated with lipid metabolism pathway of NFKB/PI3K/AKT/PTEN. When mir182 is upregulated, target gene FOO3A expression is suppressed. FOO3A suppression is activating AKT/FOXO3A cascade by blocking the dissolution of b-catenin and TGF4. Mir301 is a key miRNA associated with HCC progression. Mir301's target gene is a gene that regulates growth arrest at the specific GA home box that blocks angiogenesis by targeting NFKB. Inhibition of angiogenesis leads to apoptosis and reduced proliferation, metastasis and invasion (57,58).

4.6 MiRNAs in NAFLD diagnosis

Mir193-5p can be considered as a progressive NAFLD biomarker. As mir193-5p levels elevate, NAFLD severity aggravates. Also it is a more sensitive and accurate biomarker when compared to mir122 and ALT/AST/FIB4 panel (1). Higher levels of mir193-5p are associated with NAFLD severity as graded by biopsy by activity grade and fibrosis stage (39). Mir146 and mir214 are upregulated in NAFLD patients and participate in inflammation pathology (31). NAFLD patients have higher levels of mir103 which is involved in insulin resistance, adipogenesis by triglycerides accumulation and downregulates caveolin1 (participates in insulin receptor regulation) (20).

Mir4488 in lean individuals with NAFLD can be a diagnostic biomarker. Mir4488 controls pathways such as choline metabolism in cancer, TNF, p53 and also affects hub genes such as ARHGAP1 and SLC10A1. However, in a long-term follow up, non-lean and lean patients have the same morbidity (32).

Serum mir122 levels are severity related (33), mild steatosis is characterized by lower mir122 levels than moderate steatosis and moderate steatosis has even lower mir122 serum levels than severe steatosis (5,34). Mir122 levels are related with severity of NAFLD. Apart from diagnosing NAFLD, mir122 as a biomarker is also capable of predicting NAFLD prognosis. For 1 unit decrease of mir122 levels (mir12 levels are expressed relative to mir34a levels) it is 40% more likely for the patient to develop NASH comparatively to NAFLD (2,25). Serum mir122 are lower in mild than moderate and severe steatosis. Despite this severity correlation, mir122 is lower in severe fibrosis (***Figure 2***). Micro122 and liver enzymes are released from the hepatic cells during hepatic cell injury and membrane impairment (14). Apart from mir122, also mir192 is related with a state of

liver injury. Both these miRNAs are more associated with steatosis than fibrosis (24,25). Mir122 serum levels in NASH patients is 31-fold higher than in healthy controls (24).

Mir34a is higher in NAFLD, further increased in patient with steatosis, and if combined with other biomarkers such as patient's BMI, serum CRP and serum cholesterol levels, they can diagnose NAFLD with an accuracy of AUC 0.98 (27). Mir9 serum concentration is higher in moderate to severe NAFLD compared to mild NAFLD and even higher in steatosis (40,41).

Mir34a in early fibrosis is characterized of higher levels when compared to NAFLD, while in severe fibrosis it is distinguished for lower serum levels compared to controls (***Figure 3***). There is also a correlation with other morbidities related to NAFLD, such as hypertension and higher blood sugar during fasting (which is related to diabetes gradual progression) (36,45). NAFLD patient's mir192 levels are elevated , especially in early fibrosis (sensitivity of 63.3% and specificity of 93.3%) (45).

Fibrosis related pathways are associated with mir214, which can differentiate fibrosis and cirrhosis. Mir214 was downregulated while progressing to cirrhosis from fibrosis (48). If taken in consideration, the amount of circulating mir122 combined with stage of fibrosis, history of HCC and FIB4 index, can foretell the mortality percentages of NAFLD patients (20,51).

Patients with lower mir194 serum levels are in greater risk of developing HCC. Mir194 and mir214 are involved in the extracellular matrix development and can be used to diagnose NAFLD though all its stages (52,53).

4.7 Key findings

The key findings of this systematic review on the role of microRNAs in NAFLD spectrum are presented in table 6 ***(Table 6)***

5 Discussion

Early in NAFLD progression miRNA expression (in patients' serum and liver tissue) is altered, affecting different genes. MiRNA's expression is genetically and environmentally depended. In a percent of 50% the risk of NAFLD is hereditable. Different genes variants which contribute to lipid metabolism differ in many individuals. This diversity observed in genes reflects the diversity in the ability of lipid clearance in the liver

There are variations in miRNAs concentrations among various genders, age groups, and ethnicities. Obese women express higher levers of mir33b in their liver. Mir33b expression is related with NASH though SPEBP2 and ABCG1 gene (31). Mir122 is three-fold higher in non-Caucasians compared to Caucasians, this variability must be taken into consideration when adjusting a biomarker's normal range (7,59).

Diabetes and NAFLD are strongly related. Mir 193b-3p is associated with lipid impaired metabolism and pre-diabetes. PPAG/PGC1A is downregulated by mir193b-3p, causing reduction of VLDL secretion and glycolysis. Also, SLC2A2/GLUT2 expression is decreased when mir193b-3p serum levels are elevated. GLUT2 decreased expression is causing hyperglycemia due to lower glucose uptake (59).

Resistance to insulin causes de novo lipogenesis since glucose cannot be used as an energy source. High amounts of lipids are accumulated leading to lipotoxicity (60). Increased synthesis of triglycerides from the liver and elevated free fatty acids uptake lead to lipotoxicity. Lipotoxicity is causing hepatic mitochondria dysfunction, which results in further lipogenesis and eventually

leads to NAFLD progression. Constant oxidation of high lipid levels is causing oxidative stress, pro-inflammatory response of the immune system and eventually inflammation. An inflammatory environment is causing ballooning and fibrosis due to liver cells apoptosis. Elevated extracellular matrix production and hepatic scarring is connected to apoptosis due to liver injury (61).

Although biopsy is the gold standard method for diagnosis NAFLD, a screening method is needed in order to decide which patients have higher possibility of getting diagnosed with NAFLD and should undergo liver biopsy, which is an invasive diagnostic method. MicroRNAs can be used as a high diagnostic value screening test, especially if combined with other biomarkers. Mir181, participates in lipid metabolism in hepatocytes. FIB4 can predict severity of liver fibrosis when patients undergo liver biopsy. Also, serum mir181d, mir99a, mir197 and mir146b levels expressed in NAFLD patients (proven by biopsy) are lower than healthy individuals (62,63). Mir6881-5p is regulated by lncSPARCL1 (positive correlation). This miRNA increase along with increased HSPD1, MMP4 and ITGB1 mRNAs can lead to an accurate diagnose of NASH and also distinguish pathologies such as NAFLD, simple steatosis and NASH (64). Finally, mir122-5p, mir1290, mir27b-3p and mir192-5p can be used as a panel to identify NAFLD with high diagnostic accuracy as it is considered to be more specific than ALT and FIB4 (62). MicroRNAs can be used as a biomarker to diagnose and differentiate NAFLD, NASH and HCC.

5.1 Clinical Significance of MicroRNAs

Inherited and expressed genes that promote lipid accumulation lead easier to NAFLD and other related pathologies. Mir331-3p, mir30, mir223, mir191, mir127, mir193a-5p, mir411, mir30b are hereditable. Especially mir30 is elevated in both twins with NAFLD and also random individuals with NAFLD (8).

MiRNAs can be used as a surveillance biomarker to closely monitor NAFLD's progression and response to therapeutic measures taken. They can also set the initial diagnosis of NAFLD. NIS2+ is a screening NAFLD test that consist of two serum biomarkers (mir34a and YKL40) and it is appropriate for male and female subpopulations and for individuals with diabetes. NIS2+ achieved sensitivity of 85%, negative predictive value (NPV) of 82%, and specificity of 61% for ruling out at risk NASH. NIS2+TM specificity of 85%, positive predictive value of 77%, and sensitivity of 62% are used to rule in at-risk NASH (62). Also, there is a quantitate method of predicting hepatic fat, with an 4-5% error, by quantifying 23 serum miRNAs. Two sets of highly expressed miRNAs were identified by comprehensive microRNA sequencing of liver and serum samples (418 in the liver and 351 in the blood). According to tests using Pearson correlation, the amount of liver fat was substantially correlated with 18.5% of miRNAs in the liver and 14.5% in the blood. According to PLS-RFE models, the number of miRNAs that predicted steatosis with the lowest error in the serum and liver models was 50. When the two miRNA subsets were compared, 19 coincident miRNAs were revealed. These miRNAs were graded based on biological relevance, including guide/passenger strand, relative abundance in serum and liver, number of projected target genes for lipid metabolism, correlation significance, etc. (7).

MicroRNAs can be used as therapeutic agents. MicroRNA inhibitor molecules, obstruct microRNA's target gene inhibition leading to activation of microRNA target gene. On the other hand, microRNA mimics behave like microRNAs and inhibit their final target gene. There is a long way until miRNA mimics and inhibitors can be used as therapeutic agents but there is potential to their evolvement. Molecules that can be used as therapeutic agents are mir122 mimic molecules that reverse liver fibrosis and HCC progression. On the other hand, mir122 inhibitors can be used as HCV treatment (especially chronic infection from HCV) and can potentially restore hypercholesterolemia (65).

6 Conclusion

Several studies have evaluated the role of microRNAs in NAFLD. The most studied microRNAs are mir122 and mir34. Elevated mir34a and mir122 levels are observed in NAFLD patients. Especially, upregulation of serum mir122 is associated with NAFLD mortality and is related with steatosis severity. Mir122 controls lipid metabolism while mir34a is related to insulin resistance.

