

The Role of miRNAs and Epigenetic Factors in Non-Alcoholic Fatty Liver Disease - a Systematic Review

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ABSTRACT: Non-alcoholic liver disease is affecting approximately a quarter of the global population, with a significant morbidity and mortality rate. The present paper takes into discussion a systematic review of the literature concerning the role that microRNAs (miRNAs) and epigenetic factors can have in relation with NAFLD. Using PRISMA methodology guidelines, the systematic review offers a comprehensive image of the relevant scientific literature that explains the complex role of miRNAs in relation with NAFLD. Throughout the process of reviewing there has been highlighted seven different subtopics that can synthesize the nature and the content of the different studies: the role of miRNAs in fibrosis and liver disease progression; use of microRNAs as biomarkers for diagnosing steatotic liver disease; microRNAs and targeted therapies for NAFLD (Nonalcoholic fatty liver disease) /NASH (Non-alcoholic Steatohepatitis); metabolic regulation and obesity via microRNAs; interaction of miRNAs with other factors at the molecular level in disease pathogenesis; the role of miRNAs in systemic inflammation and effects on various other organs and the role of miRNAs as regulators and highly specialized epigenetic factors affecting NAFLD pathogenesis and evolution. Overall, this review presents diverse approaches on microRNAs and gives a background for future applied research that can explain and test their potential as biomarkers for diagnosing and understanding disease mechanisms, particularly in populations exposed to historical nutritional imbalances.

KEYWORDS: Non-alcoholic fatty liver disease, microRNAs, epigenetics, metabolic-associated fatty liver disease, biomarkers.

Introduction

Non-alcoholic fatty liver disease and its associated complications: non-alcoholic steatohepatitis (NASH), liver cirrhosis, hepatocellular carcinoma represents a major public health burden, reflected by an overall annual death rate of 25.56 per thousand person-years and a liver-specific annual mortality rate of 11.77 per thousand person-years.

Especially in Western countries there is a significant increase in the prevalence of NAFLD (estimated at 25%) and it is frequently associated with obesity, dyslipidemia, type 2 diabetes mellitus (T2DM) or metabolic syndrome. [1,2].

Initially the diagnosis of NAFLD was based on routine blood chemistry and ultrasound examination and/or liver biopsy in some cases.

But the pathophysiological mechanisms involved in NAFLD are complex, including genetic, epigenetic and environmental factors.

Hepatic insulin resistance which is common in most cases of NAFLD can cause lipid liver accumulation, generation of reactive oxygen species with activation of local inflammasomes and excess inflammatory cytokines production.

Inflammatory signals activate hepatic stellate cells leading to their activation and production of extracellular matrix [3].

The implication of epigenetic factors affecting successive generations such as DNA methylation patterns or histone acetylation in the pathogeny of NAFLD has already been demonstrated [4].

In the context of extensive study of the evolution, causes, and other conditions associated with NAFLD, Micro-RNAs have gained momentum during the last years due to their role in carbohydrate and lipid metabolism and their

value as non-invasive biomarkers for disease staging [5].

Micro-RNAs are small, non-coding RNAs (21-26 nucleotides long) that modulate gene expression by degrading mRNA or inhibiting gene translation.

Their synthesis involves a complex process: the primitive miRNA is cleaved by Drosha and DiGeorge syndrome Chromosomal Region 8 (DGCR8), exported to the cytoplasm, further cleaved by DICER family enzymes, and then attached in single-stranded form to a complex with Argonaut (Ago 1 to 4) and Transactivation Response Element (TRBP).

The resulting RNA-induced silencing complex can attach to specific 2-8 nucleotide sites on mRNA, leading to mRNA degradation [6].

Numerous experimental and clinical trials have shown the role of certain miRNAs in the pathogenesis of NAFLD by regulating gene expression involved in lipid metabolism, inflammation, insulin signaling, and fibrosis [7,8,9].

Thus, miR-33a and miR-33b are two micro-RNAs that have a considerable impact on lipid metabolism, being located in the intron of the sterol regulatory element-binding transcription factor 1.

miR-33a deficiency or inhibition in mice increases cellular cholesterol export through up-regulation of ABCA1 (ATP-binding subfamily A member 1) and the development of liver inflammation and fibrosis is suppressed.

Also, in high-fat diet mice as a model of NAFLD/NASH, (non-alcoholic fatty liver disease/non-alcoholic steatohepatitis) the use of anti-miR-33b reduced liver accumulation of free cholesterol and triglycerides and improved fibrosis [10].

Another miRNA-miR-122 is the most abundant miRNA in the liver and it is involved in liver steatosis, inflammation and development of HCC.

In a methionine-choline-deficient diet the serum level of miR-122 is increased 40-fold as compared to controls [11].

As it is highly influenced by such nutrient deficiencies one might question if we can use miR-122 as a biomarker of risk for NAFLD/NAFLD for generations of humans that might have been exposed to nutritional imbalances in the past.

In another case, for the mice fed with a diet lacking choline and folate serum, miR-192 levels are correlated with the severity of liver steatosis.

In earlier stages of liver fibrosis in humans with NAFLD, miR-192 is highly upregulated [12].

This picture indicates the possibility of formulating a research question such as: to what extent do miRNAs play an active role in diagnosing NAFLD, tracking the evolution of the disease and considering the implications from the perspective of highlighting the quality of regulators for epigenetic factors determining NAFLD.

This paper represents a systematic review of the literature in an attempt to validate the research question.

Starting from a specific methodology, based on PRISMA 2020 guidelines, 82 papers were finally selected that cover the studied issue, highlighting 7 specific subtopics.

Thus the main paper's objective is to reveal the importance of miRNAs in the pathogenesis of NAFLD/NASH and their role of biomarkers and potential therapeutic instruments in the future.

