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REVIEW

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Differentially-regulated miRNAs in COVID-19: A systematic review

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Abstract

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) is responsible for coronavirus disease of 2019 (COVID-19) that infected more than 760 million people worldwide with over 6.8 million deaths to date. COVID-19 is one of the most challenging diseases of our times due to the nature of its spread, its effect on multiple organs, and an inability to predict disease prognosis, ranging from being completely asymptomatic to death. Upon infection, SARS-CoV-2 alters the host immune response by changing host-transcriptional machinery. MicroRNAs (miRNAs) are regarded as post-transcriptional regulators of gene expression that can be perturbed by invading viruses. Several in vitro and in vivo studies have reported such dysregulation of host miRNA expression upon SARS-CoV-2 infection. Some of this could occur as an anti-viral response of the host to the viral infection. Viruses themselves can counteract that response by mounting their own pro-viral response that facilitates virus infection, an aspect which may cause pathogenesis. Thus, miRNAs could serve as possible disease biomarkers in infected people. In the current review, we have summarised and analysed the existing data about miRNA dysregulation in patients infected with SARS-CoV-2 to determine their concordance between studies, and identified those that could serve as potential biomarkers during infection, disease progression, and death, even in people with other co-morbidities. Having such biomarkers can be vital in not only predicting COVID-19 prognosis, but also the development of novel miRNA-based anti-virals and

Abbreviations: 3′ UTR, 3′ untranslated regions; 5′ UTR, 5′ untranslated regions; Ago, argonaute; ASM, asymptomatic; C, control; CAP, community acquire pneumonia; CircRNAs, circular RNAs; COVID-19, coronavirus disease of 2019; Crit, critical; DEGs, differentially expressed genes; DGRC8, DiGeorge Syndrome Critical 8; ICU, intensive care unit; INF, infected; lncRNAs, long non-coding RNAs; MI, mild; miRNAs, MicroRNAs; MM, mild/moderate; MO, moderate; NGS, next generation sequencing; nt, nucleotide; pri, primary; RAN-GTP, RAs-related nuclear protein; RE, recovered; RNA, Ribonucleic Acid; RISC, RNA-induced Silencing Complex; RITS, RNA-induce Transcriptional Silencing; RT-qPCR, reverse transcriptase quantitative polymerase chain reaction; S, severe; SARS-CoV-2, severe acute respiratory syndrome coronavirus-2; siRNAs, small interfering RNAs; snRNAs, small nuclear RNAs; snoRNAs, small nucleolar RNAs; TCZ, Tocilizumab.

therapeutics which can become invaluable in case of the emergence of new viral variants with pandemic potential in the future.

KEYWORDS
biomarkers, COVID-19, differential gene expression, disease progression, miRNAs, SARS-CoV-2

1 | INTRODUCTION

It has been estimated that up to 1.5%–2% of the total human genome transcribes protein-coding RNAs (messenger or mRNAs), whereas ~28% transcribes non-coding RNAs (ncRNAs).[1] Until now, several types of ncRNAs have been identified, including small interfering RNAs, small nuclear RNAs, small nucleolar RNAs, microRNAs (miRNAs), long non-coding (lnc) RNAs, and circular (circ) RNAs. Among these, miRNAs are a class of single-stranded non-coding RNAs which are ~20–25 nucleotide (nt) long.[2] These miRNAs are critically involved in regulating mRNA gene expression and any dysregulation in their expression affects post-transcriptional gene expression that can significantly change cellular biological processes. In general, miRNAs alter gene expression after binding to miRNA response elements (MREs) that are primarily observed within the 3′-untranslated regions (3′ UTRs) of the target mRNAs.[3] miRNAs generally act as negative gene regulators and their binding to MREs results in translation repression of the target mRNAs or its complete degradation.

1.1 | MiRNA biogenesis

Most cellular and viral miRNAs are initially produced as primary (pri)-RNAs hundreds to thousands of nucleotides long with at least one or more ~80 nt stem loop structure(s).[4–7] Over one-third of human miRNAs exist in clusters and transcribed as "polycistronic RNAs." Synthesised in the nucleus by RNA polymerase II that also transcribes other cellular genes, miRNAs are capped and polyadenylated like cellular mRNAs.[6,8] This is followed by processing of these pri-miRNAs into ~65–70 nt long pre-miRNA with 2 nt overhangs at the 3′ end by the Microprocessor Complex that comprises of the nuclear RNase III enzyme Drosha and its cofactor, Pasha/DiGeorge Syndrome Critical 8.[9] This processing maintains the imperfect stem loop structures and these partially processed substrates are then exported to the cytoplasm by the RAN-GTP transporter, Exportin-5. Once in the cytoplasm, they are further processed by another RNase III enzyme, Dicer, with the help of transactivation-responsive RNA-binding protein, which binds to dsRNA, which removes the loop part of the hairpin.[9,10] Now fully mature and ~21–24 nts in length, the miRNAs resemble siRNAs of the RNA interference pathway. Each duplex miRNA leads to the generation of two mature miRNA strands termed 5p or 3p, depending upon their location in the pre-miRNA relative to its 5′ end. Either miRNA strand can be loaded onto the RNA-induced Silencing Complex (RISC) as the "guide" strand for silencing of the target mRNA by the slicer protein, Argonaute (Ago), while the other "passenger" strand is degraded.[11] The cellular environment or cell type predominantly determines strand selection which could either be 5p or 3p exclusively, or either one equally.[8] A single miRNA can target mRNA transcripts with complementary sequences that can number in hundreds. If the complementarity is perfect between the two molecules, the mRNA is degraded (as happens mostly in plants), while if there is imperfect base pairing, the mRNA undergoes translational inhibition, as is observed mostly in the animal cells.[12]

1.2 | Mechanism of miRNA action

MiRNAs inhibit gene expression post-transcriptionally in many ways. They can remove the cap structures at the 5′ end of the transcripts, deadenylate the poly A tail at the 3′ end of the transcripts, inhibit function of ribosome during translation, or degrade the transcript itself.[7,13] Unique to yeast cells, RNA-mediated inhibition of gene expression can happen at the level of chromatin as well by the interaction of the miRNA with the "RNA-induce Transcriptional Silencing (RITS) complex.[14] In the animal cells, miRNAs function primarily by binding through incomplete complementarity with the target sequence at the 3′ UTR of the mRNAs, leading to inhibition of translation via RISC. miRNAs act by binding to and silencing target mRNAs through base pairing between a group of "seed sequences," the primary determinants of mRNA target recognition in miRNAs.[15] These are located between nts 2-8 at the 5′ end of the miRNA that interact with complementary seed sequences (MREs) found within the 3′ UTR of target genes. Other than the 3′ UTR, regions such as the 5′ UTR, the promoter region, as well as the coding region of target genes have also been observed to be targeted by miRNAs.[8,15]

1.3 | MiRNAs in SARS-CoV-2

Soon after the discovery of miRNAs in 1993 and their subsequent role in mRNA expression, several studies focussed their attention on elucidating the mode(s) of miRNA biogenesis and function.[16] Advancements in gene expression analysis made it easy to detect any change in an organism's miRNA expression between control and compromised samples. The recent coronavirus disease 2019 (COVID-19) pandemic caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) has resulted in more than 6.8 million deaths globally so far (https://covid19.who.int/). It is evident that

upon infection, viruses hijack host immune system to not only facilitate their replication, but also disable the immune response against the virus.[17,18] To ease their entry and invade host-immune system, SARS-CoV-2 has also been found to change transcriptional profile of numerous pathways associated with host-cell-defence mechanisms.[19] Recent studies have found that upon infection, SARS-CoV-2 significantly alters multiple cellular pathways in several human organs, including heart, lungs, liver, and kidneys.[20]

In most of the organs, SARS-CoV-2 infection results in destabilisation of the host cellular immune response and release of proinflammatory cytokines, dysregulated production of inflammatory cells, endothelial dysfunction, and coagulation abnormalities (Table 1).[20–43] It has been long known that upon infection, host cells produce miRNAs to counter viral attacks by regulating the host-immune response.[44,45] Moreover, viruses also transcribe miRNAs that may interfere with the host-cellular defence system.[45] Since the start of COVID-19, several studies have investigated the possible dysregulation of human miRNAs after SARS-CoV-2 infection in vivo, in vitro, and *in silico*[46–48] Initially, human miRNAs were predicted using computer-assisted techniques that were further validated through in vivo or in vitro studies.[48–50] In terms of miRNAs related to SARS-CoV-2/COVID-19, within a short span of only ~2 years, already hundreds of studies have been published. In this systematic review, we not only summarise the currently available data, but also analyse the available data from patients to examine the performance of currently enumerated miRNAs as biomarkers across the globe. Using networking techniques and cluster analysis, we identify experimentally-verified miRNAs across the globe that may act as possible disease markers for further COVID-19 investigations.

2 | METHODS

2.1 | Data collection

Most of the existing reviews on the role of miRNAs in COVID-19 have summarised the current available data based on dysregulated miRNAs either predicted using in silico techniques or observed experimentally in vivo or in vitro studies. To be considered as a valid biomarker, miRNAs should possess the same expression profiles under certain disease conditions. Unfortunately, none of the recent reviews cross-checked the existing data to validate the specificity of the current proposed biomarkers except for a review from Moatar et al[51] who predicted and grouped possible miRNA targets and associated pathways after SARS-CoV-2 infection reported in a few studies using human patients. In the current review, we only focussed on the miRNA expression results from the studies originating through analysis of human patients enrolled in different healthcare facilities during the pandemic and excluded all other in silico, in vitro and in vivo *studies*. We did this to ensure that our predicted targets reflected real life scenario. Figure 1 describes the data-search strategy and inclusion-exclusion criteria used in our study. The data included in this review was searched through the PubMed database ranging from December 2020 to November 2022 using words "COVID-19, SARS-CoV-2, miRNA". We also searched other associated databases like Google (https://www.google.com/), Google Scholar (https://scholar.google.com/), ScienceDirect (https://www.sciencedirect.com/) and PubMed Central (https://www.ncbi.nlm.nih.gov/pmc/) to ensure the inclusion of most of the current data. This data was crossed-matched to the data available in PubMed, and most of the data searched on other databases was also available in PubMed.

2.2 | Network analysis

As we were interested in miRNAs expressed at various stages of disease progression, we constructed disease stage-specific miRNAs interactions and their interacting networks using Cytoscape v. 3.9.1.[52] Briefly, miRNA lists were constructed from the given literature and grouped based upon disease severity. Healthy controls were designated as "controls" whereas infected patients were grouped into asymptomatic (ASM), mild (MI), moderate (MO), severe (S), critical (Crit) and others, as per the study. Where the authors did not further sub-group the disease severity, data was named as "infected". miRNA interaction networks were created with either overlapping miRNAs in different disease groups or among authors representing their data in similar disease groups. These interacting networks helped to sort out miRNAs that have been identified in various studies under parallel disease conditions.

2.3 | MiRNA selection criteria

The miRNAs included within this study were selected based on the differentially expressed genes (DEGs) between the healthy controls and infected group or between groups representing different disease stages specified earlier. We included all the miRNAs considered as DEGs by their publishing authors. While comparing miRNA expression, we found multiple miRNAs expressed commonly in various disease stages based on disease severity (mild, moderate, or severe). In this scenario, we chose only those miRNAs that were represented in at least 5 or more comparative groups to limit overcrowding. We also removed miRNAs showing opposite expression in the same group or the miRNA not specifying the 3p or 5p strand position. For example, if any study described miR-150 as a dysregulated miRNA and another study mentioned miR-150-5p, both of these miRNAs were not considered the same and excluded. Furthermore, we removed one-time expressing miRNAs in any group during network construction.