Although microRNAs have not been introduced in everyday clinical practice, panels of microRNAs and biomarkers are used to evaluate patients in risk of NASH and also predict hepatic fat percentage. NIS2+ is a screening NAFLD test that consist of two serum biomarkers (mir34a and YKL40) and it is appropriate for male and female subpopulations and for individuals with diabetes. NIS2+ achieved sensitivity of 85% for ruling out at risk NASH and specificity of 85% to rule in at-risk NASH. Also, there is a quantitate method of predicting hepatic fat, with an 4-5% error, by quantifying 23 serum miRNAs.

Further studies need to conclude on the role of microRNAs in the pathophysiology, progression, diagnosis and management of NAFLD spectrum.

7 References

1. Nguyen HD, Kim MS. Effects of chemical mixtures on liver function biomarkers in the Korean adult population: thresholds and molecular mechanisms for non-alcoholic fatty liver disease involved. Environ Sci Pollut Res. 2022 Nov;29(52):78555–87.

2. Kadayifci A, Tan V, Ursell PC, Merriman RB, Bass NM. Clinical and pathologic risk factors for atherosclerosis in cirrhosis: a comparison between NASH-related cirrhosis and cirrhosis due to other aetiologies. J Hepatol. 2008 Oct;49(4):595–9.

3. Patel SS, Nabi E, Guzman L, Abbate A, Bhati C, Stravitz RT, et al. Coronary artery disease in decompensated patients undergoing liver transplantation evaluation. Liver Transplant Off Publ Am Assoc Study Liver Dis Int Liver Transplant Soc. 2018 Mar;24(3):333–42.

4. Jopling CL, Yi M, Lancaster AM, Lemon SM, Sarnow P. Modulation of hepatitis C virus RNA abundance by a liver-specific MicroRNA. Science. 2005 Sep 2;309(5740):1577–81.

5. Hegazy MA, Abd ALgwad I, Abuel Fadl S, Sayed Hassan M, Ahmed Rashed L, Hussein MA. Serum Micro-RNA-122 Level as a Simple Noninvasive Marker of MAFLD Severity. Diabetes Metab Syndr Obes Targets Ther. 2021 May;Volume 14:2247–54.

6. Hendy OM, Rabie H, El Fouly A, Abdel-Samiee M, Abdelmotelb N, Elshormilisy AA, et al. The Circulating Micro-RNAs (−122, −34a and −99a) as Predictive Biomarkers for Non-Alcoholic Fatty Liver Diseases. Diabetes Metab Syndr Obes Targets Ther. 2019 Dec;Volume 12:2715–23.

7. Quintás G, Caiment F, Rienda I, Pérez-Rojas J, Pareja E, Castell JV, et al. Quantitative Prediction of Steatosis in Patients with Non-Alcoholic Fatty Liver by Means of Hepatic MicroRNAs Present in Serum and Correlating with Hepatic Fat. Int J Mol Sci. 2022 Aug 18;23(16):9298.

8. Zarrinpar A, Gupta S, Maurya MR, Subramaniam S, Loomba R. Serum microRNAs explain discordance of non-alcoholic fatty liver disease in monozygotic and dizygotic twins: a prospective study. Gut. 2016 Sep;65(9):1546–54.

9. Chen L zi, Jing X bin, Wu C fen, Zeng Y cheng, Xie Y chun, Wang M qing, et al. Nonalcoholic Fatty Liver Disease-Associated Liver Fibrosis Is Linked with the Severity of Coronary Artery Disease Mediated by Systemic Inflammation. Dis Markers. 2021 Dec 28;2021:6591784.

10. Akabame S, Hamaguchi M, Tomiyasu KI, Tanaka M, Kobayashi-Takenaka Y, Nakano K, et al. Evaluation of vulnerable coronary plaques and non-alcoholic

fatty liver disease (NAFLD) by 64-detector multislice computed tomography (MSCT). Circ J Off J Jpn Circ Soc. 2008 Apr;72(4):618–25.

11. Kazankov K, Munk K, Øvrehus KA, Jensen JM, Siggaard CB, Grønbaek H, et al. High burden of coronary atherosclerosis in patients with cirrhosis. Eur J Clin Invest. 2017 Aug;47(8):565–73.

12. Alessi MC, Bastelica D, Mavri A, Morange P, Berthet B, Grino M, et al. Plasma PAI-1 levels are more strongly related to liver steatosis than to adipose tissue accumulation. Arterioscler Thromb Vasc Biol. 2003 Jul 1;23(7):1262–8.

13. El Hadi H, Di Vincenzo A, Vettor R, Rossato M. Relationship between Heart Disease and Liver Disease: A Two-Way Street. Cells. 2020 Feb 28;9(3):567.

14. Arslan U, Kocaoğlu I, Balcı M, Duyuler S, Korkmaz A. The association between impaired collateral circulation and non-alcoholic fatty liver in patients with severe coronary artery disease. J Cardiol. 2012 Sep;60(3):210–4.

15. Mirza AJ, Taha AY, Khdhir BR. Risk factors for acute coronary syndrome in patients below the age of 40 years. Egypt Heart J EHJ Off Bull Egypt Soc Cardiol. 2018 Dec;70(4):233–5.

16. Guo Y, Xiong Y, Sheng Q, Zhao S, Wattacheril J, Flynn CR. A micro-RNA expression signature for human NAFLD progression. J Gastroenterol. 2016 Oct;51(10):1022–30.

17. Sharma H, Estep M, Birerdinc A, Afendy A, Moazzez A, Elariny H, et al. Expression of genes for micro RNA -processing enzymes is altered in advanced non-alcoholic fatty liver disease. J Gastroenterol Hepatol. 2013 Aug;28(8):1410–5.

18. Zhong D, Huang G, Zhang Y, Zeng Y, Xu Z, Zhao Y, et al. MicroRNA-1 and microRNA-206 suppress LXRα-induced lipogenesis in hepatocytes. Cell Signal. 2013 Jun;25(6):1429–37.

19. Kim TH, Lee Y, Lee YS, Gim JA, Ko E, Yim SY, et al. Circulating miRNA is a useful diagnostic biomarker for nonalcoholic steatohepatitis in nonalcoholic fatty liver disease. Sci Rep. 2021 Jul 19;11(1):14639.

20. Sendi H, Mead I, Wan M, Mehrab-Mohseni M, Koch K, Atala A, et al. miR-122 inhibition in a human liver organoid model leads to liver inflammation, necrosis, steatofibrosis and dysregulated insulin signaling. Avila MA, editor. PLOS ONE. 2018 Jul 19;13(7):e0200847.

21. Zhao Z, Zhong L, Li P, He K, Qiu C, Zhao L, et al. Cholesterol impairs hepatocyte lysosomal function causing M1 polarization of macrophages via exosomal miR-122-5p. Exp Cell Res. 2020 Feb;387(1):111738.

22. Newman LA, Useckaite Z, Johnson J, Sorich MJ, Hopkins AM, Rowland A. Selective Isolation of Liver-Derived Extracellular Vesicles Redefines Performance of miRNA Biomarkers for Non-Alcoholic Fatty Liver Disease. Biomedicines. 2022 Jan 17;10(1):195.

23. Pirola CJ, Fernández Gianotti T, Castaño GO, Mallardi P, San Martino J, Mora Gonzalez Lopez Ledesma M, et al. Circulating microRNA signature in non-alcoholic fatty liver disease: from serum non-coding RNAs to liver histology and disease pathogenesis. Gut. 2015 May;64(5):800–12.

24. Becker PP, Rau M, Schmitt J, Malsch C, Hammer C, Bantel H, et al. Performance of Serum microRNAs -122, -192 and -21 as Biomarkers in Patients with Non-Alcoholic Steatohepatitis. Schlaak JF, editor. PLOS ONE. 2015 Nov 13;10(11):e0142661.

25. Ezaz G, Trivedi HD, Connelly MA, Filozof C, Howard K, L.Parrish M, et al. Differential Associations of Circulating MicroRNAs With Pathogenic Factors in NAFLD. Hepatol Commun. 2020 May;4(5):670–80.

26. Akuta N, Kawamura Y, Arase Y, Saitoh S, Fujiyama S, Sezaki H, et al. Circulating MicroRNA-122 and Fibrosis Stage Predict Mortality of Japanese Patients With Histopathologically Confirmed NAFLD. Hepatol Commun. 2020 Jan;4(1):66–76.

27. Mohamed AA, El-Demery A, Al-Hussain E, Mousa S, Halim AA, Mostafa SM, et al. NAFLD mark: an accurate model based on microRNA-34 for diagnosis of non-alcoholic fatty liver disease patients. J Genet Eng Biotechnol. 2021 Dec;19(1):157.

28. Malakootian M, Naeli P, Mowla SJ, Seidah NG. Post-Transcriptional Effects of miRNAs on PCSK7 Expression and Function: miR-125a-5p, miR-143-3p, and miR-409-3p as Negative Regulators. Metabolites. 2022 Jun 23;12(7):588.

29. Hu MJ, Long M, Dai RJ. Acetylation of H3K27 activated lncRNA NEAT1 and promoted hepatic lipid accumulation in non-alcoholic fatty liver disease via regulating miR-212-5p/GRIA3. Mol Cell Biochem. 2022 Jan;477(1):191–203.

30. Wang DR, Wang B, Yang M, Liu Z lu, Sun J, Wang Y, et al. Suppression of miR-30a-3p Attenuates Hepatic Steatosis in Non-alcoholic Fatty Liver Disease. Biochem Genet. 2020 Oct;58(5):691–704.

31. Auguet T, Aragonès G, Berlanga A, Guiu-Jurado E, Martí A, Martínez S, et al. miR33a/miR33b* and miR122 as Possible Contributors to Hepatic Lipid Metabolism in Obese Women with Nonalcoholic Fatty Liver Disease. Int J Mol Sci. 2016 Sep 24;17(10):1620.

32. Shen N, Tang L, Qian Y, Pan J, Pan J, Miao H, et al. Serum miR-4488 as a potential biomarker of lean nonalcoholic fatty liver disease. Ann Transl Med. 2023 Feb;11(4):173–173.