Material and Methods

In order to assure the proper level of evidence and completion of information we conducted a systematic review of the scientific literature on Web of Science (WoS) database, following PRISMA 2020 guidelines (Figure 1).

In order to do so, we have also adapted the PRISMA flow diagram template for systematic reviews proposed also in Page et al, 2021 [13].

We have used the following keywords: "miRNAs" and "Steatosis Liver Disease" in order to obtain a larger initial pool of papers from the database.

We have preferred the term Steatosis instead of Steatotic, because of the wider usage within the general literature in the field.

From the point of view of the review objectives this step was intended to ensure the specificity of studies, filtering out the tangential literature. A number of 216 papers have been found with 0 duplicates.

As exclusion criteria from the initial screening, we took into consideration-papers published before 2015 (being included only papers from the interval 2015-2024), the type of record (being taken into consideration only papers, review papers and early access), the quality of "open access" type of paper, (being included only open access papers), publishing language (being selected only English language papers).

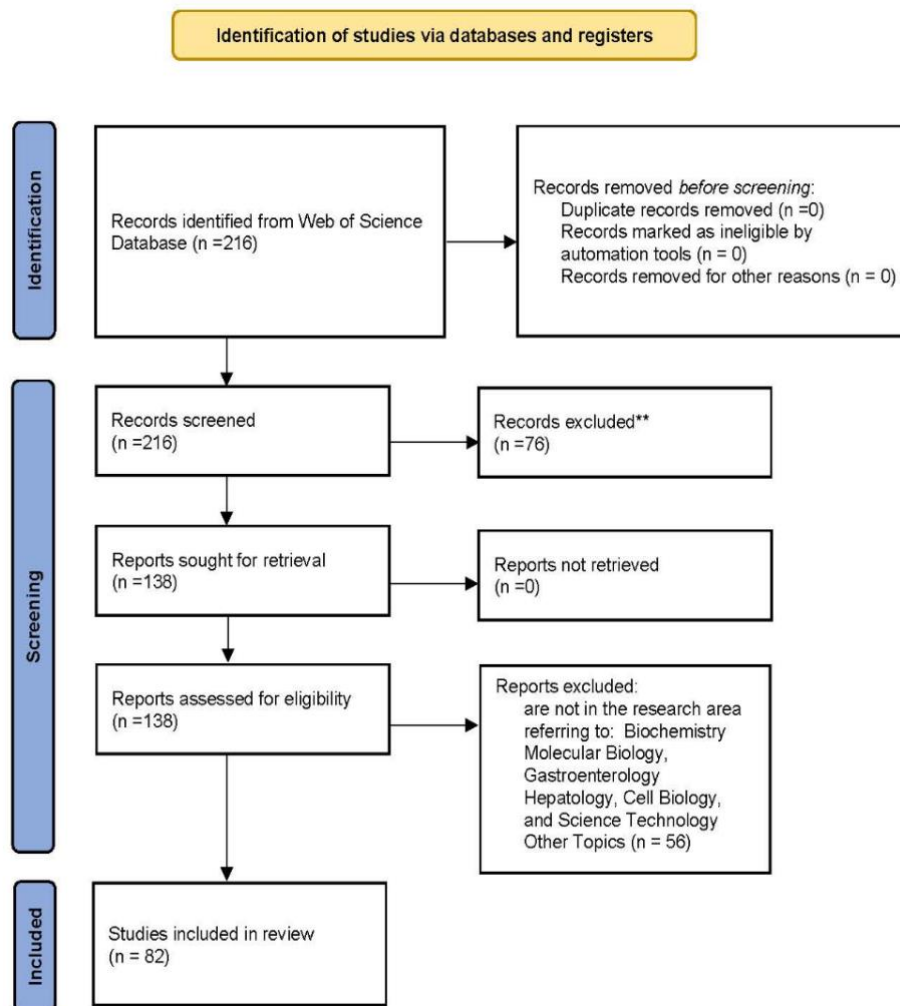


Figure 1. Flow chart describing study (papers) selection process.

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All these criteria contribute also to the objectives of the review-maintaining the currency and scientific validity of the findings (time related limitation), access to in-depth data, validated methodologies and high-quality findings (limitation to peer-reviewed papers), support transparency and full access to methodological details and results (limitation related to open-access type papers), ensuring a common scientific language in order to avoid misinterpretation (limitation to English language papers).

The total number of records that have been excluded after applying the exclusion criteria was 76 papers, remaining a number of 138 papers to be sought for retrieval.

From the total of 138 reports assessed for eligibility we have excluded a number of 56 reports as they not fall in one of the following categories of research area: Biochemistry Molecular Biology, Gastroenterology Hepatology, Cell Biology, and Science Technology Other Topics.

This final exclusion criteria were meant to assure the most relevant fields of research for our research questions, focus on biological and clinical aspects of miRNAs role in NAFLD.

Thus, the final number of studies to be included in review has been of 82 (Figure 1).

The scientific literature concerning the role that miRNAs can have in the pathology of NAFLD starts to be significant after the year 2013, with some papers that address this issue in an explicit manner.

In our systematic review, the time range start with 2015 as we considered more recent papers as being more relevant for the latest trends in the field and latest outcomes of practical research regarding the role of miRNAs.

Role of miRNAs in fibrosis and liver disease progression

miRNAs seem to be an essential factor influencing liver development and homeostasis, helping the regulation of liver diseases and in the same time the progression of hepatocarcinoma and viral infections.

Studies showed that miR-122 plays a dual role, being an essential factor for hepatitis C virus (HCV) replication, but also a potential inhibitor for hepatitis B virus (HBV) replication [14].

In 2017 the team of Eguchi et al., showed that extracellular vesicles (EVs) released by hepatocytes during liver stress are having a clear contribution to the development of alcoholic steatohepatitis (ASH).