2.4 | Clinical data selection

Most of the selected studies shared both demographic and clinical data from the patients. However, for the sake of simplicity and

TABLE 1 Associated cellular pathways and manifestations in different human organs after SARS-CoV-2 infection.

Organ(s)	Principle cellular pathways involved	Organ failure during SARS-CoV-2 infectionYes/No/Not applicable	References
Lung	ACE2 pathway dysregulation	Yes, lung failure (~5%)	20,22,24,37
	Acute respiratory distress syndrome (ARDS)		
	Interstitial inflammation		
	Immune response (circulating proinflammatory cytokine and chemokine upregulation, including tumour necrosis factor-α and interleukin 1β), hemophagocytosis (macrophage activation syndrome), immune suppression (lymphopenia), Hypoxia		
	Diffused pulmonary intravascular coagulopathy		
	TGFβ signalling		
	Oxidative stress		
	Pyruvate metabolism		
	Neutrophil extracellular trap (NET) formation		
Kidneys	ACE2 pathway dysregulation	Yes, acute kidney injury (~5%)	20,26,28,38
	High ACE2, TMPRSS2 and CTSL levels		
	Systematic inflammatory response		
	High cytokines/chemokines levels		
	Dysregulated renal hemodynamics		
	Induced MAPK and STAT3 pathways		
	Induced senescence-associated secretory phenotype		
	Induced interferon α/β pathway		
	Reduced collagen biosynthesis and integrin cell surface interactions		
	Induced ROS generation		
Blood	Lymphopenia	Venous thromboembolism (~10%)	20,29,34
	Induces inflammatory markers		
	Cytokine storm		
	Lymphocyte's apoptosis		
	Systematic thrombocytopaenia		
	Endothelial barrier disruption/dysfunction		
	Blood hypercoagulability		
	Type I interferon		
	VEGFA/Ang/Tie2 pathways		
Skin	Purpuric eruptions	Skin abnormalities (~20%)	20,36,40,43
	Livedo reticularis		
	Retiform purpura		
	Skin micro-thrombosis		
	Induce macrophagy		
	Induce inflammatory markers		
	TGFβ signalling		
	Cyclic GMP-AMP (cGAS-STING) pathway		

TABLE 1 (Continued)

Organ(s)	Principle cellular pathways involved	Organ failure during SARS-CoV-2 infectionYes/No/Not applicable	References
Brain, nervous system	Skeletal muscle injury	Not applicable	20,31,35,39
	Peripheral neuropathy		
	Taste impairment		
	Nerve pain		
	Hyper inflammation		
	Metabolic dysregulation		
	Microglia activation		
	Infected olfactory neurons		
	Olfactory and gustatory sense dysfunctions		
	Guillain-barre syndrome		
	Systematic neurological illness		
	Haemorrhagic and ischaemic strokes		
	Ocular manifestations		
	Conjunctival congestion, chemosis and epiphora		
	Matrix metalloproteinases (MMP) pathway		
	Neurofilament light chain pathway		
Heart	Upregulated ACE2 expression	Yes, acute heart failure (~2.5%) in critical patients; myocardial injury (~36%); vascular thrombosis (~16%)	20,27,30,41
	Reduced angiotensin 1-7 levels		
	Induced myocarditis/injury (induced ADAMTS13 levels)		
	Anti-fibrinolytic response		
	Hyper inflammation, hypotension		
	Reduced oxygen supply		
	Ventricular arrhythmias		
	Macrophage activation syndrome		
	Induced Activin/TGFβ signalling		
	Induced biological ageing/senescence (SASP)		
Gut	ACE2 dysregulation in the ileum and colon	Not applicable	20,23,33
	Upper tract inflammation		
	GI tract epithelial cell apoptosis		
	Elevated AST/ALT/bilirubin levels		
	Hepatocyte apoptosis		
	Hypoxia		
	Endocytosis signalling pathway		
	Macrophage induced immune response		
Endocrine	High ACE2 expression on hypothalamic and pituitary tissues	Not applicable	20,25,32,42
	High cortisol levels		
	Degeneration and necrosis of adrenal gland		
	Electrolyte imbalance (hyponatremia and hypernatraemia)		
	Hypothyroidism		

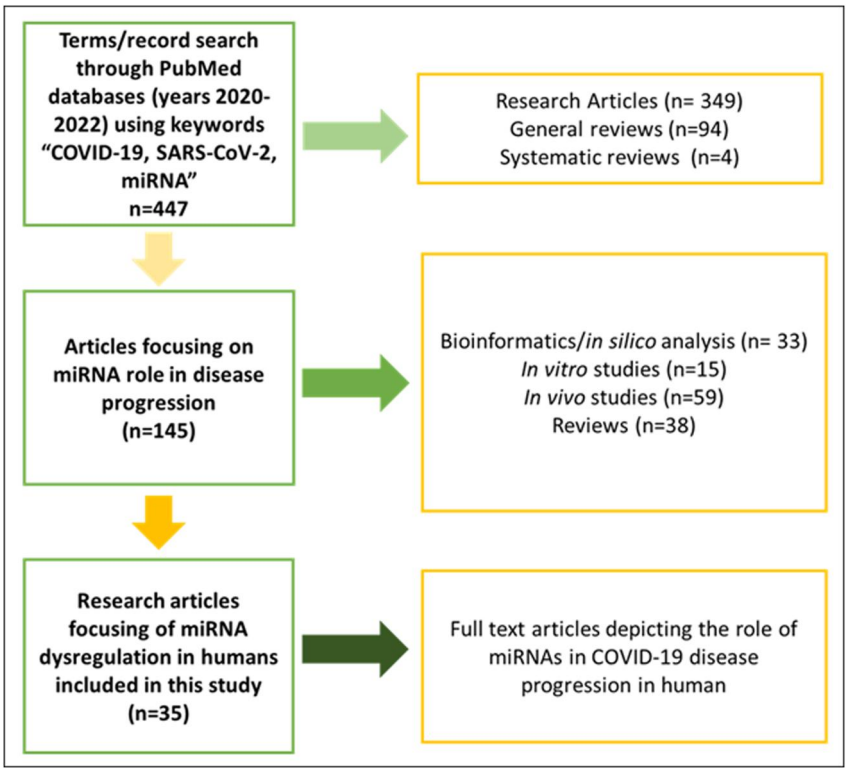


FIGURE 1 Study design. Literature search strategy and exclusion and inclusion criteria for this review.

consistency, we included only gender and age in our study which were common to all studies.

2.5 | Data analysis and statistics

Demographic and clinical data was collected from each study and was analysed using Microsoft Excel 2021 and IBM SPSS Statistics software v.26. GraphPad Prism v. 9.0.0 (121) was used to create graphs and analyse data among groups, wherever applicable.

3 | RESULTS

3.1 | MiRNA dysregulation in SARS-CoV-2-infected patients

The first step in this study was to collect suitable data. As we were interested in miRNAs dysregulated after SAR-CoV-2 infection, we found 349 research articles, 94 general reviews, and 4 systematic reviews on this topic (Figure 1). Out of these, 145 articles and reviews focussed on miRNAs in COVID-19 progression. Out of these, 33 were in silico, 59 were in vivo, and 15 were in vitro studies along with 38 reviews. Our preference was to include and analyse the data extracted from human studies from patients enrolled in a health facility around the globe. We finalised and selected 35 studies that reported dysregulation of host-miRNAs following SARS-CoV-2 infection (Figure 1).

Collected data showed that of these, most of the studies were published during the year 2021 (n = 16),[53–68] followed by 2022 (n = 15),[69–83] with the least numbers in the year 2020 (n = 4)[53,84–87] (Figure 2a). Although the selected studies were published globally, most of them were reported from China (n = 8), followed by Italy (n = 7), Spain (n = 4), and USA (n = 3). Australia, Germany, Iran and Turkey published two articles each. The remaining countries with one study included Austria, Brazil, Czech Republic, Egypt, Israel, and Lebanon (Figure 2b). The samples used for RNA extraction for miRNA analysis included, plasma (n = 14), serum (n = 9), whole blood cells/peripheral blood samples (n = 6), and nasopharyngeal swabs (n = 4). One study took both plasma and nasopharyngeal samples, whereas one study collected RNA from bones and one from placenta of pregnant women infected with SARS-CoV-2 (Figure 2c). To determine changes in miRNA expression levels, 18 studies chose reverse transcriptase quantitative PCR (RT-qPCR), whereas 17 studies used small RNA sequencing/next generation sequencing (NGS). RT-qPCR was the popular choice for validating the sequencing data. Only 7 studies used a study design with two cohorts, discovery and validation. Table 2 summarises the characteristics of the studies included in this review, whereas Table S1 contains the raw data used in this study.

3.2 | Demographic and clinical data analysis

Out of 35 studies, 30 mentioned participant ages, a mixed-age range from 3.5 to 93 years, while thirty-one mentioned gender of the

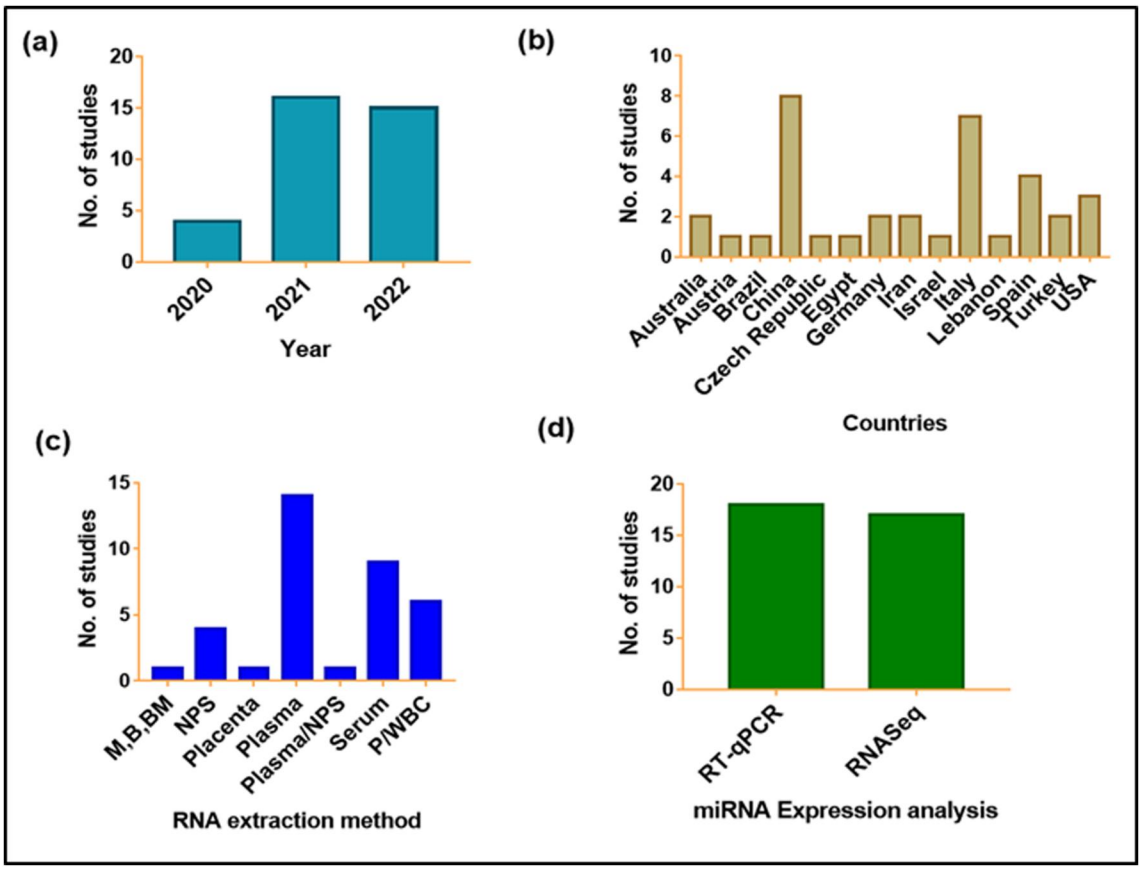


FIGURE 2 Analysis of the publishing year, regions, RNA extraction methodology, and miRNA expression analysis platform used in the studies included in this review. M, muscle; B, bone; BM, bone marrow; NPS, nasopharyngeal swabs; WBC, white blood cells.