33. Yamada H, Suzuki K, Ichino N, Ando Y, Sawada A, Osakabe K, et al. Associations between circulating microRNAs (miR-21, miR-34a, miR-122 and miR-451) and non-alcoholic fatty liver. Clin Chim Acta. 2013 Sep;424:99–103.

35. Abdelgwad M, Zakaria R, Marzouk S, Sabry D, Ahmed R, Badary HA, et al. The Emerging Role of Circular RNA Homeodomain Interacting Protein Kinase 3 and Circular RNA 0046367 through Wnt/Beta-Catenin Pathway on the Pathogenesis

of Nonalcoholic Steatohepatitis in Egyptian Patients. Rep Biochem Mol Biol. 2023 Jan;11(4):614–25.

36. Guo XY, Chen JN, Sun F, Wang YQ, Pan Q, Fan JG. circRNA_0046367 Prevents Hepatoxicity of Lipid Peroxidation: An Inhibitory Role against Hepatic Steatosis. Oxid Med Cell Longev. 2017;2017:1–16.

37. Albadawy R, Agwa SHA, Khairy E, Saad M, El Touchy N, Othman M, et al. Circulatory Endothelin 1-Regulating RNAs Panel: Promising Biomarkers for Non-Invasive NAFLD/NASH Diagnosis and Stratification: Clinical and Molecular Pilot Study. Genes. 2021 Nov 18;12(11):1813.

38. He Z, Yang JJ, Zhang R, Li HT, Wu L, Jiang F, et al. Circulating miR-29b positively correlates with non-alcoholic fatty liver disease in a Chinese population. J Dig Dis. 2019 Apr;20(4):189–95.

39. Johnson K, Leary PJ, Govaere O, Barter MJ, Charlton SH, Cockell SJ, et al. Increased serum miR-193a-5p during non-alcoholic fatty liver disease progression: Diagnostic and mechanistic relevance. JHEP Rep. 2022 Feb;4(2):100409.

40. Wang R. Gene expression profiling reveals key genes and pathways related to the development of non-alcoholic fatty liver disease.

41. Hu LK, Chen JQ, Zheng H, Tao YP, Yang Y, Xu XF. MicroRNA-506-3p targets SIRT1 and suppresses AMPK pathway activation to promote hepatic steatosis. Exp Ther Med. 2021 Oct 11;22(6):1430.

42. Huang F, Liu H, Lei Z, Li Z, Zhang T, Yang M, et al. Long noncoding RNA CCAT1 inhibits miR-613 to promote nonalcoholic fatty liver disease via increasing *LXRα* transcription. J Cell Physiol. 2020 Dec;235(12):9819–33.

43. Erhartova D, Cahova M, Dankova H, Heczkova M, Mikova I, Sticova E, et al. Serum miR-33a is associated with steatosis and inflammation in patients with non-alcoholic fatty liver disease after liver transplantation. Strnad P, editor. PLOS ONE. 2019 Nov 8;14(11):e0224820.

44. Estep M, Armistead D, Hossain N, Elarainy H, Goodman Z, Baranova A, et al. Differential expression of miRNAs in the visceral adipose tissue of patients with non-alcoholic fatty liver disease. Aliment Pharmacol Ther. 2010 Aug;32(3):487–97.

45. Ragab HM, Ezzat WM, Hassan EM, El Maksoud NA, Afify M, Abd El-Maksoud MDE, et al. Significance of MiRNA-34a and MiRNA-192 as a risk factor for nonalcoholic fatty liver disease. J Genet Eng Biotechnol. 2023 Dec;21(1):13.

46. Johnson K, Leary PJ, Govaere O, Barter MJ, Charlton SH, Cockell SJ, et al. Increased serum miR-193a-5p during non-alcoholic fatty liver disease progression: Diagnostic and mechanistic relevance. JHEP Rep. 2022 Feb;4(2):100409.

47. Chen S, Cai X, Liu Y, Shen Y, Guillot A, Tacke F, et al. The macrophage-associated microRNA-4715-3p / Gasdermin D axis potentially indicates fibrosis progression in nonalcoholic fatty liver disease: evidence from transcriptome and biological data. Bioengineered. 2022 May 2;13(5):11740–51.

48. Li CW, Chiu YK, Chen BS. Investigating Pathogenic and Hepatocarcinogenic Mechanisms from Normal Liver to HCC by Constructing Genetic and Epigenetic Networks via Big Genetic and Epigenetic Data Mining and Genome-Wide NGS Data Identification. Dis Markers. 2018 Sep 23;2018:1–22.

49. Hanson A, Piras IS, Wilhelmsen D, Still CD, Chu X, Petrick A, et al. Chemokine ligand 20 (CCL20) expression increases with NAFLD stage and hepatic stellate cell activation and is regulated by miR-590-5p. Cytokine. 2019 Nov;123:154789.

50. Rusu I, Pirlog R, Chiroi P, Nutu A, Budisan L, Puia VR, et al. Distinct Morphological and Molecular Profiles of NAFLD and NAFLD-associated HCC Revealed by Immunohistochemistry and MicroRNA Analysis. J Gastrointestin Liver Dis. 2023 Sep 28;32(3):356–66.

51. Akuta N, Kawamura Y, Suzuki F, Saitoh S, Arase Y, Fujiyama S, et al. Analysis of association between circulating miR-122 and histopathological features of nonalcoholic fatty liver disease in patients free of hepatocellular carcinoma. BMC Gastroenterol. 2016 Dec;16(1):141.

52. An MY. Potential biomarkers in NASH-induced liver cirrhosis with hepatocellular carcinoma: A preliminary work on roles of exosomal miR-182, miR-301a, and miR-373. Malays J Pathol. 2020;

53. Wang D, Hu X, Chen J, Liang B, Zhang L, Qin P, et al. Bioinformatics Analysis and Validation of the Role of Lnc-RAB11B-AS1 in the Development and Prognosis of Hepatocellular Carcinoma. Cells. 2022 Nov 6;11(21):3517.

54. Braza-Boïls A, Marí-Alexandre J, Molina P, Arnau MA, Barceló-Molina M, Domingo D, et al. Deregulated hepatic micro RNA s underlie the association between non-alcoholic fatty liver disease and coronary artery disease. Liver Int. 2016 Aug;36(8):1221–9.

55. Eikelis N, Dixon JB, Lambert EA, Hanin G, Tzur Y, Greenberg DS, et al. MicroRNA-132 may be associated with blood pressure and liver steatosis—preliminary observations in obese individuals. J Hum Hypertens. 2022 Oct;36(10):911–6.

56. Zong Y, Yan J, Jin L, Xu B, He Z, Zhang R, et al. Relationship between circulating miR-132 and non-alcoholic fatty liver disease in a Chinese population. Hereditas. 2020 Dec;157(1):22.

57. Vogt J, Sheinson D, Katavolos P, Irimagawa H, Tseng M, Alatsis KR, et al. Variance component analysis of circulating miR-122 in serum from healthy human volunteers. Mari B, editor. PLOS ONE. 2019 Jul 26;14(7):e0220406.

58. Leti F, Malenica I, Doshi M, Courtright A, Van Keuren-Jensen K, Legendre C, et al. High-throughput sequencing reveals altered expression of hepatic microRNAs

in nonalcoholic fatty liver disease–related fibrosis. Transl Res. 2015 Sep;166(3):304–14.

59. Mollet IG, Macedo MP. Pre-Diabetes-Linked miRNA miR-193b-3p Targets PPARGC1A, Disrupts Metabolic Gene Expression Profile and Increases Lipid Accumulation in Hepatocytes: Relevance for MAFLD. Int J Mol Sci. 2023 Feb 15;24(4):3875.

60. López-Sánchez GN, Montalvo-Javé E, Domínguez-Perez M, Antuna-Puente B, Beltrán-Anaya FO, Hidalgo-Miranda A, et al. Hepatic mir-122-3p, mir-140-5p and mir-148b-5p expressions are correlated with cytokeratin-18 serum levels in MAFLD. Ann Hepatol. 2022 Nov;27(6):100756.

61. Branković M, Jovanović I, Dukić M, Radonjić T, Oprić S, Klašnja S, et al. Lipotoxicity as the Leading Cause of Non-Alcoholic Steatohepatitis. Int J Mol Sci. 2022 May 5;23(9):5146.

62. Harrison SA, Ratziu V, Magnanensi J, Hajji Y, Deledicque S, Majd Z, et al. NIS2+^{TM}, an optimisation of the blood-based biomarker NIS4® technology for the detection of at-risk NASH: A prospective derivation and validation study. J Hepatol. 2023 Sep;79(3):758–67.

63. Tan Y, Ge G, Pan T, Wen D, Gan J. A Pilot Study of Serum MicroRNAs Panel as Potential Biomarkers for Diagnosis of Nonalcoholic Fatty Liver Disease. Jin DY, editor. PLoS ONE. 2014 Aug 20;9(8):e105192.

64. An X, Yang Z, An Z. MiR-149 Compromises the Reactions of Liver Cells to Fatty Acid via its Polymorphism and Increases Non-Alcoholic Fatty Liver Disease (NAFLD) Risk by Targeting Methylene Tetrahydrofolate Reductase (MTHFR). Med Sci Monit. 2017 May 16;23:2299–307.

65. Bandiera S, Pfeffer S, Baumert TF, Zeisel MB. miR-122 – A key factor and therapeutic target in liver disease. J Hepatol. 2015 Feb 1;62(2):448–57.