The mechanism was implying the fact that EVs are containing a certain, specific "barcode" of miRNAs (detectable in the blood), like miR-29a and let-7f that are specific for the liver damage related with ASH, and not to other types of hepatic tissue damage [15].

In the same line of research, the study made by Dorairaj et al. (2020) took into discussion findings of adipocyte- and hepatocyte-EVs effect on NAFLD disease development and progression [16].

The study made by Gerhard and colleagues in 2019 was going in a specific direction, contributing to the body of research that is concerning the severity of fibrosis in NASH.

miR-372-3p can regulate the expression of AEBP1, which was directly correlated with the severity of liver fibrosis [17].

Also, levels of miR-34a, miR-122, and miR-192 are strongly correlated with the severity of fibrosis, thus is possible an association between miRNAs and pathogenic factors of the disease, with miR-34a that was capable to show a

strong predictive value for the different stages of fibrosis [18].

miR-1914-5p can act as a regulator of lipid metabolism and pro-fibrotic elements in activated hepatic stellate cell.

The angiotensin-(1-7) controls the trans-differentiation of HSCs (Hepatic Stellate Cells) and can reduce hepatic fibrogenesis and steatosis [19].

Morishita et al. (2022), put emphasis on the effect of ipragliflozin, an SGLT2 inhibitor, on the development of NASH.

The results ascertain that treatment with ipragliflozin significantly reduced liver inflammation and fibrogenesis, regulating in the same time the expression of miR-19b-3p [20].

Research conducted by Qi and Lai (2022) bring into light the role of exosomes and microRNAs in the progression of NASH.

Authors come to the conclusion that exosomal miRNAs such as miR-122 and miR-34a can play an essential role regarding intercellular communication and contribute to the progression of liver inflammation and fibrosis [21].

miR-155 and miR-34a have an important role in liver inflammation, related with impairment of lipid oxidation in the liver and associated with NASH progression [22].

The research of Halász et al. demonstrated that in the case of patients having various fibrosis etiologies, one of the most abundant miRNAs from the liver-miR-122 is negatively correlated with liver fibrosis stage and FibroScan values.

Based on these results, we may assume that advanced liver fibrosis presumes a significantly reduced miR-122 which allow us to consider this miRNA as a potential biomarker for the diagnosis and monitoring process of fibrosis progression [23].

In 2020, Longo et al. discovered that there is an interaction between intestinal dysbiosis and the progression of liver fibrosis, within the evolution of NASH.

During this process, miR-122 and miR-145 are modulated contributing to liver inflammation and fibrogenesis [24].

All studies presented above are showing a clear role of miRNAs in fibrosis and liver disease progression, this characteristic being one of the most important within the study of miRNAs implication in NAFLD etiology.

Use of miRNAs as biomarkers for diagnosing steatotic liver disease

Moving back in time, the research made in 2015 by Pirola et al. was showing also that miR-122, miR-192, miR-19a, and miR-19b were

strongly upregulated in NASH patients compared to those with simple steatosis (SS) or the control group.

Therefore, in their effort to investigate the circulating signature of microRNAs associated with non-alcoholic fatty liver disease, authors demonstrate the fact that miR-122 and miR-192 can be used as biomarkers for the diagnosis and monitoring of disease progression [25].

Results of Pirola et al can be completed by the ones of Mattis et al. (2015), that are showing that miR-29a can be seen as a regulator of lipoprotein lipase (LPL), an essential factor in hepatic lipid metabolism.

This research was showing that if we have decreased levels of miR-29a levels, we could assess lipid accumulation in the liver, which can worsen steatosis [26].

Research by Yu et al. in 2019 found that miR-122 and miR-34a, were correlated with level of development and progression of hepatic steatosis, thus they can be used as means of early diagnosis of NAFLD [27].

Zhang et al. (2020), have identified miR-193a-5p and miR-122-5p, as potential biomarkers for fatty liver disease.

These miRNAs are involved in pathological screening that is showing the progression of liver diseases.

Also, they are associated with hepatic steatosis and fibrosis. [28].

In the same context, we can take into consideration the research made in 2017 by Austermann et al. that have investigated the role of miR-200a as a biomarker for steatosis and liver damage in HIV patients, providing another insight into how miRNAs can serve as indicators of disease progression.

They demonstrated that miR-200a levels increase depending on the stage of steatosis and can predict the evolution of liver damage, which validates and complements the results obtained by other authors until then, investigating the role of microRNAs in liver pathology [29].

There are also different studies showing the potential role of biomarkers for the assessment and evolution of NAFLD, MAFLD (Metabolic dysfunction-Associated Fatty Liver Disease), NASH etc.

Thus, miR-103-3p is able to ameliorate lipid accumulation and oxidative stress in NAFLD, being capable to target the enzyme ACOX1 that is involved in fatty acid oxidation [30].

miR-22-3p can be used as a biomarker for stratifying MAFLD patients, because his relation

with hepatic steatosis that was induced by the exosomes that are containing it [31].

miR-26a regulates lipid homeostasis and protects liver cells from NAFLD progression, thus it can be used as a biomarker involved in preventing the development of the disease [32].

The effects of miR-206, miR-181b and miR-21 in patients with hypothyroidism and associated hepatic steatosis showed that these miRNAs can be used as biomarkers for the early diagnosis of metabolic dysfunctions [33].

Another research showed that miR-193a-5p levels are significantly increased in advanced stages of NAFLD, and this miRNA can be used as a biomarker to assess fibrosis severity and disease activity.

They demonstrated that miR-193a-5p is associated with oxidative stress and fibrosis progression [34].

The miRNAs: miR-27, Let-7, and miR-106a, are downregulated during the progression from cirrhosis to steatosis and then to hepatocellular carcinoma, thus they can play an important role in regulating the hepatic response to Hepatitis C.

Virus infection and in the pathogenesis of HCC (hepatocellular carcinoma) [35].