participants (Table 2). In all of these studies, the ethnicity of the participants was not disclosed and a written consent form approved from ethical committees was signed by each participant. In most of the studies, infected patients after testing positive for COVID-19 infection were further categorised into the following stages: (i) mild, (ii) moderate, (iii) severe, (iv) critical, and (v) asymptomatic. Other common stages were patients with or without mechanical ventilation, recovered, or deceased. Mechanical ventilation was defined as falling within the severe stage, intensive care unit patients without ventilation as moderate, and asymptomatic as mild, in this review. Some studies combined these stages as one group, as given in Table 2. A few studies also examined the effect of co-morbidities like diabetes, cerebrovascular issues, pregnancy, common cold, influenza, and bone fractures in infected patients.

3.3 | MiRNA dysregulation in "Inf *vs* C" studies

Our data showed that 13 studies[53,54,56,57,66,70–72,80–83,85] compared miRNA regulation in SARS-CoV-2 infected patients without further sub-staging of the disease severity, whereas 22 studies reported their results as an overall and also in sub-groups (Table 2). A total of 404 miRNAs were reported in these studies that showed significant dysregulation in infected patients. As we were interested in those miRNAs that were reported in at least two studies, 70 miRNAs were selected from 16 studies[53,55–57,63,66–68,71,73,78,79,81–83,85] to create an miRNA-study network in infected (Inf) *versus (vs)* control (C) patients. This network contains 86 nodes and 160 edges with 3721 average number of neighbours (Figure 3a). The red or green edges (connecting lines) represent up- or down-regulation of miRNAs in any study. We further filtered out 30 miRNAs which showed inconsistent expression patterns within studies. The final list for the present study contained 29 up-regulated and 11 down-regulated miRNAs with consistent expression (Figure 3b, Table S2). In the up-regulated miRNAs, miR-320b and miR-320c appeared in four studies, while miR-1290, miR-15a-5p, and miR-27b-3p appeared in three studies. In down-regulated miRNAs, miR-150-5p appeared in six studies.

3.4 | MiRNA dysregulation in "severe (S)" versus (*vs*) "control (C)" studies

Review of current literature revealed that there were five studies[59,69,78,84,86] that compared miRNA expression in severely infected patients with healthy controls. These also included studies where authors combined two or more disease stages due to similar miRNA expression trends. These five studies collectively reported

TABLE 2 Characteristics of the 35 studies included in this review.

Author(s) main conclusions	Year	Country	Tissue sample	Expression analysis methodology	No. of participants Inf, C, ASM, MI, MO, S, Crit	Gender (M/F) Age (Ave ± SD) or Age (low-high)	Comparative groups	miRNAs detected	Up-regulated	Down-regulated
Akula et al[69] Decline in the miR-150-5p plasma levels in COVID-19 patients may support enhanced SARS-CoV-2 infection.	2022	USA	Plasma	RNASeq	12 Inf 8 C	6M/6F Inf 3M/5F C 47.8 ± 9.8 Inf 46.0 ± 7.3	Inf *vs* C MO/S *vs* C	8	4	4
Bagheri-Hosseinabadi et al[54] miR-10b is downregulated in the COVID-19 patients and might result in increased levels of IL-2 and IL-8; hence, contributing to cytokine storm.	2021	Iran	Plasma	RT-qPCR	33 Inf 29 C	13M/20F Inf 9M/20F C 62.4 ± 3.7 Inf 56.6 ± 1.6 C	Inf *vs* C	1	0	1
Chen et al[84] Identified genes, proteins, and exRNAs as potential biomarkers that might assist in predicting the prognosis of SARS-CoV-2 infection.	2020	China	Whole blood cells	RNASeq	Total 280 190 MI/58 S/32C Final 66 Inf (50 MI, 16S) 14 C	98M/92F MI 44M/14F S 17M/15F C	MI/S *vs* C	234	176	58
de Gonzalo-Calvo et al[55] Plasma miRNA signature emerges as a novel tool to assist in the early prediction of vital status deterioration among ICU patients.	2021	Spain	Plasma	RT-qPCR	**Discovery Cohort** 79 Inf (43 Ward/36 ICU) **Validation Cohort** 36 Inf ICU (20 alive/16 Dead)	**Discovery Cohort** 44M/35F 68Y (56.6–77) 18M/25F (Ward) 68Y (56.5–84) 26M/10F (ICU) 68Y (56.8–72.2) **Validation Cohort** 15M/5F (alive) 60Y (48–68.2) 11M/5F (Dead) 70.5Y (68–73.2)	ICU Dead versus Survived	10	5	5
Demiray et al[53] Result revealed that the increase in miR-190a level may be a prognostic factor related to COVID-19.	2021	Turkey	Serum	RT-qPCR	40 Inf 10 C	23M/17F Inf <55Y=22 >55Y = 18 11M/9F C <55Y=6 >55Y = 4	Inf *vs* C	9	2	7
Eichmeier et al[71] The most differentially expressed miRNA was miR-21, which is likely linked to the presence of SARS-CoV-2 infection of nasopharynx tissues.	2022	Czech Republic	Nasal Swab	RNASeq	10 Inf 10 C	N/A	Inf *vs* C	6	6	0

TABLE 2 (Continued)

Author(s) main conclusions	Year	Country	Tissue sample	Expression analysis methodology	No. of participants Inf, C, ASM, MI, MO, S, Crit	Gender (M/F) Age (Ave ± SD) or Age (low-high)	Comparative groups	miRNAs detected	Up-regulated	Down-regulated
Farr et al[56] SARS-CoV-2 infection induces a robust host miRNA response that could improve COVID-19 detection and patient management.	2021	Australia	Plasma	RNASeq	10 Inf 10 C	4M/6F Inf 53.5 ± 17.2 4M/6F C 53 ± 17.6	Inf *vs* C	60	28	32
Farr et al[72] Characterises the host miRNA response to SARS-CoV-2 infection and identifies candidate biomarkers for improved COVID-19 detection.	2022	Australia	Nasal Swab	RNASeq	12 In 8 C	N/A	Inf *vs* C	8	5	3
Fayyad-Kazan et al[57] Plasma miR-19a-3p, miR-19b-3p, and miR-92a-3p expression levels could serve as potential diagnostic biomarker and/or putative therapeutic targets during SARS-CoV-2-infection.	2021	Lebanon	Plasma	RT-qPCR	33 Inf 14MI/13MO/6S 10 C	20M/13F Inf N/A C 45Y (30–60) Inf	Inf *vs* C	12	8	4
Fernandez-Pato et al[73] SARS-CoV-2 infection severely disturbs the plasma miRNome from an early stage of COVID-19, making miRNAs highly valuable as early predictors of severity and mortality.	2022	Spain	Plasma	RNASeq	52 MO 32 S 12 AM 13 C	26M/26F MO 59.4Y (53.0–71.8) 23M/9F S 63.4Y (52.9–78.3 4M/8F AM 66.2Y (44.4–72.6) 7M/6F C 66.7Y (57–68.9)	Inf *vs* C MO/S *vs* MI S *vs* MO	200 75 137	142 19 79	58 56 58
Gambardella et al[58] A significant association linking endothelial cells (EC) and extracellular vesicles (EV) which could be valuable in understanding the mechanisms underlying the pathophysiology of CBV complications in COVID-19.	2021	Italy	Plasma	RT-qPCR	37 CBV (no CoV) 57 C (no CoV) 263 CoV- no CBV 58 CoV- CBV	22M/15F CBV (no CoV) 65.3 ± 12.7Y 29M/28F C (no CoV) 59.5 ± 14.7Y 143M/120F CoV-no CBV 61.5 ± 14.2Y 31M/27F CoV-CBV 63.6 ± 14.6Y	Inf *vs* C cerebrovascular (CBV) versus no CBV	1	0	0
Gambardella et al[74] Data indicate that exosomal miR-145 and miR-885 are essential in modulating thromboembolic events during COVID-19.	2022	Italy	Serum	RT-qPCR	26 Inf 10 C	N/A	Inf *vs* C Survivors' versus non-survivors	4	1	3

(Continues)

TABLE 2 (Continued)

Author(s) main conclusions	Year	Country	Tissue sample	Expression analysis methodology	No. of participants Inf, C, ASM, MI, MO, S, Crit	Gender (M/F) Age (Ave ± SD) or Age (low-high)	Comparative groups	miRNAs detected	Up-regulated	Down-regulated
Garcia-Giralt et al[75] Study that shows that miR-369-3p was altered in patients with mechanical ventilation requirement in comparison with COVID-19 patients without this requirement.	2022	Spain	Serum	RNASeq	48 Inf **Discovery Cohort** 28 (15 Ventilated) **Validation Cohort** 20 (10 Ventilated)	**Discovery Cohort** 16M/12F 15MV 49 ± 8.7Y 13NMV 44 ± 12Y **Validation Cohort** 11M/9F 10MV 63±9Y 10NMV 55 ± 12Y	Ventilated (S) vs Non-ventilated (MI)	170	68	102
Garg et al[59] miRNA profiles that are able to differentiate between severely ill, mechanically-ventilated Influenza-ARDS and COVID-19 patients, indicating a rather specific response and cardiac involvement of COVID-19.	2021	Germany	Serum	RT-qPCR	**Discovery Cohort** 18 Inf, Ventilated 15 C **Validation Cohort** 20 Ventilated 13 influenza ARDS (I-ARDS) 32 C	**Discovery Cohort** 17M/1F Inf 59Y (47–69) 14M/1F C 31Y (Median) **Validation Cohort** 14M/6F Vent 59.5Y (46–68) 11M/2F I-ARDS 56Y (49–58) 20M/12F C	Ventilated (S) *vs* C I-ARDS versus S *vs* C	5	4	1
Gedikbasi et al[76] microRNA-155 facilitates immune inflammation via targeting SOCS1, thus, establishing its association with disease prognosis.	2022	Turkey	Whole blood cells	RT-qPCR	73 Inf (37 MO/25 S/11 CRIT) 10 C	19M/18F MO 56.05 ± 13.72Y 18M/7F S 59.6 ± 14.8Y 7M/4F CRIT 51.27 ± 16.9 N/A C	MO *vs* C S *vs* C CRIT *vs* C	1	1	0
Giuliani et al[77] High levels of circulating miR-320b and miR-483-5p can be useful as minimally invasive biomarkers to stratify older COVID-19 patients with an increased risk of in-hospital mortality.	2022	Italy	Plasma	RNASeq	**Discovery Cohort** 12 (6 alive/6 dead) **Validation Cohort** 116 Inf (75 alive/41 dead)	**Discovery Cohort** **Total 12** 75.4 ± 5.9Y 3M/3F alive 74.4±3Y 3M/3F Dead 76.2 ± 8.2 **Validation Cohort** 39M/77F 86.5Y (82–91) 25M/50F alive 84Y (81–90)) 14M/27F Dead 90Y (85–93)	SR *vs* DS	34	9	25