8 Appendix

8.1 Figure legends

Figure 1: PRISMA Flow Chart

From the 1925 articles screened, 44 were used for this review. Records were identified from 2 databases (PubMed and Cochrane) and the number of total registers was 1925. Before screening, 19 duplicate records were excluded. Also, 1.591 other articles were removed. From those 1.591 articles, 287 articles were reviews, while 6 were systematic reviews. The population studied in 34 related articles was pediatric and 256 studies concerned women only population, 983 were animal studies and finally 25 articles weren't written in English language so they were excluded. All articles were retrieved, 11 articles weren't included due to insufficient efficacy endpoints and unclear methods.

From: Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372: n71.

Figure 2: Serum mir122 fold change compared to control group. Mir122 is downregulated in NASH and further reduced in fibrosis.

Figure 3: Serum Mir34a levels fold change compared to control group. Mir34a is severity related .Mir34a is downregulated in severe fibrosis.

8.2 Tables Index

Figure 1: PRISMA Flow Chart

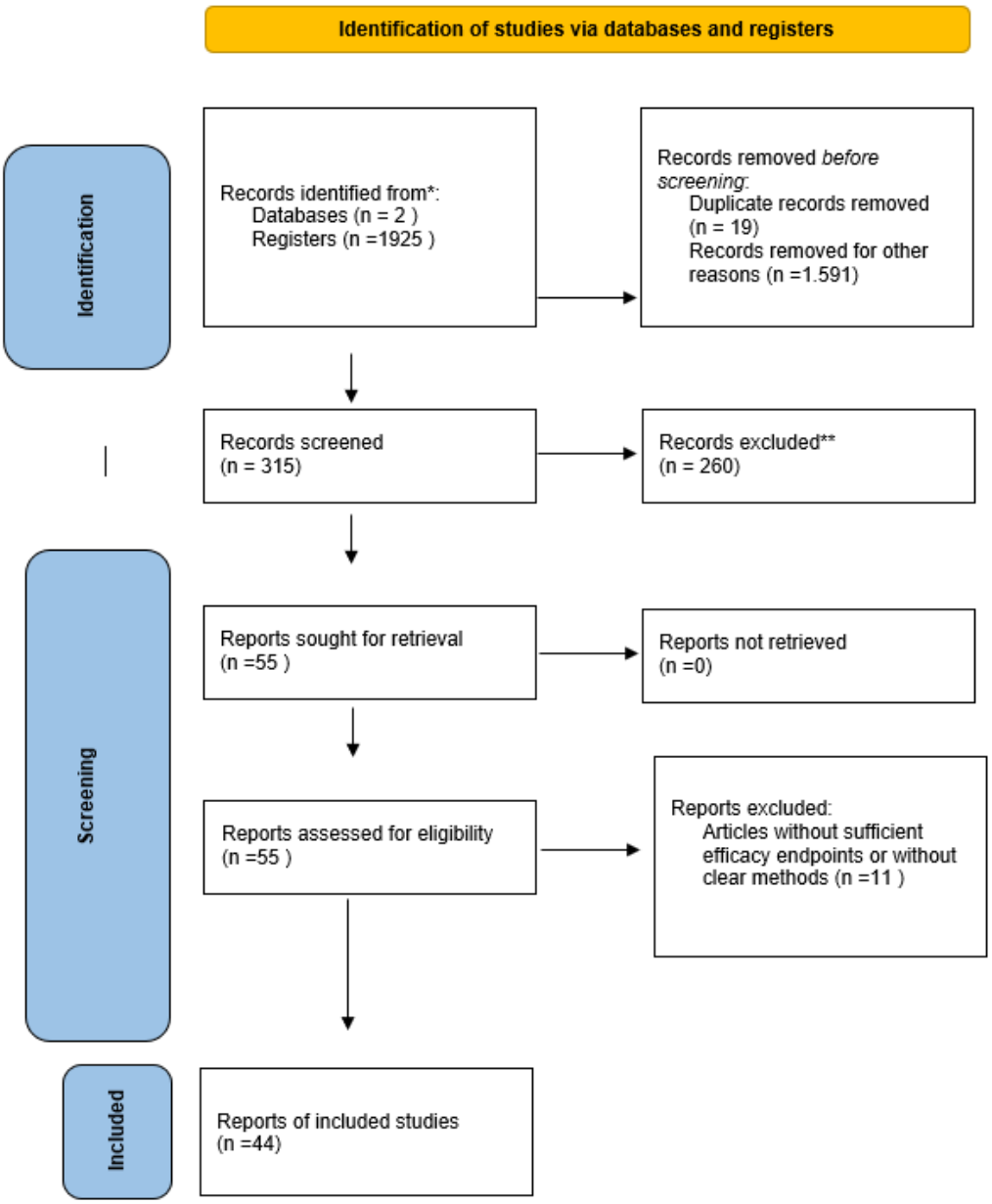

Figure 2: Serum mir122 fold change compared to control group. Mir122 is downregulated in NASH and further reduced in fibrosis.

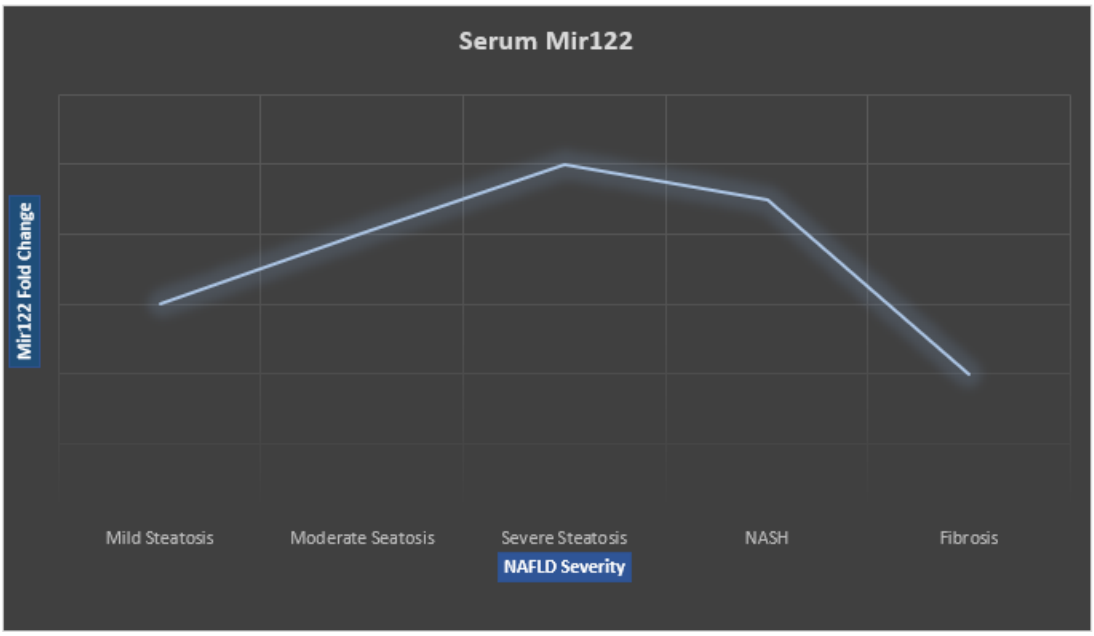

Figure 3: Serum Mir34a levels fold change compared to control group. Mir34a is severity related .Mir34a is downregulated in severe fibrosis.

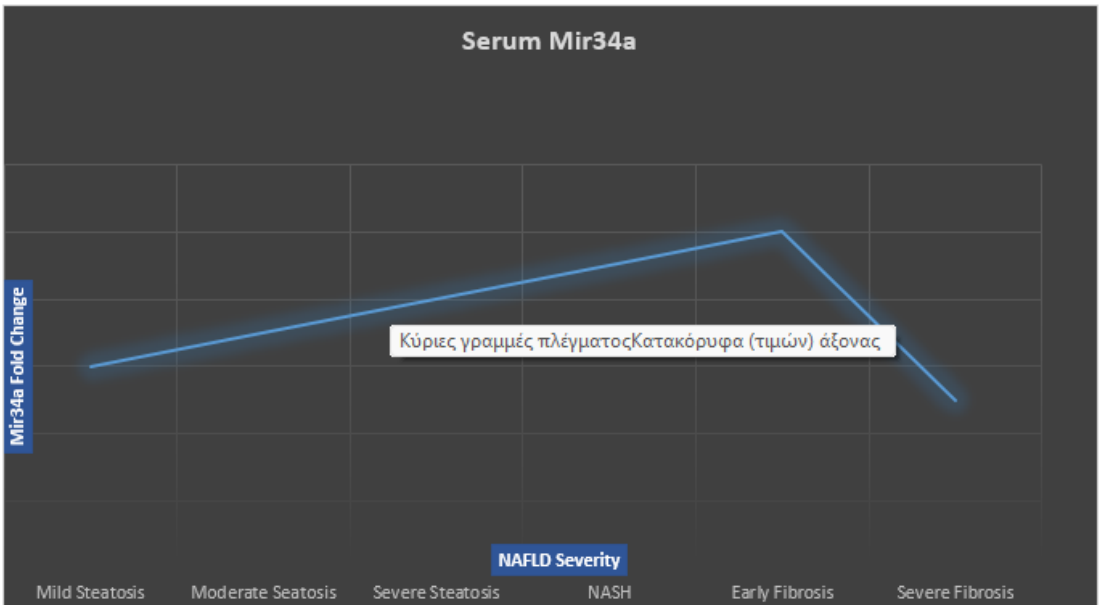

Table 1: MiRNAs mentioned in the reports studied categorized by tissue of origin		
MiRNA tissue of origin	**Number of articles concerning different NAFLD spectrum Pathologies**	**MiRNAs examined in the articles**
Serum miRNA	14 articles about NAFLD in General	Mir122, mir34, mir141, mir125a-5p, mir142-3p, mir409-3p, mir2916-3p, mir199-5p, mir2125p, mir30a-5p, mir30a-3p, mir146, mir149, mir325p, mir4488.
	18 articles about Steatosis	Mir122, mir34a, mir29a, mir193a-5p, mir38d, mir38e, mir185, mir29, mir126, mir3941, mir4517, mir4672, mir132, mir103, mir9, mir50-30p, mir613, mir615, mir33a .
	8 articles about fibrosis	Mir122, mir34a, mir193-5p, mir192, mir451, mir214, mir590-5p.
	10 articles about HCC	Mir34, mir192, mir122, mir191, mir9, mir9-5p, mir30a, mir194, mir214.
Tumoral Tissue miRNA	2 articles about HCC	Mir21, mir215-5p, mir34a-5p, mir130a-3p, mir130a-5p, mir155-3p, mir4726-5p, mir426-5p.
Liver Tissue miRNA	4 articles about steatosis	Mir122, mir192, mir21, mir34a-5p, mir122-5p, mir29c-3p.
	2 articles about HCC	Mir193-5p, mir192.
Visceral Adipose Tissue miRNA	2 articles about steatosis	Mir19, mir99, mir99a, mir132.
Ascetic Fluid miRNA	2 articles about HCC	Mir182,mir301a, mir373.