The team of Castaño et al, complete the previous results with research that shed a light upon the contribution of obesity-associated exosomes contain miR-122 and other miRNAs to glucose intolerance and hepatic steatosis.

This results also support the idea that miRNAs can be used as markers in the early diagnosis of metabolic and liver dysfunctions [36].

The results obtained by Bandiera et al. can be complemented with those obtained by Becker et al, in the same year (2015), who investigated the performance of miR-122, along with miR-192 and circulating miR-21, as biomarkers for the non-invasive diagnosis of non-alcoholic steatohepatitis (NASH).

Thus, a combination of microRNA profiles and traditional markers can increase diagnostic accuracy, being a more practical tool with the quality of non-invasiveness [37].

López-Pastor et al. in their study from 2022, demonstrate that miR-26b-5p, miR-34a-5p, and miR-122-5p are essential in promoting insulin resistance, autophagy, and progression to NASH.

These results are confirming the potential of these miRNAs as biomarkers for advanced stages of the disease, in the context of NAFLD progression in the case of apolipoprotein E-deficient mouse [38].

Salvoza et al. made an important discovery that suggest the capacity of miR-34a and

miR-122 to be effective biomarkers capable to identify and monitor NAFLD progression.

In their research they investigate the role that these miRNAs can have in dyslipidemia associated with patients diagnosed with NAFLD.

The levels of these miRNAs have been correlated with very low-density lipoprotein (VLDL-C) and triglyceride levels, being present in high density in the serum of NAFLD patients [39].

Quintás et al. have demonstrated in 2022, that several microRNAs, including miR-10a-5p, miR-98-5p, and miR-19a-3p, are strongly correlated with the percentage of liver fat in NAFLD patients.

Because of this they can be considered biomarkers for the evaluation of hepatic steatosis, providing a non-invasive predictive model [40].

Ghosh et al. (2020) have demonstrated that miR-c12, a new type of miRNA at that time, is capable to modulate fatty acid oxidation and plays an important protective role in chronic hepatitis C.

This miRNA is capable actually to regulate the lipid metabolism because is interfering with PPAR α sequestration and, in a natural manner is limiting the hepatic steatosis induced by HCV [41].

In their 2023 research, Infante-Menéndez et al. demonstrated that miR-7d-5p is increased in NAFLD-affected liver and contributes to insulin resistance.

They proposed the use of this miRNA as a blood biomarker for the early diagnosis of NAFLD [42].

Back in 2016, the team of Liu et al, demonstrate the possibility to assess miR-34a as a specific diagnostic marker for non-alcoholic steatohepatitis (NASH) in a Chinese population.

Their research was showing that miR-34a, along with other miRNAs such as miR-122 and miR-192 present a strong correlation with steatosis and liver inflammatory activity.

Finally, their results demonstrate that miR-34a serum levels can act as non-invasive biomarker for the diagnosis of NASH, giving the possibility of a more accurate method of diagnosis than the traditional one [43].

In 2022, López-Sánchez et al. carried out a research that was showing a close correlation between serum levels of cytokeratin-18 (CK-18) (that is an important biomarker of NAFLD progression) with hepatic expression of miR-122-3p, miR-140-5p, and miR-148b-5p.

In this way, these miRNAs can be considered as potential biomarkers for early and advanced stages of the disease [44].

Okamoto et al. we're exploring miRNAs in the maternally imprinted 14q32.2 region in NAFLD progression.

Results were showing a number of miRNAs involved in the regulation of inflammation and hepatocyte apoptosis, information that can suggest the potential use of these miRNAs as biomarkers for the different stages of the disease [45].

Another research from the same year showed the efficacy of serum microRNAs used for the diagnosis of NASH.

The conclusion if their study was that miR-34a can exhibit high diagnostic accuracy for NASH and also is capable to help doctors to distinguish simple steatosis from NASH [46].

The results of Malakootian et al. (2022), are showing that miR-125a-5p, miR-143-3p and miR-409-3p are negative regulators of PCSK7 expression, which is an important factor of the hepatic triglyceride metabolism regulation.

Thus, the results are demonstrating that the above miRNAs are playing a crucial role in the development of hepatic steatosis [47].

Another research, made by Qu et al. (2023) shows new insights into NAFLD pathogenic mechanisms, identifying five biomarkers and 11 miRNAs, including miR-21 and miR-122, as significantly associated with lipid metabolism and liver inflammation.

Actually, authors have been explored the relationship between propionate metabolism and immune function in NAFLD.

They identified five key biomarkers (JUN, LDLR, CXCR4, NNMT, and ANXA1) and provided a comprehensive regulatory network involving 11 miRNAs-such as miR-21 and miR-122-significantly associated with lipid metabolism and liver inflammation." [48].

The scientific literature in the field is comprising also recent research such as the one carried by Li et al. (2024), that is an important point of view regarding the way in which how circulating microRNAs are involved in NAFLD in severely obese adolescents.

The results have been showing that there are several miRNAs that are associated with hepatic steatosis, inflammation, and fibrosis, thus that can be used for the early diagnosis of NASH in case of young subjects [49].

All of the above sustain the idea that miRNAs are biomarkers useful for diagnosing steatotic

liver disease, evolution of treatment and disease prognostic.

Actually, the role of biomarkers is one of the most highlighted in the specialized literature, this being an intensively used research direction in recent years.

miRNAs and targeted therapies for NAFLD (Non-alcoholic fatty liver disease) /NASH (Non-alcoholic Steatohepatitis)

In the research made by Roos et al. (2016), we can see that, authors are highlighting the central role-played microRNAs in the regulation of inflammation associated with obesity. The results showed how miR-146a suppresses the inflammatory response in human adipose cells, thus being linked to the increased risk of developing hepatic steatosis [50].

miRNAs are having an active role in the regulation of CYP2E1 enzyme, which is connected with the alcohol metabolism and the degree of liver damage induction.