TABLE 2 (Continued)

Author(s) main conclusions	Year	Country	Tissue sample	Expression analysis methodology	No. of participants Inf, C, ASM, MI, MO, S, Crit	Gender (M/F) Age (Ave ± SD) or Age (low-high)	Comparative groups	miRNAs detected	Up-regulated	Down-regulated
Grehl et al[60] Analysis of circulating human miRNAs reveal differentially expressed miRNAs, discriminating mild from severe disease.	2021	Germany	Plasma	RNASeq	8 Inf 2 C	4M/6F Inf 2F C	S *vs* MI	31	11	20
Gutmann et al[78] Circulating miRNAs of different tissue origin, including several known cardiometabolic biomarkers, rise with COVID-19 severity.	2022	Austria	Plasma	RNASeq	**Discovery Cohort** 18 MI/18 S/11 C **Validation Cohort** 6 MI/39 MO/16 S	**Discovery Cohort** 10M/8F MI 55Y (36–66 8M/10F S 58Y (39–66) 5M/6F C 40Y (30–46) **Validation Cohort** 1M/5F 60.5Y (40.5–80.5) 30M/9F MO 60. 5Y (35.5–85.5) 3M/13F S 62Y (47.5–76.5)	Total S *vs* C S *vs* MI MI *vs* MO *vs* S	91 53 35 12	70 35 32 12	21 18 3 0
Kassif-Lerner et al[79] Found that miR-155 is a good predictor of COVID-19 mortality.	2022	Israel	Serum	RT-qPCR	37 Inf, (22 MI/15 S) 15 C	22MI (14M/8F) 77.5Y (62.2–88) 15S (12M/3F) 55Y (48–66) 4M/11F C 46Y (42–57)	MI *vs* C S *vs* MI	2	0	2
Keikha et al[68] Identification of neuroinflammatory miRNAs in COVID-19 patients.	2021	Iran	Serum	RT-qPCR	21 MI 20 MO 20 S 21 Crit 21 ASM 20 C	10M/11F (MI) 50 ± 10Y 10M/10F (MO) 50 ± 10Y 9M/11F (S) 50 ± 10Y 10M/11F (Crit) 50 ± 10Y 10M/11F (ASM) 50 ± 12Y 10M/10F (C) 50 ± 12Y	Crit *vs* S *vs* MO *vs* MI *vs* ASM *vs* C	4	1	3

(Continues)

TABLE 2 (Continued)

Author(s) main conclusions	Year	Country	Tissue sample	Expression analysis methodology	No. of participants Inf, C, ASM, MI, MO, S, Crit	Gender (M/F) Age (Ave ± SD) or Age (low-high)	Comparative groups	miRNAs detected	Up-regulated	Down-regulated
Latini et al[80] The involvement of hsa-let7b-5p in the regulation of genes necessary for SARS-CoV-2 infection and its putative role as a therapeutic target for COVID-19.	2022	Italy	Nasal Swab	RT-qPCR	35 Inf 25 C	26M/9F Inf 62 ± 16 (20–92) Y 59 ± 16 Y Male 66 ± 17 Y Female 19M/6F C 58 ± 16 (27–84) Y 58 ± 16 Y Male 59 ± 21 Y Female	Inf *vs* C	1	0	1
Li et al[85] Differential miRNA expression found in COVID-19 patients may regulate immune responses and viral replication during viral infection.	2020	China	Serum	RT-qPCR	10 Inf 4 C	4M/6F Inf 44.9 ± 19.9 Y 2M/2F C 44.7 ± 11.8 Y	Inf *vs* C Total Given	73 32	35 13	38 19
Li et al[61] Induction of the IFN system appears to be particularly important in controlling SARS-CoV-2 infection.	2021	China	Whole blood cells	RNASeq	30 MM (Mild/Moderate) 16 SC (Severe/Critical) 24 C	16M/14F MM 48Y (37–59.3) 13M, 3F SC 54Y (49.3–65.5) 10M/14F C 36Y (25.8–54.3)	SC *vs* MM	2	0	2
Li et al[81] MiR-125b-5p, miR-155-5p, STAT3, and TRIM32 could be useful biomarkers to predict the time nodes of the acute, turn-negative, and recovery stages of COVID-19.	2022	China	Whole blood cells	RT-qPCR	16 Inf 16 C	9M/7F Inf 49.3 ± 16.6Y 52.9 ± 14.8 Y Male 45.8 ± 18.6 Y Female 9M/7F C 48 ± 17.8Y 52.3 ± 15.7 Y Male 43.8 ± 19.8 Y Female	Total Inf *vs* C Acute versus recovered	6 2 4	4 2 2	2 0 2

TABLE 2 (Continued)

Author(s) main conclusions	Year	Country	Tissue sample	Expression analysis methodology	No. of participants Inf, C, ASM, MI, MO, S, Crit	Gender (M/F) Age (Ave ± SD) or Age (low-high)	Comparative groups	miRNAs detected	Up-regulated	Down-regulated
Martinez-Fleta et al[62] Compared differential plasma miRNAs and cytokine profiles between COVID-19 and community-acquired pneumonias (CAP).	2021	Spain	Plasma	RT-qPCR	123 SARS-Covid (SARS), 33 Community acquired pneumonia (CAP) **Discovery Cohort** 38 SARS (20 MI/18 S) 9 CAP **Validation Cohort** 85 SARS (43 MI/42 S) 24 CAP	**Discovery Cohort** 23M/15F SARS 59.5Y (51–69) 20 MI (12M/8F) 59Y (49.5–69.5) 18 S (11M/7F) 59.5Y (51-0.38) 4M/5F CAP 62Y (59–72) **Validation Cohort** 35M/50F SARS 64Y (55–76) 43 MI (13M/30F) 58Y (51–68) 42 S (22M/20F) 72Y (61–83) 14M/10F CAP 66.5Y (60.5–80)	CAP versus SARS	35	19	16
McDonald et al[63] This study demonstrated that miR-2392 is present in the blood and urine of patients positive for COVID-19, but is not present in patients negative for COVID-19.	2021	USA	Plasma and Nasal swabs	RNASeq	Serum samples 10 Inf incubate 10 Inf outpatients 10 C Nasal Swab Samples 10 Inf 6 common cold virus 6 CoV NL63	Serum samples 5M/5F Inf incubated 5M/5F Inf outpatients 5M/5F C Age (27–85) Y all Nasal Swab Samples N/A	Inf versus common cold virus versus CoV NL63	8	1	7
Medhat et al[70] Data suggest that miRNA-142-5p could affect the severity of cytokine storm through its effect on Nrf2/Keap1 axis.	2022	Egypt	Plasma	RT-qPCR	35 Inf Ward (n = 20) ICU (n = 15) 15 C	21M/14F Inf Ward (11M/9F) 54.6 ± 13.9 Y ICU (10M/5F) 61.6 ± 14.9 Y 8M/7F C 48.2 ± 17.5 Y	Inf *vs* C	1	1	0

(Continues)

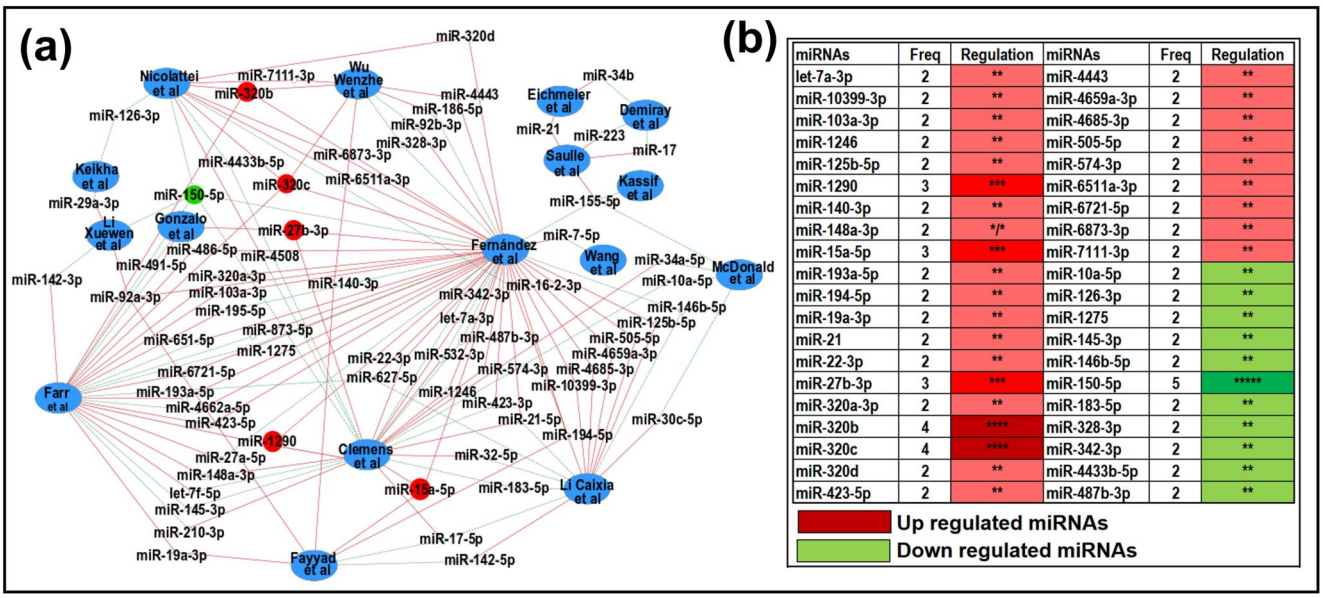
TABLE 2 (Continued)