Table 2: Diagnostic Value of serum MiRNAs associated with NAFLD			
NAFLD Spectrum **Pathology**	**MiRNA**	**Diagnostic Value**	
		Initial Diagnosis	***Follow up***
NAFLD	Mir193-5p	NAFLD biomarker	Severity related
	Mir146, mir214, mir103, mir194	Upregulated in NAFLD	
	Mir4488	Elevated especially in lean individuals	
	Mir122	Prognosis related	Severity related
	Mir34a	Upregulated	Further increase in steatosis

Table 3: Diagnostic Value of serum MiRNAs associated with steatosis			
NAFLD Spectrum **Pathology**	**MiRNA**	**Diagnostic Value**	
		Initial Diagnosis	***Follow up***
Steatosis	Mir122		Downregulation of mir122 levels is related to NASH development
	Mir192, mir34a, mir9	Upregulated, related with a state of liver injury	

Table 4: Diagnostic Value of serum MiRNAs associated with fibrosis			
NAFLD Spectrum **Pathology**	**MiRNA**	**Diagnostic Value**	
		Initial Diagnosis	***Follow up***
Fibrosis	Mir122		Downregulated
	Mir34a		Higher levels than NAFLD in early fibrosis, lower levels than NAFLD in severe fibrosis
	Mir192		Upregulated in early fibrosis
	Mir214	Can differentiate fibrosis from cirrhosis (downregulated while progressing from fibrosis to cirrhosis)	

Table 5: Main findings of the studies included in the systematic review					
Study Title	**Demographic features**	**Hepatic enzymes**	**Mirna**	**Qualitive Results**	**Quantitate Results**
Diagnostic potential and pathogenic performance of circulating miR-146b, miR-194, and miR-214 in liver fibrosis	70 patients, 34 NAFLD patients, the mean age was 46.8 ± 1.9, with 61.7% being male.	No significant differences observed between ALT and AST in the patient groups, but there was a significant difference in total cholesterol levels ($p = 0.03$)	miR-146b, miR-194, miR-214	Positive correlation between age and disease severity within the population. Most of the patients were classified as overweight rather than obese.	**miR-146b and miR-214 were significantly up-regulated in NAFLD patients, while miR-194 expression level was notably decreased** ($p < 0.05$). MiR-146b relative expression in NASH, fibrosis, and cirrhosis was 5.03 ± 1.36, 6.41 ± 1.07, and 5.68 ± 2.47, respectively. For miR194, the expression level in NASH, fibrosis, and cirrhosis was 0.29 ± 0.1, 0.37 ± 0.053, and 0.33 ± 0.06. The relative expression of miR-214 was 63.73 ± 16.69, 106.7 ± 13, and 57.79 ± 4.6 in NASH, fibrosis, and cirrhosis. **Moreover, miR-214 expression decreased significantly during progression from fibrosis to cirrhosis ($p < 0.05$).**
Circulating MicroRNA-122 and Fibrosis Stage Predict Mortality of Japanese Patients With histopathologically Confirmed NAFLD	441 Japanese patients with histopathologically confirmed NAFLD after a median follow-up period of 4.7 years	Parameters (e.g., age, AST, ALT, platelet count, NAS, and low-density lipoprotein cholesterol) that indicated a strong correlation with others were considered confounding factors and excluded from statistical analysis.	Mir122	The rate with a FIB-4 index of <1.30 was significantly higher than that of 2.67-3.24 ($P < 0.001$) and ≥3.25 ($P < 0.001$) by multiple comparisons.	**Low serum level of miR-122 as a significant and independent risk factor of mortality.**

Analysis of association between circulating miR-122 and histopathological features of nonalcoholic fatty liver disease in patients free of hepatocellular carcinoma	serial liver biopsies from 36 hepatocellular carcinoma (HCC)-free Japanese patients with histopathologically-proven NAFLD.	There were also significant and strong associations between serum miR-122 ratio and changes in other clinical parameters, including aspartate aminotransferase and alanine aminotransferase.	Mir122	Controversial results due to small number of patients who showed progression of steatosis	Patients with improvement and progression of ballooning scores had significantly low serum miR-122 levels at second biopsy, and that serum miR-122 levels correlated with improvement in steatosis and fibrosis scores but neither with progression of steatosis nor fibrosis scores.
Potential biomarkers in NASH-induced liver cirrhosis with hepatocellular carcinoma: A preliminary work on roles of exosomal miR-182, miR-301a, and miR-373	26 HCC and 26 non-hcc patients	-	Mir182, miR-301a, miR-373	serum, and ascitic fluid exosomal miRNAs may be potential resources of biological markers for NASH-related liver cirrhosis with HCC.	**Serum levels of serum exosomal miR182, miR-301a and miR-373 were significantly up regulated in patients with NASH-related liver cirrhosis with HCC** as compared to patients with NASH-related liver cirrhosis without HCC.
Performance of Serum microRNAs -122, -192 and -21 as Biomarkers in Patients with NonAlcoholic Steatohepatitis	Serum profiles of four microRNAs were evaluated in two cohorts consisting of 137 NAFLD patients and 61 healthy controls.	miR-122 and miR-192 had a significant and positive correlation with serum ALT (miR-122: R = 0.53, p< 0.0001; miR-192: R = 0.45, p< 0.0001)	miR-21, miR-122, miR-192	Combined microRNA expression profiles with CK18Asp396 fragment level scoring model had a higher potential of NASH prediction compared to other risk biomarkers (AUROC = 0.83, 95% CI = 0.754–0.908; p<0.001).	Different profile of circulating miR-21 in NASH patients (p<0.0001). miR-122 and miR-192 are differentially regulated in NAFL and NASH.
Deregulated hepatic microRNAs underlie the association between non-alcoholic fatty liver disease and coronary artery disease	133 cases of autopsies with SCD and established CAD (patient group, CAD-SCD) and 106 cases of non-CAD sudden death	-	miR-34a-5p mir-29c-3p miR-122-5p	Patients more frequently exhibited NAFLD and necroinflammatory steatohepatitis (NASH) than controls (62% vs 26%, P = 0.001 and 42% vs 26%, P = 0.001	**An increase in miR-34a-5p and a decrease in miR-122-5p and 29c-3p in patients with NASH** vs controls without NAFLD were observed (P < 0.05). Finally, **significant correlations between**

	(control group, non-CAD-SD) were included. miRNAs were quantified in frozen liver tissues. Males predominated in both groups.			respectively). In both groups, the presence of NAFLD correlated with body mass index and abdominal circumference (P < 0.05).	**miR-122-5p and unfavourable lipid profile** and also hs-CRP and miR-34a-5p were noted.
MicroRNA-132 may be associated with blood pressure and liver steatosis—preliminary observations in obese individuals	Obese individuals undergoing bariatric surgery for weight loss (n = 19) Participants (aged 39 ± 8.1 years) had a body mass index (BMI) of 42 ± 4.5 kg/m2 and presented with 2.2 ± 1.2 metabolic abnormalities	Subcutaneous fat miRNA-132 expression was correlated to plasma triglycerides (r = 0.51, P < 0.01) and liver enzymes (ALT: r = −0.52; AST: r = −0.48, P < 0.05 for all).	miR-132	Reduction of miR132 may be a target for the regulation of liver lipid homeostasis and control of obesity-related blood pressure.	**Hepatic and visceral fat expression of miR-132 were correlated** (r = 0.59, P = 0.033). There was no correlation between subcutaneous and visceral expression of miR-132 (r = −0.31, P = 0.20). Hepatic and visceral fat miR-132 expression were associated with BMI (r = 0.62 and r = 0.68, P = 0.049 respectively) and degree of liver steatosis (r = 0.60 and r = 0.55, P < 0.05, respectively). Subcutaneous fat **miRNA-132 expression was correlated to office systolic BP** (r = 0.46, P < 0.05), several aspects of 24 h BP (24 h systolic BP: r = 0.52; day systolic BP: r = 0.59, P < 0.05 for all), plasma triglycerides (r = 0.51, P < 0.01) and liver enzymes (ALT: r = −0.52; AST: r = −0.48, P < 0.05 for all). We found an association between miR-132 and markers of cardiovascular and metabolic disease.