Actually, their results indicate the fact that miR-132 and miR-378 modulate CYP2E1 activity thus, being possible to have a role into the treatment of liver injury [51].

In addition to all this, the study of Auguet et al. (2016) showed that the expression of miR-33a/b* and miR-122 is closely related to hepatic lipid metabolism in obese women with NAFLD, and circulating levels of these miRNAs can predict disease severity, thus providing a potential non-invasive for the diagnosis of NASH [52].

The team conducted by Escutia-Gutiérrez in a research made in 2021, succeed to show the role that pirlfenidone can have in the alteration of the hepatic expression of miRNAs in a model of MAFLD/NASH.

Their results specifically shown that miR-21a-5p, miR-34a-5p, miR-122-5p, and miR-103-3p are involved in disease progression, and the treatment based on pirlfenidone can reduce their expression in a great extent, indicating a potential treatment for NASH [53].

Lack of the miR-379/miR-544 cluster protects mice against high-fat diet-induced obesity and prevents triglyceride accumulation in the liver.

miR-379 silencing could ameliorate hepatic steatosis, indicating a potential therapeutic target for NAFLD [54].

The study by Khalifa et al. (2023) presents results that are sustaining the possible role of miRNAs in the treatment of NAFLD.

Thus, they show the way in which GLP-1RA exendin-4, a glucagon-like peptide-1 receptor agonist, modulates the expression of several

miRNAs involved in reducing hepatic steatosis, such as miR-219a and miR-378c.

In other words, this receptor can regulate the expression of miRNAs involved in lipid metabolism, being a possible venue for the treatment of NAFLD [55].

In completion to these results, we may consider the ones of Khan et al. (2023) that are showing the capacity of miR-98-5p to regulate the gluconeogenesis and lipogenesis in hepatocytes by targeting PPP1R15B.

Their results actually demonstrated that miR-98-5p overexpression reduces fat accumulation and glucose production in HepG2 cells, which may be another path to explored regarding therapeutic solutions for treating NAFLD and diabetes [56].

Wang et al. have been demonstrating that miR-130a-3p is capable to target TGF- β receptors and negatively regulates hepatic stellate cell activation thus can provide a possible therapeutic instrument for the treatment of NAFLD [57].

The research conducted by Liu et al. (2023) highlight the improvement of lipid and glucose metabolism as a direct effect of natural products.

In this way, the researchers made a point about the capacity of natural products to treat metabolic diseases, including NAFLD [58].

Another study that was pointing out the way in which lipid metabolism and apoptosis were regulated by a network comprising circ_0004535/miR-1827/CASP8 within the context of NAFLD associated with type 2 diabetes was the one made by Li et al. (2023).

The study was providing new insights into pathogenetic mechanisms and potential therapeutic targets [59].

Kim et al. (2021) made a research regarding the way in which miRNAs and transcription factors can regulate autophagy in NAFLD.

Their study shows that miRNAs such as miR-199a-5p and miR-34a play a central role in autophagic processes that contribute to disease progression, so analyzing this process can help to the diagnose of the disease evolution [60].

A research made in 2023 by Lu et al. demonstrate that adipose tissue-derived exosomes can carry miR-103 and are inhibiting autophagy, thus exacerbating non-alcoholic steatohepatitis (NASH).

These findings can be used to ameliorate hepatic steatosis and inflammation so providing a possible treatment for NASH [61].

Zhao et al. (2021) demonstrated that by regulating the expression of miR-21a-5p with the

help of aerobic exercise, hyperlipidemia can be significantly improved and the metabolism of lipids is also influenced.

These results, along with previous ones suggests that miR-21a-5p may be used to elaborate a treatment that can prevent hepatic steatosis and hyperlipidemia [62].

A more recent paper, written by Zhu et al. in 2023, emphasize the therapeutic effects of miRNAs on NAFLD and NASH.

Authors have done a meta-analysis and concluded that miR-34a can be considered a very promising therapeutic intervention, that is able to improve the level of total cholesterol and triglycerides [63].

All of the above present the potential of miRNAs to contribute to the development of targeted therapies for NAFLD or NASH with improved results and a better capacity of disease evolution assessment.

Metabolic regulation and obesity via miRNAs

Another perspective can be considered the one given by the study of Castaño et al, that highlighted in 2022 that the metabolic dysfunction associated with obesity can be treated (and ameliorated) with the help of EV-miRNAs (that are derived from extracellular vesicles).

These results are supporting other previous ones and contribute to a new perspective about treatments based on miRNAs in case of metabolic or liver diseases [64].

A year later we can find the research made by, Atic et al. (2023) that complement these results with the idea that miR-122 and other circulating miRNAs can be altered by weight-loss or insulin-sensitizing treatments.

Thus, that can be considered as another evidence that they can be used as biomarkers capable to monitor NAFLD and NASH.

In the same time, this line of research is demonstrating how important can be to fully understand the mechanism referring to the capacity of miRNAs to contribute to liver dysfunctions [65].

Later on, the research carried out by Tobaruela-Resola et al. in 2024 showed that miR-122-5p, miR-151a-3p and miR-126-5p can provide a non-invasive model for the diagnosis of steatosis.

Thus, these miRNAs can be considered predictive markers for evaluating hepatic steatosis and liver stiffness in patients with MANAFLD (Metabolic Dysfunction-Associated Steatotic Liver Disease) [66].

Very recently, in 2024, the team of Díez-Sainz et al. made a groundbreaking contribution for the use of miRNAs in the treatment of NAFLD showing that plant-based miRNAs like miR8126-3p and miR8126-5p are able to reduce lipid accumulation in human hepatocytes by modulating metabolic genes [67].

So, we can highlight a distinct od of research that can demonstrate the capacity of miRNAs to regulate the metabolism and obesity.