Author(s) main conclusions	Year	Country	Tissue sample	Expression analysis methodology	No. of participants Inf, C, ASM, MI, MO, S, Crit	Gender (M/F) Age (Ave ± SD) or Age (low-high)	Comparative groups	miRNAs detected	Up-regulated	Down-regulated
Mi et al[64] Up-regulation of miR-4485 is responsible for the suppression of osteogenic differentiation in COVID-19 patients.	2021	China	Muscle, bone, bone marrow	RT-qPCR	Fractured patients 35 Inf 50 C Expression analysis 30 Inf 30 C	25M/28F 55.8 ± 18.3 y 61.4 ± 17.1 Y Male 51.17 ± 18.4 Y Female	Fractured Inf *vs* C	1	1	0
Nicoletti et al[82] The miRNAs identified in this study might be used as possible biomarkers for the diagnosis and severity of COVID-19.	2022	Brazil	Plasma	RNASeq	8 Inf (4 MI/4 S) 4 C	4M/4F Inf 2M/2F MI 61.8 ± 11.7 Y 2M/2F S 64 ± 8.6 Y 2M/2F C 62.8 ± 14.9	MI/S *vs* C S *vs* MI	18 42	13 0	5 42
Sabbatinelli et al[65] Data show that a blood-based biomarker, such as miR-146a-5p, can provide clues about the molecular link between inflammation and COVID-19 clinical course.	2021	Italy	Serum	RT-qPCR	TCZ treated COVID-19 patients **Responders** 16 **Non- responders** 13 29 C	**Responders** 11M/5F 65.9 ± 10.6 Y **Non- responders** 6M/7F 69.4 ± 12.8 Y	TCZ versus C	3	1	2
Saulle et al[66] Following SARS-CoV-2 infection, the transcriptomic profile of pregnant women is significantly altered in different anatomical sites, even in the absence of clinical symptoms and vertical transmission.	2021	Italy	Plasma/ Placenta	RT-qPCR	15 Inf (SIPW) 6 C (UPW)	All pregnant female SIPW 32Y (21–39) UPW 33.5Y (28–40)	Plasma Inf *vs* C Placenta Inf *vs* C	13 8	13 8	0 0
Tang et al[86] Identified potential contributors to the disease pathogenesis, possibly serving as biomarkers of severe COVID-19 and as candidate therapeutic targets.	2020	China	Whole blood cells	RNASeq	6 MO 6 S 4 C	4M/2F MO Age (20–89) Y 5M/1F S Age (60–89) Y 2M/2F C Age (50–69) Y	Total MO *vs* C S *vs* C S *vs* MO	823 375 351 97	299 127 115 57	524 248 236 40

TABLE 2 (Continued)

Author(s) main conclusions	Year	Country	Tissue sample	Expression analysis methodology	No. of participants Inf, C, ASM, MI, MO, S, Crit	Gender (M/F) Age (Ave ± SD) or Age (low-high)	Comparative groups	miRNAs detected	Up-regulated	Down-regulated
Wang et al[67] Circulating exosomal miRNAs can directly inhibit SARS-CoV-2 replication and may provide a possible explanation for the difference in response to COVID-19 between the young and the elderly or people with co-morbidities	2021	China	Serum	RNASeq	**Cohort 1** 15 Young 15 Old 15 healthy 15 diabetic **Cohort 2** 20 Young 20 Old 20 healthy 20 diabetic **Cohort 3 Exercise study** Total = 36 Exercise = 18 Control = 18	**Cohort 1** Young (7M/8F) 20.5 ± 0.5 Y Old (8M/7F) 76.5 ± 10.9 Y Healthy (10M/5F) 48.0 ± 6.5 Diabetic (8M/7F) 51.0 ± 19.8 **Cohort 2** Young (8M/12F) 20.3 ± 1.2 Y Old (9M/11F) 72.5 ± 9.6 Y Healthy (10M/10F) 49.6 ± 9.2Y Diabetic (10M/10F) 54.2 ± 20.1Y **Cohort 3** Control (18M/0F) 20.3 ± 1.9 Y Exercise (18M/0F) 20.6 ± 1.9 Y	C *vs* Inf w or w/o diabetes Young/old	4	0	4
Wu et al[83] Results demonstrated the involvement of tRFs in COVID-19 and reveal new SARS-CoV-2 svRNAs.	2022	USA	Nasal swab	RNASeq	6 Inf 7 C	Total (n = 13) Inf (49.2 ± 10.5 Y) C (51.7 ± 13.7 Y) Seq Inf (n = 4) 54.3 ± 4 Y Seq C (n = 4) 50.5 ± 10.2 Y	Inf *vs* C	42	36	6
Zheng et al[87] These findings uncovered the dynamic pattern of immune responses and observed a key role of T cell immunity in the creation of immune protection against COVID-19.	2020	China	Whole blood cells	RNASeq	18 total 6 MI 7 MO 5 S	11M/7F43.0 ± 18.2 YMI (3M/3F) Age (3.5–40) YMO (3M/4F) Age (32–58) YS (4M/1F)Age (49–69) Y	MI *vs* MO *vs* S	4	0	4

Abbreviations: ASM, asymptomatic; CRIT, critical; ICU, intensive care unit; IFN, interferon; Inf vs C, Infected versus Control; MI vs C, Moderate versus Control; MI vs MO vs S, Mild vs Moderate Severe; MI/S vs C, Mild/Severe versus Control; MO vs C, Moderate versus Control; MO/S vs C, Moderate /Severe versus Control; S vs C, Severe versus Control; S vs MI, Severe versus Mild; S vs MO, Severe versus Moderate; SC vs MM, Severe/Critical versus Mild/Moderate; SR vs DS, Survived versus Deceased; TCZ, Tocilizumab.



miRNAs	Freq	Regulation	miRNAs	Freq	Regulation
let-7a-3p	2	**	miR-4443	2	**
miR-10399-3p	2	**	miR-4659a-3p	2	**
miR-103a-3p	2	**	miR-4685-3p	2	**
miR-1246	2	**	miR-505-5p	2	**
miR-125b-5p	2	**	miR-574-3p	2	**
miR-1290	3	***	miR-6511a-3p	2	**
miR-140-3p	2	**	miR-6721-5p	2	**
miR-148a-3p	2	*/*	miR-6873-3p	2	**
miR-15a-5p	3	***	miR-7111-3p	2	**
miR-193a-5p	2	**	miR-10a-5p	2	**
miR-194-5p	2	**	miR-126-3p	2	**
miR-19a-3p	2	**	miR-1275	2	**
miR-21	2	**	miR-145-3p	2	**
miR-22-3p	2	**	miR-146b-5p	2	**
miR-27b-3p	3	***	miR-150-5p	5	*****
miR-320a-3p	2	**	miR-183-5p	2	**
miR-320b	4	****	miR-328-3p	2	**
miR-320c	4	****	miR-342-3p	2	**
miR-320d	2	**	miR-4433b-5p	2	**
miR-423-5p	2	**	miR-487b-3p	2	**

FIGURE 3 MiRNAs dysregulated after SARS-CoV-2 infection in "Infected *versus* Control" group. (a) miRNA Interaction network among miRNAs published in literature, showing commonly found miRNAs from various studies. Cytoscape 3.9.1 was used to construct the network. The red and green connecting lines (edges) show up- or down-regulation of miRNAs reported from each study. (b) Heatmap of the 40 dysregulated miRNAs with consistent expression profile in infected patients. The red and green highlighted miRNAs represent the reported up (U)- or down (D)-regulated miRNAs, respectively, with the number of Us or Ds reflecting the intensity of dysregulation. See text for details.

513 differentially-regulated miRNAs. To construct a miRNA-study network, we selected 85 miRNAs that appeared in at least two of the five studies. Furthermore, we had to remove two studies from the network analysis since they showed no overlapping/common miRNAs. Thus, the network consisted of 88 nodes (85 common miRNAs and three studies), 170 edges and 3864 average number of neighbours (Figure 4). There were no shared miRNAs among the three selected studies; however, Akula et al[69] and Chen et al[84] revealed two shared miRNAs (miR-375 and miR-150-5p) with suppressed expression in severe disease when compared to uninfected individuals. We also observed 83 differentially-regulated miRNAs that were reported from Tang et al[86] and Chen et al.[84] While 12 and 15 of the shared miRNAs from both studies showed the same expression profile (up- or down-regulation, respectively; Table S3); interestingly, a majority of these shared miRNAs (67%; $n = 56$) showed an opposite expression profile in these studies. This suggests the need for further study of expression of these miRNAs in the severe group compared to uninfected individuals since these results came from only three studies.

3.5 | Unique miRNAs that distinguish "severe" from "infected" patients

During data analysis, we observed that most of the differentially-regulated miRNAs found in the available data were not present in all studies, filtering out many miRNAs that could have been of importance. We were especially interested in those miRNAs that could distinguish severe disease in infected patients without further disease sub-groupings like mild or moderate. To achieve this goal, we first compared and removed those miRNAs from "Inf *vs* C" and "S *vs* C" groups that showed opposite expression within the group from various studies. Such a strategy removed 30 miRNAs from the "Inf *vs* C" and 56 miRNAs from "S *vs* C" groups. The outcome was 156 miRNAs shared by both groups, meaning these miRNAs could be used to distinguish patients with severe COVID-19 from infected individuals (Figure 5a, Table S4). Out of these 156 miRNAs, 51 were up-regulated and 43 were down-regulated in both groups. The remaining 62 miRNAs (Figure 5b) likely distinguish "severe" cases from "non-severe patients" due to their opposite expression in both conditions (18 up-regulated and 44-down regulated miRNAs in severe disease condition). Interestingly, from this list miR-15a-5p and miR-31-5p appeared in two or more studies[56,57,73,78,82,84,86] and could potentially serve as biomarkers for disease severity. Table 3 summarises the targets and principle cellular pathways associated with miRNAs regulated during Inf *vs* C and S *vs* C stages and cited previously.[46,56,73,88–120] Most of the miRNAs were involved in targeting virus-host interactions, viral replication, and host-immune responses.

3.6 | MiRNA expression analysis in all stages of disease severity after SARS-CoV-2 infection

As mentioned earlier, most of the studies included in this review either represented their miRNA dysregulation data as "Inf *vs* C" ($n = 13$) or "S *vs* C" ($n = 5$) comparison. However, some of the studies also further elaborated their results based on disease severity. To