Serum miR-33a is associated with steatosis and inflammation in patients with nonalcoholic fatty liver disease after liver transplantation	116 liver transplant recipients who underwent post-transplant protocol liver biopsy.	Steatosis was independently associated with serum miR-33a, ALT, glycaemia and waist circumference.	miR-33a	Circulating miR-33a is an independent predictor of liver steatosis and inflammation inpatients after liver transplantation	Liver graft steatosis and inflammation, but not ballooning or fibrosis, were significantly associated with serum miR-33a, dyslipidemia and insulin resistance markers on univariate analysis. Inflammation was independently associated with miR-33a, HbA1 and serum triglyceride levels.
Differential expression of miRNAs in the visceral adipose tissue of patients with non-alcoholic fatty liver disease	Patients with biopsy-proven NAFLD were divided into non-alcoholic steatohepatitis (NASH) (n = 12) and non-NASH (n = 12)	-	hsa-miR-132, hsa-miR-150, hsa-miR-433, hsa-miR-28-3p, hsa-miR-511, hsa-miR-517a, hsa-miR-671, miR-197, hsa-miR-99	miRNA expression from VAT may contribute to the pathogenesis of NAFLD – a finding which may distinguish relatively simple steatosis from NASH.	MiRNA differentially expressed between NASH patients and nonNASH patients ($P < 0.05$). Of these, seven remained significant after multiple test correction (hsa-miR-132, hsa-miR-150, hsa-miR-433, hsa-miR-28-3p, hsa-miR-511, hsa-miR-517a, hsa-miR-671). Two miRNA species, **hsa-miR-197 and hsa-miR-99, were significantly associated with pericellular fibrosis in NASH patients** ($P < 0.05$)
Differential Associations of Circulating MicroRNAs With Pathogenic Factors in NAFLD	132 subjects with biopsy-proven NAFLD.	-	miR-34a, miR-122, miR-191, miR-192, and miR-200a	miR-34a, miR-122, miR-192, and miR200a demonstrate strong associations with NAFLD severity by histology, but differential associations with pathogenic factors.	miR-34a, miR-122, miR-192, miR-200a, but not miR-191, strongly correlate with fibrosis in NAFLD by increases of 0.20 to 0.40 SD ($P < 0.005$) with each stage of fibrosis. In multivariate analysis, miR-34a, miR-122, and miR-192 levels are independently associated with hepatic steatosis and fibrosis, but not lobular

					inflammation or ballooning degeneration, whereas miR-200a is only associated with fibrosis. Among the four miRNAs, **miR-34a, miR-122, and miR-192 are associated with pathogenic factors of NAFLD**, including insulin resistance, miR-200a is only associated with the TM6SF2 E167K variant. **Finally, miR-34a has the strongest predictive value for various stages of fibrosis.**
Evaluation of the severity of nonalcoholic fatty liver disease through analysis of serum exosomal miRNA expression	Exosomal miRNAs were isolated from the sera of 41 patients with NAFLD (diagnosed using liver biopsy) for microarray profiling.	ALP, ALT, AST, BUN, Cr, CRP, Hb, GGT, PT, PLT, WBC, and NAS scores were positively correlated with the expression of other miRNAs	let-7b-5p, miR-378h, -1184, -3613-3p, -877-5p, -602, -133b, and 509-3p	Serum exosomal miRNAs can be used to evaluate NAFLD severity and identify potential targets for NAFLD treatment.	Eight miRNAs (let-7b-5p, miR-378h, -1184, -3613-3p, -877-5p, -602, -133b, and 509-3p) showed anticorrelated patterns with the corresponding mRNA expression.
circRNA_0046367 Prevents Hepatoxicity of Lipid Peroxidation: An Inhibitory Role against Hepatic Steatosis	Five patients with biopsy-proven hepatic steatosis (5 of nonalcoholic fatty liver disease (NAFLD), age: 51.60 ± 12.10; male/female: 3/2) and 3 nonsteatosis controls (2 of chronic hepatitis B (CHB), 1 of primary biliary cirrhosis (PBC), age: 55.00 ± 18.19; male/female: 1/2)	-	Mir-34a	Here, we uncover circRNA_0046367 to be endogenous modulator of miR-34a that underlies hepatic steatosis.	**circRNA_0046367 normalization abolished miR-34a's inhibitory effect on peroxisome proliferator-activated receptor α (PPARα) via blocking the miRNA/mRNA interaction with miRNA response elements (MREs).**

A micro-RNA expression-signature for human NAFLD progression	36 liver biopsy specimens stratified by disease severity. Class III obese subjects schedule for elective bariatric surgery and between 26 and 59 years.	-	miR-301a-3p, miR-34a-5p, miR-375, miR-375	NAFLD severity is associated with a specific pattern of altered hepatic microRNA expression that may drive the altered lipid and carbohydrate metabolism hallmark of this disorder.	The abundance of 3 miRNAs **(miR-301a-3p and miR-34a-5p increased and miR-375 decreased) found to be differentially regulated with disease progression** was validated by RT-PCR.
NIS2+TM, an optimisation of the blood-based biomarker NIS4® technology for the detection of at-risk NASH: A prospective derivation and validation study	198 included patients from the GOLDEN-505 trial. The validation (n = 684) and test (n = 2,035) cohorts included patients from the RESOLVE-IT trial.	NIS2+TM exhibited a statistically higher area under the receiver operating characteristic curve (0.813) vs. NIS4® (0.792; p = 0.0002), Fibrosis-4 (0.653; p <0.0001), and alanine aminotransferase (0.699; p <0.0001).	miR-34a-5p	NIS2+TM constitutes a robust optimisation of NIS4® technology for the detection of at-risk NASH.	Performance of NIS2+TM was compared with that of NIS4®, Fibrosis-4, and alanine aminotransferase using area under the receiver operating characteristic curve, and robustness was analyzed through score distribution.
Circulating miR-29b positively correlates with non-alcoholic fatty liver disease in a Chinese population	276 individuals, 67 individuals with both NAFLD and T2DM, 73 individuals with NAFLD but no T2DM, 68 individuals with T2DM but no NAFLD, and 68 controls with neither of these two diseases, respectively.	In the non-diabetic group, NAFLD patients had significantly higher levels of aspartate transaminase (AST), alanine aminotransferase (ALT), γ-glutamyltransferase (γ-GT), than participants without NAFLD (all P < 0.05). While in the diabetic group, higher levels of AST, ALT, γ-GT, TG, TC, and low-density lipoprotein cholesterol	miR-29a-c	Serum miR-29b was associated with intrahepatic lipid content and NAFLD in a Chinese population-based study.	Serum miR-29b, but not miR-29a or miR-29c, was positively associated with NAFLD (odds ratio [OR] 2.04 [1.16- 3.58], P = 0.013). **MiR-29b level was positively correlated with intrahepatic lipid content** (β ± SE = 6.055 ± 2.630, P = 0.024) after adjusted for age, sex, and body mass index.

		(LDL-C) were observed in patients with NAFLD than in those without (all P < 0.05).			
Serum Micro-RNA-122 Level as a Simple Noninvasive Marker of MAFLD Severity	120 obese Egyptian patients with MAFLD, which were diagnosed and classified according to ultra-sonographic liver findings.	Serum micro-RNA-122 was significantly positively correlated with BMI, LDL (Figures 3 and 4), TG, ALT, AST, and GGT with a p-value <0.001. On the contrary, it was significantly negatively correlated with HDL with a p-value <0.001.	micro-RNA-122	Serum micro-RNA-122 could be a useful predictor of assessing MAFLD severity regarding level of steatosis or fibrosis.	There was a significant **increase in levels of micro-RNA-122 in obese patients with MAFLD compared to controls** (p<0.001). Micro-RNA-122 level was lower in patients with mild liver steatosis than patients with moderate or severe steatosis (p<0.001). It was lower in patients with a mild degree of fibrosis than those with mild or moderate fibrosis (p<0.001). Micro-RNA -122 was significantly positively correlated with low-density cholesterol and triglycerides level, and liver enzymes, and negatively correlated to high-density cholesterol (p<0.001).
The Circulating Micro-RNAs (−122, −34a and −99a) as Predictive Biomarkers for Non-Alcoholic Fatty Liver Diseases	210 patients with NAFLD (124 patients of simple steatosis (SS) and 86 of non-alcoholic steatohepatitis (NASH). Apparently matched for age and gender, healthy participants (n= 90) were enrolled as a control group.	There was a statistically significant increase in the BMI, waist circumference, fasting glucose, fasting insulin, HOMA-IR, AST, ALT, and GGT among NAFLD patients compared to the control group, while lipid profile, ALP, and serum	mir−122, mir−34a mir−99a	The integration of a circulating mi-RNA panel to diagnose NAFLD cases and to discriminate between SS and NASH.	Compared with the control subjects, **both mi-RNA-122 and −34a levels were increased in NAFLD** (p< 001) and at a cut-off = 1.261, mi-RNA-122 had 92% sensitivity, 85% specificity to differentiate NAFLD from healthy controls, while mi-RNA-99a were significantly decreased in NAFLD patients with an observed

		albumin levels showed non-significant differences between both groups.			decrease in disease severity, and at a cut-off = 0.46, **miRNA-99a had 94% sensitivity and 96% specificity to discriminate SS from NASH.**
Acetylation of H3K27 activated lncRNA NEAT1 and promoted hepatic lipid accumulation in non-alcoholic fatty liver disease via regulating miR-212-5p/GRIA3	Ten patients with NAFLD and ten healthy controls.	-	miR-212-5p	LncRNA NEAT1 and GRIA3 was upregulated, while miR212-5p was downregulated in NAFLD patients.	LncRNA NEAT1 knockdown upregulated miR-212-5p level and inhibited FFA-induced lipid accumulation while suppressing GRIA3 expression. Such function was antagonized by miR-212-5p inhibition and GRIA3 knockdown counteracted with miR-212-5p inhibition. H3K27 acetylation was enriched within the promoter of lncRNA NEAT1 and promoted lncRNA NEAT1 transcription. LncRNA NEAT1 could then interact with miR-212-5p and suppress its cellular concentration.
Long noncoding RNA CCAT1 inhibits miR-613 to promote nonalcoholic fatty liver disease via increasing LXRα transcription	NAFLD (n = 17) and normal liver tissues (n = 10) or serum samples	-	miR-613	CCAT1 increases LXRα transcription by serving as a competing endogenous RNA for miR-613 in an LXRE-dependent manner, thereby promoting lipid droplet formation and NAFLD. CCAT1 and LXRα might be potent targets for NAFLD treatment.	CCAT1 was induced by OA and upregulated in NAFLD clinical samples. CCAT1 silencing significantly suppressed lipid droplet accumulation in vitro. LXRα was positively correlated with CCAT1. By inhibiting miR613, CCAT1 increased the transcription of LXRα and promoted LXRα expression. The expression of LXRα was significantly increased in NAFLD