Interaction of miRNAs with other factors at the molecular level in disease pathogenesis

A promising result was obtained by Rusu et al. in 2023, regarding a link between a panel of four microRNAs (including miR-21 and miR-34a) and the progression of liver carcinogenesis.

The team was investigating the molecular profiles of patients with NAFLD and NAFLD-associated HCC, showing that is significantly dysregulated in non-tumor and tumor tissues [68].

The research of Torres et al. (2018) suggested that miR-34a, miR-122 and miR-155 are involved in the pathogenesis of alcoholic and non-alcoholic liver diseases.

In the same time, research results strongly indicate that these miRNAs can be considered as biomarkers for the diagnosis and monitoring of diseases progression [69].

Gong et al. (2018), were able to investigate the role that miR-17 can have in the process of hepatic steatosis.

This miRNA (being a miRNA in the miR-17-92 cluster) is targeting the CYP7A1 gene that is an important regulator of lipid metabolism.

Because of this, the miR-17 can increase the risk of steatosis.

These results, among the previous ones contribute to new insights regarding the potential involvement of miRNAs in the initiation and progression of NAFLD (non-alcoholic fatty liver disease) [70].

Another potential proof for the potential of NAFLD treatment that can imply miRNAs is given by the results of Li et al. from research conducted in 2018 that miR-199a-5p seems to play an essential role in the regulation of insulin sensitivity, because is able to inhibit ATG14-mediated autophagy in the liver.

The mechanism also implied the significant improvement of insulin sensitivity with the help of miR-199a-5p and in the same time the reduction of lipid accumulation which means a potential way to treat NAFLD [71].

The study made by Lv et al. in 2021 shows the role of small extracellular vesicles (exosomes) in modulating liver inflammation through miR-122 and other microRNAs.

In their experiment, the liver injury was changing the biological characteristics of the exosomes in serum and reprograms liver macrophages, contributing to the progression of NAFLD [72].

Wu et al. (2017), showed that miRNAs can ameliorate hepatic steatosis and hepatocyte injury with the help of a mechanism that is suppressing the expression of FABP1, a key protein in hepatic lipid metabolism. Actually, it was all about three specific miRNAs-miR-3941, miR-4517, and miR-4672 who were able to suppress FABP1 expression in hepatocytes, leading to significant resistance to free fatty acid-induced steatosis and liver injury [73].

miRNAs can have a complex interaction with different factors along NAFLD pathogenesis, future research is called upon to elucidate to a greater extent the practical way in which control over this interaction can be exercised.

Role of miRNAs in systemic inflammation and effects on various other organs

Exosomes and circulating miRNAs have a particular role in the connection between extrahepatic organs and the liver in the context of obesity and NAFLD. miRNAs play an essential role in intercellular communication that contributes to disease progression [74].

The particular results of this research have demonstrated also by another team conducted in 2021 by Gim et al [75].

IRE1 α is capable to degrade some microRNAs such as miR-34 and miR-200.

This enzyme, actually is capable to regulates lipid homeostasis by promoting the degradation of these miRNAs.

As an effect, the expression of genes involved in fatty acid oxidation and triglyceride lipolysis can be suppressed [76].

miR-21 can have a role in the regulation of inflammation and lipid metabolism in NAFLD patients.

This miRNA was capable to modulate the expression of genes involved in fatty acid metabolism and the regulation of inflammatory processes, thus contributing to the progression of hepatic steatosis [77]

Mehta et al. (2016) showed that miR-143 and miR-145 are correlated with disease severity, in case of patients with NAFLD and associated coronary artery disease (CAD).

Thus, it was highlighted a potential link between hepatic steatosis and cardiovascular risk [78].

Pervez et al. have demonstrated the effects that delta-tocotrienol and alpha-tocopherol can have on patients with NAFLD.

Their research showed that these supplements significantly reduced the expression of miR-122, miR-34a and miR-375, contributing to hepatocytic protection by reducing inflammation and apoptosis [79].

Liver-derived micro-RNAs can be exported through exosomes into the bloodstream influencing pancreatic function, the cardiovascular system (promoting atherosclerosis in case of miR-122), neuronal loss and cognitive impairment and muscle metabolism [80].

This is due to their systemic regulatory role and inter-organ communication functions, which can be attributed to several key mechanisms like: circulation of miRNAs (are secreted in the bloodstream and the act like endocrine-like messengers reaching distant targets like adipose tissue, pancreas, heart, gut, or skeletal muscle), shared metabolic signaling pathways, the cross-talk between liver and other organs (adipose tissue, gut microbiota, pancreas etc.), extracellular vesicles (liver cells under stress may be able to release EVs containing miRNAs that can modulate gene expression in remote tissues) [81].

Role of miRNAs as regulators for highly specialized epigenetic factors affecting steatotic liver disease

Along with the above studies that are reflecting the role of miRNAs in the diagnostic and evolution assessment of the NAFLD, we may add some relevant papers that are talking also about the role of these miRNAs in the regulation of highly specialized epigenetic factors upon the same disease.

Belloni et al, 2018 have been highlighting the role of miR-21 and the STAT3 pathway in lipid accumulation in hepatocytes.

Through inhibition, miR-21 shows reduced expression and hepatic steatosis.

The same research shows that long-term treatment with metformin decreased the activity of STAT3 and miR-21, highlighting the epigenetic influence on the evolution of NAFLD and providing a new therapeutic direction [82].

miR-33a/b, play an important role in lipid metabolism and regulation of insulin resistance, the progression of the NAFLD being related with epigenetic dysregulations, being possible the stratification of NAFLD patients.

These findings confirm the impact of genetic and epigenetic factors regulated by miRNAs [83].