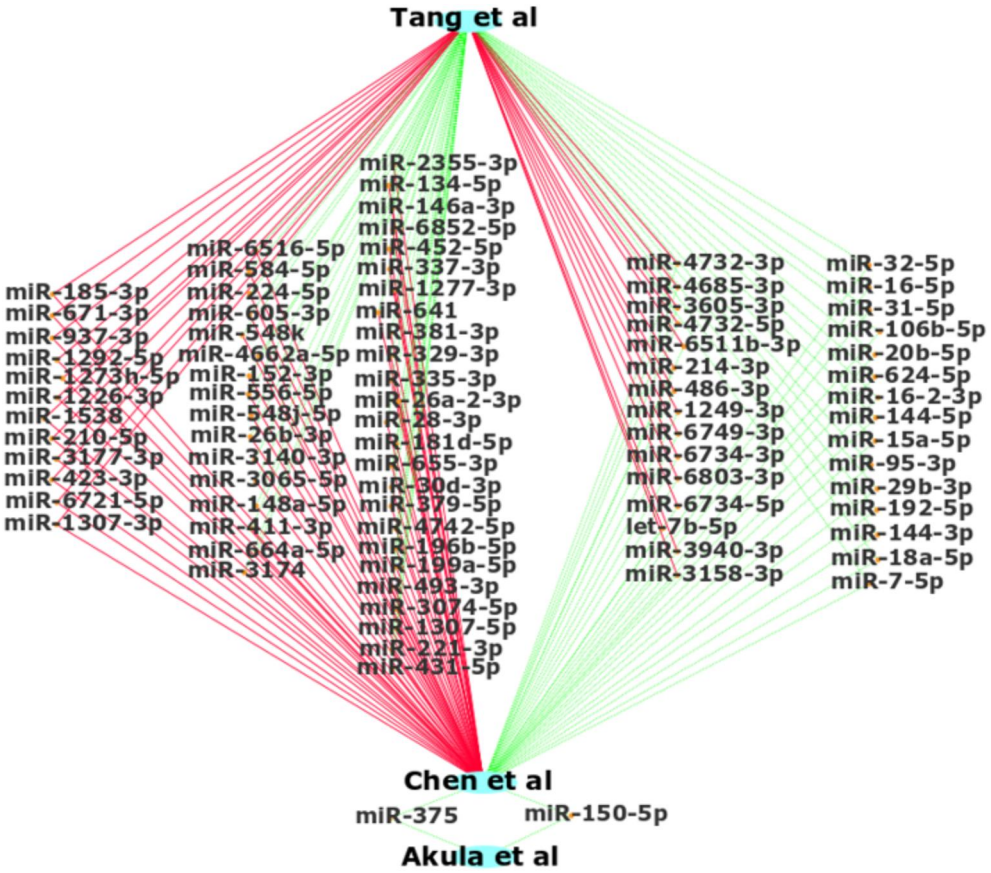
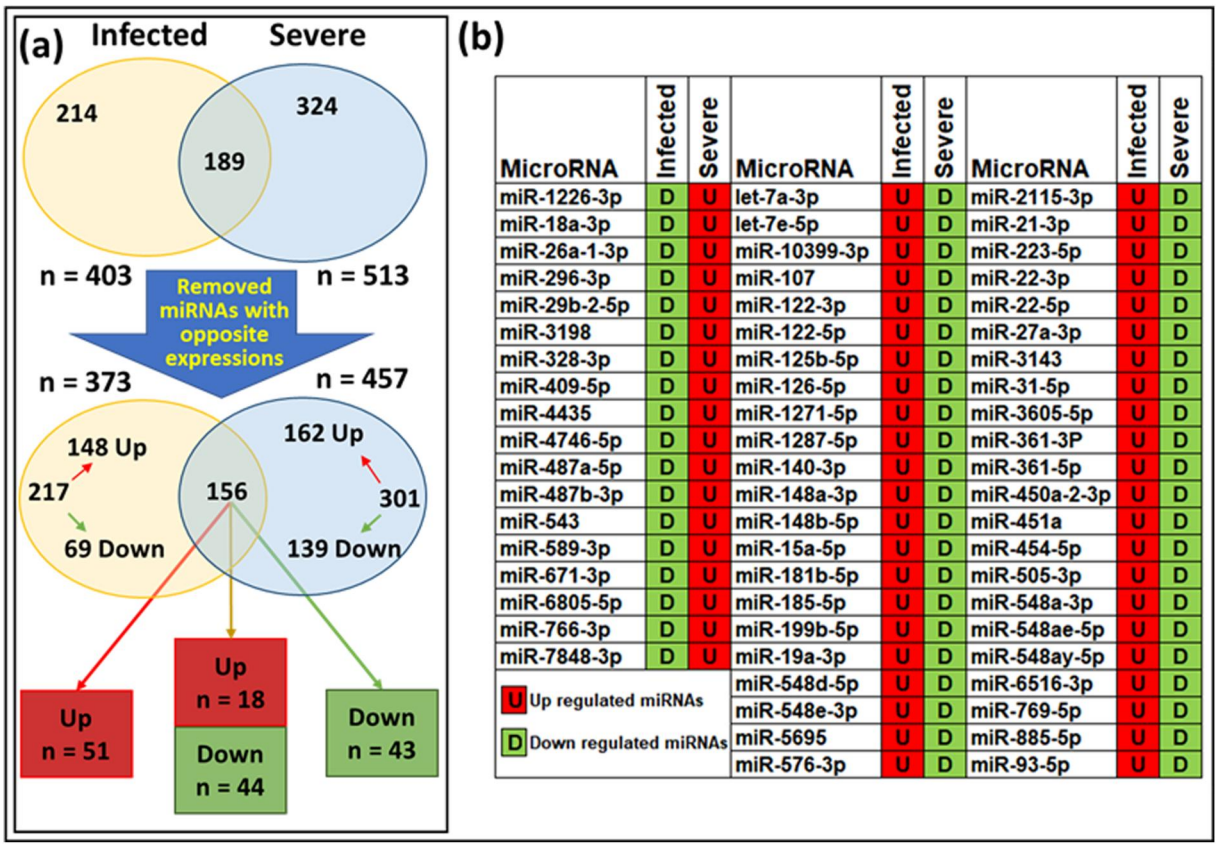


FIGURE 4 Dysregulation of miRNAs after SARS-CoV-2 infection in "Severe *versus* Control" group. The red and green lines connecting the miRNAs (edges) show up (red)- or down (green)-regulation of miRNAs reported from each study.

gain further insights into how miRNAs dysregulate after SARS-CoV-2 infection, we combined the data from all such studies ($n = 23$) and constructed a network of "miRNA-disease severity" to find abundantly expressed miRNAs in literature since these miRNAs may be able to distinguish disease severity in the infected patients. Towards this end we combined the miRNAs from the following groups: "Inf *vs* C", "Severe/Moderate *versus* Control (S/MO *vs* C)", "Severe *versus* Moderate (S *vs* MO)", "Moderate *versus* Control (MO *vs* C)", "Survived *versus* Deceased (SR *vs* DS)", "Severe/Critical *versus* Mild/Moderate (SC *vs* MM)", "Severe *versus* Moderate *versus* Mild (S *vs* MO *vs* MI)", "Severe *versus* Mild (S *vs* MI)", "Severe/Mild *versus* Control (S/MI *vs* C)", Severe *versus* Control (S *vs* C)" and "Recovered *versus* Infected (RE *vs* Inf)". Table S5 represents the summary of the total number of reported miRNAs in different COVID-19 severity stages. It should be noted that most of the groups included in this network were reported in only one or two studies: S/MO *vs* C,[69] S *vs* MO,[73,86] MO *vs* C,[86] SR *vs* DS,[77] SC *vs* MM,[61] S *vs* MO *vs* MI,[78] MO *vs* MI,[73] S *vs* MI,[60,75,78,82] S/MI *vs* C,[84] RE *vs* Inf[81]) except "Inf *vs* C" and "S *vs* C". Initially, this network contained 71 miRNAs from 23 studies. However, we removed 23 miRNAs that showed opposite regulation in the same group. The final network comprised of 48 miRNAs and 10 disease stages from 17 studies (Figure 6; Table S5). This network consisted of 58 nodes, 273 edges and 8034 average number of neighbours. Seventeen miRNAs in this network showed consistent expression in the different disease groups of which 8 were up-regulated (miR-127-3h-3p, miR-1307-3p, miR-193-5p, miR-423-5p, miR-1292-5p, miR-320c, miR-1273h-5p, and miR-1290), and 9 were down-regulated (miR-106b-5p, miR-342-3p, miR-548j-3p, miR-28-5p, miR-96-5p, miR-144-3p, miR-144-5p, miR-146b-5p, and miR-29b-3p) in the infected samples. The remaining 31 showed altered expression in distinct disease groups (Figure 6). A heatmap of these 48 miRNAs in various studies showed that similar expression profile of many miRNAs was observed (Figure 7).

3.7 | MiRNA regulation in "deceased" versus patients that "survived"

There were four studies[55,59,74,77] that observed 47 differentially-regulated miRNAs in COVID-19 patients that died *versus* that survived (Table S6). Interestingly, there were no overlapping/common miRNAs observed among these studies. However, during comparative analysis of these miRNAs with the ones observed among other groups, we detected 10 unique down-regulated miRNAs that were only present in the deceased group: miR-145, miR-17-p, miR-208a, miR-24, miR-422a, miR-499, miR-8061, miR-885, miR-101-5p, and



MicroRNA	Infected	Severe	MicroRNA	Infected	Severe	MicroRNA	Infected	Severe
miR-1226-3p	D	U	let-7a-3p	U	D	miR-2115-3p	U	D
miR-18a-3p	D	U	let-7e-5p	U	D	miR-21-3p	U	D
miR-26a-1-3p	D	U	miR-10399-3p	U	D	miR-223-5p	U	D
miR-296-3p	D	U	miR-107	U	D	miR-22-3p	U	D
miR-29b-2-5p	D	U	miR-122-3p	U	D	miR-22-5p	U	D
miR-3198	D	U	miR-122-5p	U	D	miR-27a-3p	U	D
miR-328-3p	D	U	miR-125b-5p	U	D	miR-3143	U	D
miR-409-5p	D	U	miR-126-5p	U	D	miR-31-5p	U	D
miR-4435	D	U	miR-1271-5p	U	D	miR-3605-5p	U	D
miR-4746-5p	D	U	miR-1287-5p	U	D	miR-361-3P	U	D
miR-487a-5p	D	U	miR-140-3p	U	D	miR-361-5p	U	D
miR-487b-3p	D	U	miR-148a-3p	U	D	miR-450a-2-3p	U	D
miR-543	D	U	miR-148b-5p	U	D	miR-451a	U	D
miR-589-3p	D	U	miR-15a-5p	U	D	miR-454-5p	U	D
miR-671-3p	D	U	miR-181b-5p	U	D	miR-505-3p	U	D
miR-6805-5p	D	U	miR-185-5p	U	D	miR-548a-3p	U	D
miR-766-3p	D	U	miR-199b-5p	U	D	miR-548ae-5p	U	D
miR-7848-3p	D	U	miR-19a-3p	U	D	miR-548ay-5p	U	D
			miR-548d-5p	U	D	miR-6516-3p	U	D
			miR-548e-3p	U	D	miR-769-5p	U	D
			miR-5695	U	D	miR-885-5p	U	D
			miR-576-3p	U	D	miR-93-5p	U	D

FIGURE 5 Possible miRNA as biomarkers that distinguish severe disease from mild or moderate. (a) Selection criteria for differentially-regulated miRNAs distinguishing severe disease cases from uninfected. Red boxes show up-regulated while green boxes show down-regulated miRNAs. (b) Up- (U)- and down- (D) regulated miRNAs in severe condition. The red boxes show up-regulated while green shows down-regulated miRNAs.

miR-132-3p. In addition, we also observed up-regulation of miR-1285-5p and decreased expression of miR-185-3p, miR-21, miR-323a-3p, miR-378f, and miR-410-3p in the deceased patients when compared to the remaining SARS-CoV-2-infected groups. These miRNAs may be helpful in the further prognosis of infected patients and their appearance may suggest deterioration of the patient, an aspect that needs further investigation.

3.8 | MiRNA regulation in SARS-CoV-2 infected patients with other co-morbidities

Among the studies analysed, there were six studies[62–67] that also examined the role of other co-morbidities and conditions in SARS-CoV-2-infected patients. These included patients with bone fractures, community acquire pneumonia, common cold, diabetes, pregnancy, patients recovered from SARS-CoV-2 infection, and any patient treated with Tocilizumab (TCZ). We found 110 miRNAs being differentially-regulated within these groups. First, we filtered out 14 miRNAs that appeared in more than one of these groups followed by 61 more miRNAs that were also present in infected patients only. The remaining 35 miRNAs were unique and can potentially be considered as being regulated owing to the presence of other conditions in COVID-19 infected patients (Table 4).