					tissues and was positively correlated with CCAT1.
Increased serum miR-193a-5p during non-alcoholic fatty liver disease progression: Diagnostic and mechanistic relevance	183 patients with NAFLD of varying severity together with 10 population controls. he findings for miR-193a-5p were replicated in a cohort of 372 additional NAFLD cases.	-	miR-193a-5p	miR-193a-5p consistently increased. We confirmed this increase in a second group of cases with NAFLD.	Plasma levels of **miR-193a-5p were significantly increased in patients with advanced fibrosis**, high NAS scores, or high SAF scores.
Circulating miRNA is a useful diagnostic biomarker for nonalcoholic steatohepatitis in nonalcoholic fatty liver disease	12 NAFL patients and 12 NASH patients.	-	miR-21-5p, miR-151a-3p, miR-192-5, miR-4449	NASH represents significantly distinct miRNA expression profile compared with NAFL.	Only four miRNAs (miR-21-5p, miR-151a-3p, miR-192-5p, and miR-4449) showed significant area under the receiver operating characteristic curve (AUC) values for NASH diagnosis. The combination of the four miRNAs showed satisfactory diagnostic accuracy for NASH (AUC 0.875; 95% CI 0.676–0.973). External validation revealed similar diagnostic accuracy for NASH (AUC 0.874; 95% CI 0.724–0.960)
High-throughput sequencing reveals altered expression of hepatic miRNAs in non-alcoholic fatty liver disease-related fibrosis	Frozen liver tissue from 30 NAFLD patients with stage F0 or F3/4 fibrosis. 15 individuals without NAFLD fibrosis (F0) and 15 individuals with severe NAFLD fibrosis or cirrhosis (F3-	Individuals with NAFLD fibrosis had higher levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) compared to individuals without fibrosis.	miR-182	Significant role for hepatic miRNAs in the pathogenesis of NAFLD-related fibrosis	FOXO3 and FBXW7 as potential targets of miR-182, and found that levels of FOXO3, but not FBXW7, were significantly decreased in fibrotic samples.

	4), matched for age, sex, BMI, T2D status, HbA1c, and use of diabetes medications.				
Hepatic mir-122-3p, mir-140-5p and mir-148b-5p expressions are correlated with cytokeratin-18 serum levels in MAFLD	6 patients of both sexes, aged between 18-60 years	-	hsa-miR-122-3p, hsa-miR-140-5p, hsa-miR-148b-3p	There is a correlation between elevated tissue expression of hsa-miR-122-3p, hsa-miR-140-5p, and hsa-miR-148b-3p with plasma levels of CK-18 in patients with simple steatosis compared with patients without the disease.	Circulating levels of CK-18 showed a significant difference in patients with MAFLD, and a strong correlation was found between hsa-miR-1223p, hsa-miR-140-5p, and hsa-miR-148b-5p versus the CAP value.
Circulating miRNA in patients with non-alcoholic fatty liver disease and coronary artery disease	44 patients with NAFLD and with or without CAD. Patients undergoing elective coronary angiography	ALT and AST levels were similar in both groups	miR-132 miR-143 miR-145 miR-211 miR-146a miR-30c miR-161 miR-241	The study shows an opposite trend in levels of circulating miRNA than previously reported intracellular levels in the tissues affected by pathogenic conditions. Altered circulating levels of miRNAs may serve to balance intracellular levels of miRNA in target tissues. Circulating miRNA as a link between multi-tissue crosstalk.	When compared to patients with NAFLD without CAD, patients with NAFLD and CAD had lower circulating levels of miR-132 (0.24±0.16 vs 0.30±0.11, p=0.03), while the circulating levels of miR-143 were higher (0.96±0.90 vs 0.64±0.77, p=0.02). The levels in circulation demonstrated trends opposite to previously observed intracellular levels in patients with CAD. In obese patients with NAFLD, lower circulating levels of miR-145 (1.42±1.00 vs 2.41±1.80), miR-211 (41.26 ±20.40 vs 57.56±25.45), miR-146a (2.13±1.40 vs 2.90 ±1.36) and miR-30c (6.92±4.99 vs 11.0±6.92) were detected when compared to lean patients with NAFLD. For

					miR-161 (0.59±1.19 vs 0.15±0.14) and miR-241 (0.28±0.29 vs 0.16±0.13), higher circulatory levels were detected in the obese patients with NAFLD
NAFLD mark: an accurate model based on microRNA-34 for diagnosis of non-alcoholic fatty liver disease patients	314 Egyptian individuals were included (100 healthy individuals and 214 NAFLD patients)	The multivariate analysis was used to evaluate the NAFLD-associated variables (CRP, cholesterol, body mass index (BMI), ALT had p< 0.0001	microRNA-34	The NAFLD mark is a non-invasive and highly sensitive and specific model for NAFLD diagnosis	**MiRNA-34 levels were higher in NAFLD patients than healthy individuals with a significant** difference (P< 0.0001). The multivariate analysis was used to evaluate the NAFLD-associated variables (CRP, cholesterol, body mass index (BMI), ALT had p< 0.0001 while mRNA-34 had (p=0.0004). The AUCs (CI) of candidate NAFLD markers were in the order of miRNA-34 0.72 (0.66–0.77) < ALT 0.73 (0.67–0.79) < BMI 0.81 (0.76–0.86) < cholesterol < 0.85 (0.79–0.90) < CRP 0.88 (0.84–0.92).
Selective Isolation of Liver-Derived Extracellular Vesicles Redefines Performance of miRNA Biomarkers for Non-Alcoholic Fatty Liver Disease	NAFLD patients (NAFL n = 8; biopsy proven NASH n = 6) and healthy donors (n = 14) matched for age and sex.	-	miR-122, mir-192, mir-128-3p	This study demonstrates the capacity for liver-specific isolation to transform the performance of EV-derived miRNA biomarkers for NAFLD, robustly distinguishing patients with NAFL and NASH.	The expressions of miR-122, -192, and -128-3p were quantified in total cell-free RNA, global EVs, and liver-specific EVs from control, NAFL, and NASH subjects. In ASGR1+ EVs, each miRNA biomarker trended positively with disease severity and expression was significantly higher in NASH subjects compared with controls. The c-statistic defining the

					performance of ASGR1+ EV derived miRNAs was invariably >0.78.
Circulating microRNA signature in non-alcoholic fatty liver disease: from serum non-coding RNAs to liver histology and disease pathogenesis	51 NAFLD patients and the liver of 14 subjects without NAFLD (near-normal liver histology (NNLH)	miR-122 did much better than CK-18 and slightly better than ALT or AST in distinguishing patients with NASH and in predicting liver fibrosis.	miR-122, miR-192, miR-19a miR-19b, miR-125b, miR-375	miR-122 plays a role of physiological significance in the biology of NAFLD; circulating miRNAs mirror the histological and molecular events occurring in the liver	miR-122, miR-192, miR-19a and miR-19b, miR-125b, and miR-375 were upregulated >2-fold ($p<0.05$) either in simple steatosis (SS) or nonalcoholic steatohepatitis (NASH). **The most significant fold changes were observed in the serum levels of miR-122 (7.2-fold change in NASH vs controls and 3.1-fold change in NASH vs SS)** and miR-192 (4.4-fold change in NASH vs controls); these results were replicated in the validation set. The majority of serum miR-122 circulate in argonaute2-free forms. Circulating miR-19a/b and miR-125b were correlated with biomarkers of atherosclerosis. Liver miR-122 expression was 10-fold ($p<0.03$) downregulated in NASH compared with SS and was preferentially expressed at the edge of lipid-laden hepatocytes.

Quantitative Prediction of Steatosis in Patients with Non-Alcoholic Fatty Liver by Means of Hepatic MicroRNAs Present in Serum and Correlating with Hepatic Fat	human livers from organ donors (n = 20), and human sera from biopsy-proven NAFLD patients (n = 23), both with a wide range of steatosis quantified in their liver biopsies.	-	miR-10a-5p, miR-98-5p, miR-19a-3p, miR-30e-5p, miR-32-5p miR145-5p	It is possible to identify serum miRNAs, with biological significance, that correlate with the % of liver fat in NAFLD patients.	**miR-10a-5p, miR-98-5p, miR-19a-3p, miR-30e-5p, miR-32-5p and miR145-5p showed the highest biological relevance. PLS regression models with serum levels of 2–3 of these miRNAs predicted the % of liver fat with errors <5%**
Significance of MiRNA-34a and MiRNA-192 as a risk factor for nonalcoholic fatty liver disease	60 NAFLD patients, and the control group included 30 healthy subjects. The patient group was further divided into two groups: the first group included 28 NAFLD patients at an early stage of fibrosis (F0-F2), and the second group included 32 NAFLD patients with advanced stage of fibrosis (F3-F4). All participants are between 18 and 60 years old. The mean	-	miRNA-34a MiRNA-192	Ultimate role of miRNA-34a in lipid metabolism dysregulation may lead to NAFLD. MiRNA-34a and MiRNA-192 are broadly stated to play a role in the development of NAFLD and NASH.miRNA-34a was positively correlated with metabolic disorders associated with NAFLD such as hypertension and diabetes. However, their expression showed no association with advanced fibrosis.	**miRNA-34a and MiRNA-192 are upregulated in NAFLD patients with early fibrosis compared to controls** [with a fold change of 4.02 ± 11.49 (P = 0.05) and 18.43 ± 47.8 (P = 0.017), respectively]. However, miRNA-34a is downregulated in NAFLD patients with advanced fibrosis compared to controls, with fold expression of 0.65 ± 1.17 (P = 0.831).