Within research conducted by Torres et al. (2019), the results have shown that expression of miR-34a and miR-194 can be modulated with green tea treatment, meaning that it was exercised an epigenetic influence over lipid metabolism and prevention of fat accumulation in the liver [84].

In another research setting, it was demonstrated that miRNAs delivered by extracellular vehicles (EVs) contribute to NAFLD progression.

This was another proof that miRNAs can regulate epigenetic factors that are causing development of liver inflammation and fibrosis [85].

Vulf et al. found that miR-195-5p and miR-16-5p are dysregulated and influence mitochondrial function and apoptosis in case of patients with steatosis and NASH (Non-Alcoholic Steatohepatitis).

This was another example of correlation between epigenetic mechanisms regulated by miRNAs and NAFLD progression [86].

In case of hepatocellular carcinoma (HCC) associated with NAFLD, studies have shown that miRNAs can provide essential information about the stage of the disease through their role of epigenetic biomarkers that can be used for detection and monitoring [87].

All of the above are showing that miRNAs are accountable for the regulation of epigenetic factors that influence the progression and development of metabolic liver disease (NAFLD) and, by extension, lipid accumulation, liver inflammation, and the fibrogenesis and apoptosis processes.

Discussion

The current review offers a complex image about the role that miRNAs can have within the context of steatotic liver disease.

This role may comprise not only the diagnostic or prognostic capabilities as biomarkers but also the possibility to be real drug candidates.

The change in nomenclature from NAFLD (Non-Alcoholic Fatty Liver Disease) to MAFLD (Metabolic Dysfunction-Associated Fatty Liver Disease) comes as a direct effect of the complexity and systemic nature of the condition that is in discussion and express in the same time the necessity for a broader frame of conditions assessment, not only liver-specific pathologies but also possible associated cardiometabolic comorbidities [88].

Therefore, the approach for a combined clinical management has to be interdisciplinary with a combination of fields like diabetology, cardiology, and nutrition.

1. Essential Regulatory Roles of miRNAs in NAFLD Pathophysiology

Among the miRNAs that have been highlighted within different studies, the miR-122 seems to be the one that has the most prominent and significant role in the regulation of hepatic glucose and lipid metabolism.

In the same time, there are also other several miRNAs like miR-379, miR-33a/b, miR-103-3p, miR-98-5p, and miR-19a-3p that are playing an important role in the regulation of fat accumulation at the level of the liver [37,32,54].

Interestingly, while some miRNAs aggravate steatosis, others, such as miR-103-3p and miR-98-5p, have hepatoprotective effects through the reduction of lipid deposition.

The development of MAFLD is significantly determined by the oxidative stress and systemic inflammation.

The accumulation of fat in the liver cause insulin resistance, with a whole range of inflammatory events afterwards.

MicroRNA-122, along with miR-26a, miR-199a-5p, miR-34a-5p, and miR-33a/b, plays a central role in this disease process [71,44].

Within this framework, there is an important development of inflammatory cytokines derived from the adipose tissue, that are enhancing liver pathology.

Chronic insulin resistance typically leads to type 2 diabetes mellitus (T2DM), especially when combined with obesity-a frequent comorbidity in patients with NAFLD.

2. miRNAs as Biomarkers for Disease Stratification

Enhanced liver fibrosis, an intrinsic feature of Metabolic Dysfunction-Associated Fatty Liver Disease (MAFLD), has been associated with various microRNAs, namely miR-146-5p, miR-192, and miR-34a.

Studies have been showing that an entire range of serum miRNAs panels like miR-122/miR-192/miR-21 and miR-122/miR-192/miR 34a offers the possibility to make diagnostic and prognostic for the condition [53,68,27].

In previous research, our scientific team has validated a panel consisting of miR-122, miR-33a, miR-33b, and miR-192 that present during experiments a great potential for both diagnosis staging [89].

In the same time, intestinal dysbiosis has been identified as a key driver of liver fibrosis with the impact mediated by the dysregulation of miR-122 and miR-145 [24].

The rampant application of antibiotics-often underestimated-has lasting impacts on the gut microbiota, which further influences liver health.

3. Modulation of miRNA Expression by Lifestyle and Pharmacological Interventions

Similar to other epigenetic marks, miRNAs are sensitive to changes in lifestyle. For instance, miR-122 expression is influenced by weight loss. Physical activity also modulates miRNAs such as miR-212, which is involved in lipogenesis and NAFLD progression via targeting FGF-21. Such mechanisms form the basis of the new concept of "liver fitness," whereby mitochondrial function and epigenetic regulation combine to promote better hepatic outcomes.

Despite this evidence, diet and exercise are still first-line treatments in NAFLD. Treatment outcomes, however, are very much reliant on patient compliance.

Pharmacological agents also impact the expression of miRNAs. GLP-1RA exendin-4 modulates miR-219a and miR-378c, while SGLT2 inhibitor ipraglifozin modulates the expression of miR-19b-3p [55,20].

These findings point towards the potential of miRNAs as pharmacodynamic markers.

4. Emerging Therapeutic Directions

Resmetirom, the first FDA-approved drug for NASH, is a THR- β agonist and shows marked effectiveness in lowering liver fat and improving lipid metabolism [90].

It is, however, contraindicated in patients with cirrhosis or concomitant alcohol use and should be monitored for interaction with statins and thyroid abnormalities.

Antagomirs-antagonists of miRNA-are therapeutically promising but also present oncological concerns because inhibition of certain miRNAs would elevate the risk for certain cancers like hepatocellular carcinoma [91].

Second-generation RNA-based therapies like Inclisiran (a siRNA against PCSK9) reduce LDL-C and are based on the natural miRNA mechanism [92].

Other compounds with promise are firsocostat, a liver-directed ACC inhibitor, and aramchol, an inhibitor of stearyl-CoA desaturase-both demonstrating utility in the reduction of fibrosis [93].