4 | DISCUSSION

MiRNAs have been reported as potential biomarkers in various diseases, including COVID-19, that may be able to differentiate disease severity in SARS-CoV-2 infected patients. Any change in miRNAs expression can depict the molecular modification(s) at the cellular level as these non-coding RNAs control the expression of genes involved in the diverse cellular pathways.[121] Due to their small structure, miRNAs are more stable and reliable, and have a longer half-life in the collected samples.[122] Although several diagnostic tests are routinely performed to detect the viral infection, these tests have limitations and are not able to predict the overall damage or next stage of the disease or predict disease prognosis.[123–125] These tests included nasal swab or saliva samples for SARS-CoV-2 detection using RT-qPCR, serological tests based on SARS-CoV-2 antibodies, including IgM and IgG, and tests for clinical markers, such as chest X-ray, changes in inflammatory, haematologic or biochemicals markers. Thus, the need for COVID-19-specific biomarkers exists that should

TABLE 3 Differentially-regulated miRNAs in infected and severe stage and their targets/pathways in SARS-CoV-2 infection.

MiRNA	Inf vs C	S vs C	Potential target(s)	Cellular pathway(s)
let-7a-3p	Up	Down	STAT3, WNT, mTOR	Cell cycle, cell survival, proliferation
let-7b-3p	Up	Up	STAT3, WNT, mTOR	Cell cycle, cell survival, proliferation
let-7d-3p	Up	Up	STAT3, WNT, mTOR	Cell cycle, cell survival, proliferation
let-7e-5p	Up	Down	3′ UTR of TMPRSS	Virus-TMPRSS2 binding activation
let-7f-2-3p	Up	Up	STAT3, WNT, mTOR	Cell cycle, cell survival, proliferation
let-7g-5p	Down	Down	STAT3, WNT, mTOR	Cell cycle, cell survival, proliferation
miR-10399-3p	Up	Down		Immune responses
miR-103a-3p	Up	NA	3′ UTR of Spike mRNA	Viral protein interactions
miR-106b-5p	Down	Down	ACE2	Virus-ACE2 binding
miR-107	Up	Down	NMDA receptors	anti-NMDA receptor encephalitis
miR-122-3p	Up	Down	Hepatic acute response	Inflammation
miR-122-5p	Up	Down	ADAM17	Viral replication/life cycle
miR-1246	Up	Up	3′ UTR of ACE2	Virus-ACE2-TMPRSS2 binding
miR-125b-5p	Up	Down	3′ UTR of ACE2	Virus-ACE2 binding
miR-126-3p	Down	Down	NF-Kβ	INF-β pathway
miR-126-5p	Up	Down	5′ UTR of viral NS mRNA	Viral proteins interactions
miR-1273h-3p	Up	Up	RISC complex	PTM silencing of SARS CoV-2
miR-1273h-5p	Up	Up	RISC complex	PTM silencing of SARS CoV-2
miR-1287-5p	Up	Down	IL6R and RIG-I regulation	Immune responses
miR-1301-3p	Up	Up	Vial nucleocapsid	Viral protein interactions
miR-132-5p	Down	Down		Virus-ACE2-TMPRSS2 binding
miR-133a-3p	Up	Up		Immune response
miR-144-3p	Down	Down	EGF/IL-10	Immune response
miR-144-5p	Down	Down	EGF/IL-10	Immune response
miR-145-3p	Down	Down	D-dimer	Thrombosis
miR-146a-5p	Down	Down	MAPK, NF-Kβ	Inflammation, Jak/STAT
miR-146b-5p	Down	Down	Target IRAK1/TRAF6	Immune responses
miR-148a-3p	Up	Down	Viral ORF1a, E, S, and M mRNAs	Viral protein interactions
miR-150-5p	Down	Down	Blocks viral Nsp10	Immune responses, apoptosis
miR-151a-5p	Up	Up	Viral spike mRNA	Viral protein interactions
miR-155-5p	Down	Down	SOCS1 expression regulation	Virus-ACE2-TMPRSS2 binding
miR-15a-5p	Up	Down	IFN signalling	Immune responses
miR-16-5p	Down	Down	APP/CALM1/CAV1/CBL	Thrombosis
miR-181a-2-3p	Down	Down	TMPRSS2	Virus-TMPRSS2 binding
miR-181b-5p	Up	Down	ACE2	Virus-ACE2 binding
miR-183-5p	Down	Down	ITGB1	Viral replication/life cycle
miR-185-5p	Up	Down	ACE2	Virus-ACE2 binding
miR-18a-3p	Down	Up	DICER/VFGFA/VGFD	ACE2 expression
miR-18a-5p	Down	Down	DICER/VFGFA/VGFD	ACE2 expression
miR-194-5p	Up	NA	FOXP3/CCL20/IL-17/Th-17	Immune responses

(Continues)

TABLE 3 (Continued)

MiRNA	Inf vs C	S vs C	Potential target(s)	Cellular pathway(s)
miR-197-3p	Up	Up	ACE2	Virus-ACE2 binding
miR-199a-3p	Down	Down	ACE2/TMPRSS2	Viral replication
miR-199b-3p	Down	Down	ACE2/TMPRSS2	Viral replication
miR-199b-5p	Up	Down	ACE2/TMPRSS2	Viral replication
miR-19a-3p	Up	Down	TGFβ	Immune response
miR-20a-5p	Down	Down	TL4/TXNIP/TNF/CCL2/CXCL9/IL10	Inflammation
miR-21	Up	Up	MAPK, NF-Kβ	Inflammation, Jak/STAT
miR-223-3p	Down	Down	STMN1	Viral replication
miR-223-5p	Up	Down	STMN1	Viral replication
miR-24-3p	Down	Down	Spike mRNA	Immune response
miR-26a-1-3p	Down	Up	PGE2/COX-2	Inflammation
miR-27a-3p	Up	Down	ALB/CAV1/COL1A1	Thrombosis
miR-27b-3p	Up	NA	PPRS regulation	Virus-ACE2-TMPRSS2 binding
miR-27b-5p	Up	Up		Virus-ACE2-TMPRSS2 binding
miR-29b-2-5p	Down	Up	POU2F2	Inflammation
miR-3143	Up	Down	RISC complex	PTM silencing of SARS CoV-2
miR-31-5p	Up	Down	TNFα	Inflammation
miR-320a-3p	Up	NA	CRP, IL-6, D-dimer	Inflammation
miR-320b	Up	Up	CRP, IL-6, D-dimer	Inflammation
miR-320c	Up	NA	CRP, IL-6, D-dimer	Inflammation
miR-320d	Up	NA	CRP, IL-6, D-dimer	Inflammation
miR-320e	Up	Up	CRP, IL-6, D-dimer	Inflammation
miR-326	Up	Up	CEBPA regulation	Inflammation
miR-328-3p	Down	Up	Suppresses type I interferon	Immune responses
miR-331-3p	Up	Up	HER2/PI3-AKT/ERK1/2	Apoptosis
miR-340-5p	Down	Down	MAP3K2/MAPK/ERK	Immune response, cell migration
miR-342-3p	Down	Down	Nucleocapsid	Viral proteins interactions
miR-342-5p	Down	Down	Nucleocapsid	Viral proteins interactions
miR-3613-5p	Down	Down	TGFβ Signalling	Regulate FGF2/VCAM1
miR-361-3p	Up	Down	P53	Apoptosis
miR-374a-5p	Down	Down	CCL2	Immune responses
miR-423-5p	Up	Up		Immune responses
miR-451a	Up	Down	Cytokine/chemokine synthesis	Immune responses
miR-454-3p	Down	Down	TGFβ2	Immune responses
miR-454-5p	Up	Down	TGFβ2	Immune responses
miR-4659a-3p	Up	NA		Immune responses
miR-4685-3p	Up	NA	ZBTB16	Immune responses
miR-548d-5p	Up	Down	SP1	Immune response, apoptosis
miR-659-5p	Down	Down		Viral proteins interactions
miR-6741-3p	Up	Up	ACE2	Virus-ACE2 binding

TABLE 3 (Continued)

MiRNA	Inf vs C	S vs C	Potential target(s)	Cellular pathway(s)
miR-760	Up	Up		Immune responses
miR-769-5p	Up	Down	3′ UTR of ACE2	Virus-ACE2 binding
miR-885-5p	Up	Down	3′ UTR of S protein, D-dimer	Blocks viral entry, thrombosis
miR-93-5p	Up	Down	ACE2	Virus-ACE2 binding
miR-98-3p	Up	Up	3′ UTR of TMPRSS	Virus-TMPRSS2 binding
miR-99a-5p	Down	Down	PTEN/AKT/mTOR	Autophagy
miR-99b-3p	Up	Up	PTEN/AKT/mTOR	Autophagy

Abbreviations: IFN, interferon; Inf *vs* C, Infected versus Control; S *vs* C, Severe versus Control.

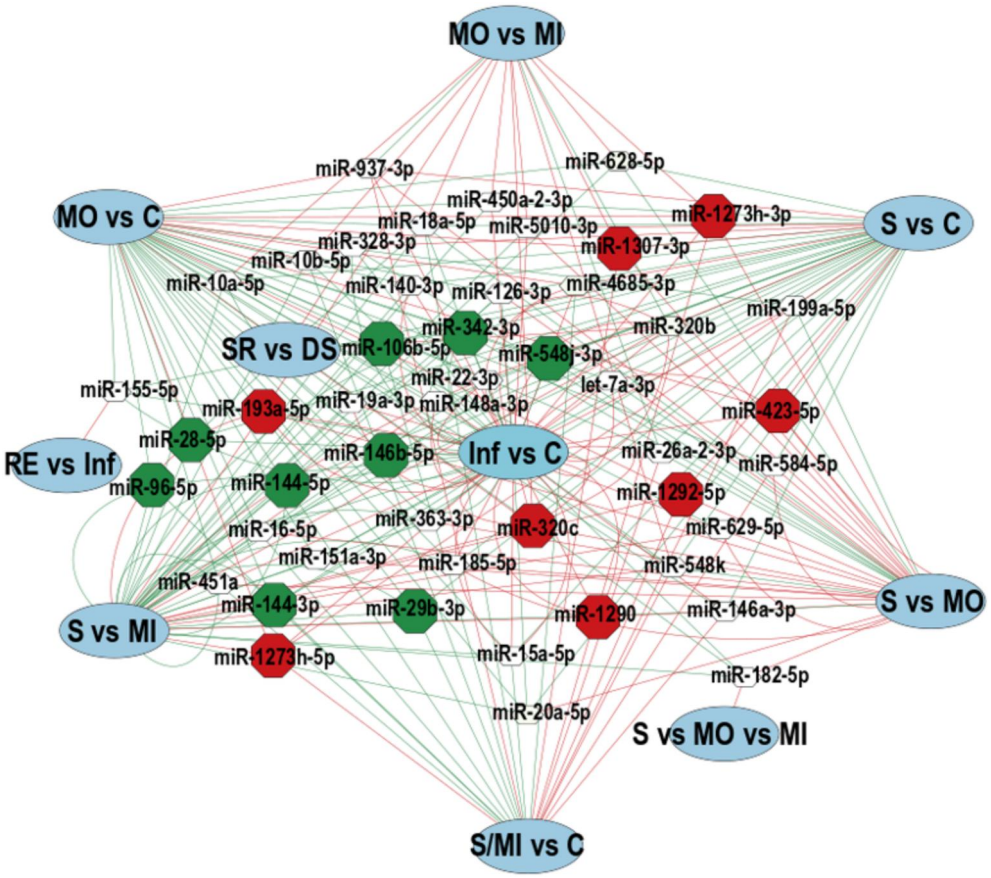


FIGURE 6 Network analysis showing differentially expressed miRNAs in various disease stages after SARS-CoV-2 infection. The red and green connecting lines (edges) show up- or down-regulation of miRNAs reported from each study. The highlighted red or green octagon boxes highlight either up- or down-regulated miRNAs from various disease groups with the similar trend. The remaining miRNAs show altered expression in the subsequent disease groups. C, control uninfected; MI, mild; MO, moderate; S, sever; RE, recovered; SR, survived; DS, deceased.

reliably and effectively predict disease prognosis, especially considering that COVID-19 patients can quickly take a turn for the worse and come down with severe disease with a considerably high rate of mortality.