	age of participants was 40.08 ± 13.3 years.				
Distinct Morphological and Molecular Profiles of NAFLD and NAFLD-associated HCC Revealed by Immunohistochemistry and MicroRNA Analysis	14 NAFLD-associated HCC and 41 NAFLD patients.	-	miR-21-5p, miR-130a-3p, miR-34a-5p	(miR-21-5p, miR-130a-3p and miR-34a-5p) are overexpressed in p53 signaling pathway, in proteoglycans processing pathway in cancer and in hepatitis B. Three miRNA, miR-21-5p, miR-130a-3p and miR-34a-5p are involved in fatty acids metabolism and cell cycle progression. Additionally, miR-34a-5p and miR-130a-3p are overexpressed in multiple solid malignancies and in oncogenic pathways.	**MiR-21-5p and miR-34a5p were overexpressed in HCC tissue samples compared with normal control samples (p<0.0001). MiR-130a-3p was downregulated in HCC samples compared with normal controls (p<0.0001)** and for miR-155-3p no statistically significant differences between HCC samples and controls were seen (p=0.86)
Expression of genes for microRNA-processing enzymes is altered in advanced non-alcoholic fatty liver disease	All patients had biopsy-proven NAFLD (NASH patients [n = 12] and non-NASH NAFLD [n = 12]).	-	pri-miR-7-1 primiR-16-2 pri-miR-26a-1 pri-miR-125b-2	VAT-derived miRNA may contribute to the pathogenesis of NASH in obese patients.	Expression of Dicer1, Drosha and DGCR8 was significantly increased within the NASH cohort along with expression of pri-miR-7-1. The presence of focal necrosis on the liver biopsy correlated significantly with levels of Dicer1 and DGRC8. Both NASH and ballooning degeneration of hepatocytes correlated negatively with the expression levels of hsa-miR-125b. Histologic NASH correlated positively with the expression levels of primiR-16-2 and pri-miR-7-1. The presence of the hepatocyte's ballooning

					degeneration in the liver biopsy correlated positively with pri-miR-26a-1 and pri-miR-7-1. The expression profile of pri-miR-125b-2 also correlated positively with body mass index.
Serum miR-4488 as a potential biomarker of lean nonalcoholic fatty liver disease	498 patients with NAFLD and 98 healthy controls were included to compare the clinical characteristics of lean NAFLD patients [LNs: body mass index (BMI) <23 kg/m2], nonlean NAFLD patients (NLNs: BMI ≥23 kg/m2) and normal healthy individuals (HIs). A total of 14 serum samples were collected from 4 LNs, 6 NLNs and 4 HIs for high-throughput profiling to identify altered miRNA expression patterns in lean NAFLD.	-	miR-4488	LNs were older and had a smaller waist circumference, lower levels of alanine aminotransferase, glutamyl transpeptidase, fasting insulin, and uric acid, lower HOMA-IR score, and higher levels of total cholesterol, high-density lipoprotein cholesterol, and hemoglobin (P<0.05)	**The serum level of miR-4488 was increased in LNs** compared with HIs (P<0.0001) and NLNs (P=0.025). miR-4488 had acceptable performance in predicting [area under the curve (AUC) =0.794, 0.698] lean NAFLD.
A Pilot Study of Serum MicroRNAs Panel as Potential Biomarkers for Diagnosis of Nonalcoholic Fatty Liver Disease	465 participants (healthy controls and NAFLD patients)	BMI, ALT, AST and platelets levels of NAFLD patients were significantly different from those of the normal controls.	hsa-miR-122-5p, hsa-miR-1290, hsa-miR-27b-3p, and hsa-miR-192-5p	Serum microRNA panel with considerable clinical value in NAFLD diagnosis. The results indicate that the miRNA panel is a more sensitive and specific biomarker for NAFLD than ALT and FIB-4.	miRNA panel (hsa-miR-122-5p, hsa-miR-1290, hsa-miR-27b-3p, and hsa-miR-192-5p) with a high diagnostic accuracy for NAFLD.

Variance component analysis of circulating miR-122 in serum from healthy human volunteers	42 individuals (19 females, 23 males, ranging from 29 to 62 years of age)	-	miR-122	miR-122 demonstrated higher than expected variability in serum from healthy volunteers, which has implications for its potential utility as a prospective biomarker of liver damage or injury.	High variability of miR-122 in serum appeared to be due in part to ethnicity, as 95% confidence reference intervals were approximately three-fold lower in volunteers that identified as Caucasian relative to those that identified as Non-Caucasian.
Gene expression profiling reveals key genes and pathways related to the development of non-alcoholic fatty liver disease	27 healthy obese samples, 14 steatosis samples and 18 nonalcoholic steatohepatitis (NASH) samples.	-	hsa-miR-330-3p, hsa-miR-126	The identified DEGs (PRKCA,EGFR,CDC42,VEGFA), disturbed pathways (ribosome, ubiquitin mediated proteolysis, focal adhesion, FcγR-mediated phagocytosis, etc.) and miRNAs (hsa-miR-330-3p, hsa-miR-126, etc.) might be closely related to NAFLD progression.	In PPI networks, PRKCA interacted with EGFR and CDC42. Besides, hsa-miR-330-3p and hsa-miR-126 modulated focal adhesion through targeting VEGFA and CDC42.
Associations between circulating microRNAs (miR-21, miR-34a, miR-122 and miR-451) and non-alcoholic fatty liver	443 persons who attended health examinations in August 2010 in Hokkaido Japan	The sera of men participants with NAFLD contained higher concentrations of AST, ALT, triglyceride and total cholesterol than participants without the NAFLD; the sera of women participants with NAFLD contained higher concentrations of TP, albumin, ALT, γ-GTP, triglyceride, and LDL-cholesterol than participants without the NAFLD.	miR-21, miR-34a, miR-122, miR-451	Serum levels of miRNAs, particularly miR-122, may be a useful biomarker for NAFLD.	Serum levels of miRNAs, miR-21, miR-34a, miR-122, and miR-451 were higher in participants with NAFLD. **The serum level of miR-122 was correlated with the severity of liver steatosis**.

Serum microRNAs explain discordance of non-alcoholic fatty liver disease in monozygotic and dizygotic twins: a prospective study	Cohort study of 40 (n=80) twin-pairs residing in Southern California. Among the 40 twin-pairs, there were 6 concordant for NAFLD, 28 were concordant for non-NAFLD and 6 were discordant for NAFLD. The prevalence of NAFLD was 22.5% (18/80).	-	miR-331-3p and miR-30c	Discordancy in liver fat content between the twins can be explained by miRNAs, and that they are heritable.	miR-331-3p and miR-30c, were also among the 21 miRNAs that were different between NAFLD and non-NAFLD groups (for miR-331-3p: 7.644±0.091 vs 8.057 ±0.071, respectively, p=0.004; for miR-30c: 10.013 ±0.126 vs 10.418±0.086, respectively, p=0.008). **Both miRs were highly heritable (35.9% and 10.7%,** respectively) and highly correlated with each other (R=0.90, p=2.2×10−16) suggesting involvement in a common mechanistic pathway. **An interactome analysis of these two miRNAs showed seven common target genes.**
Relationship between circulating miR-132 and non-alcoholic fatty liver disease in a Chinese population	140 non-diabetic and 134 diabetic patients	Serum miR132 was positive correlated with ALT, TG, FPG, γ-GT, APOE, HOMA-IR.	miR-132	Serum miR-132 was found to be associated with NAFLD risk in a Chinese cross-section study.	Serum miR-132 was positively associated with NAFLD in non-type 2 diabetes mellitus (T2DM) groups by logistic regression (OR = 3.082 [1.057, 8.988], P = 0.039) after adjusting age, sex, and body mass index (BMI). Additionally, in non-T2DM subgroup, after adjusting age, sex, bmi, serum miR-132 was significantly associated with ALT (β ± SE = 0.005 ± 0.002, P = 0.018), TG (β ± SE = 0.072 ± 0.029, P =0.015), FPG (β ± SE = 0.123 ± 0.058, P =0.036), γ-GT (β ± SE = 0.002 ±

					0.001, P = 0.047), apoE (β ± SE = 0.038 ± 0.002, P = 0.017)

Table 6: Key findings
Lean NAFLD patients express higher levels of mir4488 (26).
MiR-331-3p and miR-30c are inheritable and share 7 common target genes (8).
Elevated mir34a and mir122 levels are observed in NAFLD patients (6).
Elevated serum mir122 is associated with NAFLD mortality and is related with steatosis severity (6).
Mir122 controls lipid metabolism while mir34a is related to insulin resistance (27).
An elevated mir132 expression can lead to systolic blood pressure elevation (25).
Mir193-5p is a more accurate biomarker than mir122 for NAFLD staging (1).
Steatosis can be discriminated from NASH with mir99a (6).
Mir214 was downregulated while progressing to cirrhosis from fibrosis. Mir197 and mir99 are elevated when pericellular fibrosis occurs in NASH (28).
In early stages of fibrosis, mir34a and mir192 are overexpressed, while advanced fibrosis is characterized by elevated mir193a-5p (29,30).
Hcc is characterized by high mir21-5p and mir34a-5p levels, while usually patients with HCC are characterized by lower mir130a-3p levels (31)
In early stages of fibrosis, mir34a and mir192 are overexpressed, while advanced fibrosis is characterized by elevated mir193a-5p (29,30).
Hcc is characterized by high mir21-5p and mir34a-5p levels, while usually HCC patients are characterized by lower mir130a-3p levels (31)