In order to synthesize the results from the main areas incorporated within our systematic review we propose in the following table a general view upon the main studies extracted from the literature review (Table 1).

Table 1. Summary Table of Main Studies Included on miRNAs in NAFLD.

Nr crt.	Study/Author	miRNA(s)	Isolation/Detection Method	Disease/Disease Stage	Main Role/Finding
1.	Pirola et al., 2015 [25]	miR-122, miR-192, miR-19a, miR-19b	Circulating, serum	NASH vs. Simple Steatosis	Diagnostic biomarkers
2.	Mattis et al., 2015 [26]	miR-29a	Circulating	Steatosis	Lipid accumulation regulator
3.	Yu et al., 2019 [27]	miR-122, miR-34a	Serum	Hepatic Steatosis	Early diagnostic markers
4.	Zhang et al., 2020 [28]	miR-193a-5p, miR-122-5p	Serum	Steatosis and Fibrosis	Progression markers
5.	López-Pastor et al., 2022 [38]	miR-26b-5p, miR-34a-5p, miR-122-5p	Mouse model	NASH	Biomarkers for IR and autophagy
6.	Salvoza et al., 2016 [39]	miR-34a, miR-122	Serum	NAFLD	Linked with dyslipidemia and VLDL-C
7.	Quintás et al., 2022 [40]	miR-10a-5p, miR-98-5p, miR-19a-3p	Serum	NAFLD	Non-invasive prediction of liver fat
8.	Infante-Menéndez et al., 2023 [42]	miR-7d-5p	Liver tissue	NAFLD	Linked to insulin resistance
9.	Liu et al., 2016 [43]	miR-34a, miR-122, miR-192	Serum	NASH	Non-invasive biomarkers
10.	Okamoto et al., 2016 [45]	miRNAs from 14q32.2	Liver tissue	NAFLD	Linked to inflammation and apoptosis
11.	Malakootian et al., 2022 [47]	miR-125a-5p, miR-143-3p, miR-409-3p	Liver tissue	Steatosis	Targeting PCSK7, affecting triglyceride metabolism
12.	Qu et al., 2023	miR-21, miR-122	Bioinformatics, liver	NAFLD	Regulate lipid metabolism and inflammation
13.	Li et al., 2024	Multiple	Serum	NAFLD	Biomarkers for early NASH diagnosis
14.	Xin et al., 2020 [46]	miR-34a	Serum	NASH vs. Steatosis	High diagnostic accuracy (AUROC 0.91), non-invasive biomarker

15.	Wang et al., 2023 [74]	Various (exosomal and organokines-associated miRNAs)	Serum/exosomes	Obesity-related NAFLD	Explains cross-talk between organs and liver, therapeutic implications
16.	Wang et al., 2017 [57]	miR-130a-3p	Liver tissue (mouse model + patient validation)	Fibrosing NASH	Inhibits TGFBR1/2, suppresses HSC activation and fibrosis
17.	Wu et al., 2017 [73]	miR-3941, miR-4517, miR-4672	Hepatocytes	NAFLD	Suppress FABP1, reduce steatosis and hepatocyte injury
18.	Wang et al., 2018 [76]	miR-200 family, miR-34 family	Liver tissue (mouse + human)	NAFLD progression	Regulated by IRE1 α ; targets PPAR α and SIRT1, key lipid regulators
19.	Yu et al., 2019 [27]	miR-122, miR-34a	Serum	NAFLD	Review of miRNA regulatory role in hepatic lipid dysregulation and diagnosis

As we can see within the table, miR-122 is the most frequently mentioned and validated biomarker in NAFLD and NASH. Also, there is a consensus on the usefulness of multiple miRNA panels for diagnosis, prognosis, and monitoring.

Finally, we may add that the role of miRNAs is confirmed both as molecular indicators and as potential therapeutic targets.

Looking at the ideas and information extracted throughout our systematic review we may state that microRNAs have a role beyond biomarkers. They hold great therapeutic promise, although more trials are required to assess long-term risk.

Limitations

This review is constrained by the main limitations that have been put in place-usage of one database (Web of Science), a set time period (2015–2024), and strict inclusion criteria that narrowed the total number of studies included.

Practical Applications

The potential applications of miRNAs in diagnosis, disease staging, and monitoring of therapeutic response portend their eventual use in day-to-day clinical practice.

Their transition from theory and laboratory testing to actual practice, however, needs to overcome some hurdles like developing standardized detection methodologies, validation of diagnostic cut-offs in populations, and incorporation of miRNA panels into clinical decision-making algorithms.

Future Directions

Taking account of the presented results and types of research employed by various authors within the analyzed papers, we may propose as future potential directions for more detailed research the following:

- Standardizing miRNA-based diagnostic and prognostic panels for NAFLD;

- Exploring miRNA roles in pediatric and adolescent populations;

- Clarifying the cross-talk between miRNAs, gut microbiota, and systemic inflammation;

- Advancing personalized medicine approaches by targeting specific miRNA-regulated pathways;

- Conducting large-scale, longitudinal studies and clinical trials assessing safety, efficacy, and long-term outcomes of miRNA-based therapies.

Conclusions

Our review demonstrates the importance of microRNAs as epigenetic factors involved in the pathogenesis of NAFLD.

We have highlighted several mechanisms of action for microRNAs in glucose, lipid metabolism, liver inflammation and fibrosis with a great potential of diagnostic and prognosis biomarkers.

As liver biopsies are invasive clinical investigations, serum microRNAs are important not only as diagnostic tools allowing risk stratification and evaluation of response to therapy but also as indicators for other organs involvement in the context of MAFLD.

There is an urgent necessity to standardize the detection methods for serum microRNAs and to select specific panels for NAFLD/MAFLD.

Judicious use of specific liver-targeted agomirs or antagomirs might represent the next step in the personalized therapy of NAFLD.

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Conflict of interests

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