There is convincing evidence that miRNA spectrum and expression levels are dependent upon the functional state of the body and a change in their expression may reflect different stages of disease condition(s). SARS-CoV-2-infected patients may or may not develop COVID-19, with their symptoms ranging from being asymptomatic at one end of the spectrum to developing from mild, to moderate, to severe disease. The severe patients could exhibit critical symptoms requiring mechanical ventilation or other type of aids to remain alive. There are 24 studies that were included in this review which investigated any change in miRNA expression in

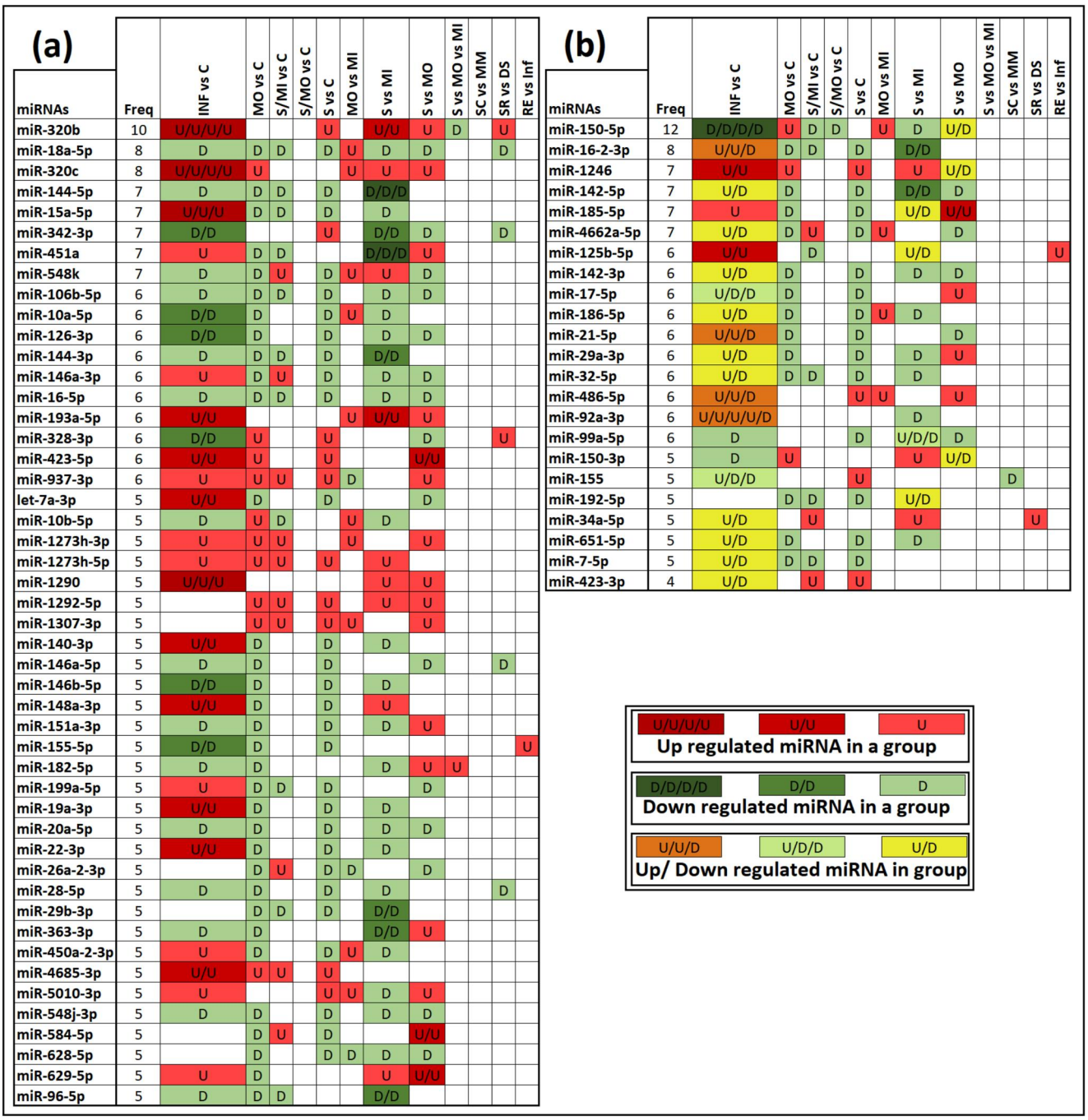


FIGURE 7 Heatmap of significantly up- or down-regulated 71 miRNAs in different stages of disease severity. (a) Forty-eight miRNA showing similar trends within the individual group. (b) Twenty-three miRNAs showing altered expression within the individual group. The boxes with multiple "Us and Ds" depict the appearance of any miRNA expression from various studies. The abbreviations "U" and "D" represent the up- and down-regulated miRNAs, respectively. Boxes become darker when more studies with similar dysregulation were observed for any miRNA.

SARS-CoV-2 infected patients. A few of them also sub-staged the disease severity and tested their findings in a validation cohort, whereas, the remaining studies tested the change of miRNA expression in the presence of other diseases in SARS-CoV-2 infected patients.

The overall goal of this study was to find miRNAs that could act as biomarkers in SARS-CoV-2-infected patients, especially to differentiate between disease stage/severity. Although we found many miRNAs that were reported in multiple studies, we chose the ones with consistent expression profiles across studies. Our efforts identified 40 miRNAs that were differentially-regulated in SARS-CoV-2 infected patients compared to healthy controls (Figure 3). The frequently reported up-regulated miRNAs included miR-1299, miR-15a-5p, miR-27b-3p, miR-320b, and miR-320c, while the

TABLE 4 MiRNAs expressed due to the presence of co-morbidities in COVID-19 patients.

MiRNAs	Expression	Reference	Study Group
miR-362-3p	Up	Martinez-Fleta et al., 2021[62]	CAP *vs* COVID-19
miR-376a-3p	Down	Martinez-Fleta et al., 2021[62]	CAP *vs* COVID-19
miR-382-5p	Down	Martinez-Fleta et al., 2021[62]	CAP *vs* COVID-19
miR-451ab	Up	Martinez-Fleta et al., 2021[62]	CAP *vs* COVID-19
miR-99b-5p	Down	Martinez-Fleta et al., 2021[62]	CAP *vs* COVID-19
miR-2392	Down	McDonald et al., 2021[63]	Common cold *vs* COVID-19
miR-1247-5p	Up	Mi et al., 2021[64]	Fractured *vs* no fracture
miR-133b	Down	Mi et al., 2021[64]	Fractured *vs* no fracture
miR-1-3p	Down	Mi et al., 2021[64]	Fractured *vs* no fracture
miR-194-5p	Down	Mi et al., 2021[64]	Fractured *vs* no fracture
miR-1973	Up	Mi et al., 2021[64]	Fractured *vs* no fracture
miR-200a-3p	Up	Mi et al., 2021[64]	Fractured *vs* no fracture
miR-33b-3p	Up	Mi et al., 2021[64]	Fractured *vs* no fracture
miR-365b-3p	Up	Mi et al., 2021[64]	Fractured *vs* no fracture
miR-378e	Up	Mi et al., 2021[64]	Fractured *vs* no fracture
miR-378f	Up	Mi et al., 2021[64]	Fractured *vs* no fracture
miR-378g	Up	Mi et al., 2021[64]	Fractured *vs* no fracture
miR-383-3p	Down	Mi et al., 2021[64]	Fractured *vs* no fracture
miR-4485	Up	Mi et al., 2021[64]	Fractured *vs* no fracture
miR-4536-3p	Up	Mi et al., 2021[64]	Fractured *vs* no fracture
miR-511-5p	Up	Mi et al., 2021[64]	Fractured *vs* no fracture
miR-548qr-3p	Down	Mi et al., 2021[64]	Fractured *vs* no fracture
miR-5582-3p	Down	Mi et al., 2021[64]	Fractured *vs* no fracture
miR-5699-3p	Up	Mi et al., 2021[64]	Fractured *vs* no fracture
miR-6859-5p	Down	Mi et al., 2021[64]	Fractured *vs* no fracture
miR-146	Up	Saulle et al., 2021[66]	Pregnant *vs* non-pregnant women
miR-146a	Up	Saulle et al., 2021[66]	Pregnant *vs* non-pregnant women
miR-150	Up	Saulle et al., 2021[66]	Pregnant *vs* non-pregnant women
miR-190	Up	Saulle et al., 2021[66]	Pregnant *vs* non-pregnant women
miR-21b	Up	Saulle et al., 2021[66]	Pregnant *vs* non-pregnant women
miR-23b	Up	Saulle et al., 2021[66]	Pregnant *vs* non-pregnant women
miR-28	Up	Saulle et al., 2021[66]	Pregnant *vs* non-pregnant women
miR-29a	Up	Saulle et al., 2021[66]	Pregnant *vs* non-pregnant women
miR-346	Up	Saulle et al., 2021[66]	Pregnant *vs* non-pregnant women
miR-92	Up	Saulle et al., 2021[66]	Pregnant *vs* non-pregnant women

down-regulated miRNAs included miR-150-5p that could act as possible biomarkers to distinguish infected from non-infected participants. Furthermore, we identified those miRNAs that were exclusively dysregulated during severe disease and found 18 up- and 44 down-regulated miRNAs in severe condition that showed opposite expression in other disease stages (Figure 5). Of these, miR-15a-5p and miR-31-5p appeared in most of the studies. The expression of miR-15a-5p was up-regulated upon infection and gradually decreased upon increasing disease severity; thus, having the potential to serve as a COVID-19 disease severity marker. However, it was tricky to identify miRNA markers that could differentiate early-stage patients with the patients that recovered.

As miRNA expression is associated with disease progression, we were also interested in identifying miRNAs that could distinguish intermediate disease stages. Towards this end, we identified 71 miRNAs which appeared in more than 5 comparative disease severity groups (Figures 6 and 7). Our analysis showed that the expression of miR-320b, miR-18a-5p, miR-320c, miR-144-5p, miR-15a-5p, miR-342-3p, miR-451a, and miR-548k was consistent during progression of disease severity. Interestingly, miR-150-5p was reported 12 times in seven comparative groups; however, its expression was not consistent within groups. Similar findings were observed for miR-16-2-3p, miR-1246, miR-142-5p, miR-185-5p, and miR-4662a-5p that failed to show expression consistency within groups (Figures 6 and 7). Although most of the infected patients recovered fully, unfortunately, a small minority could not bear the brunt of the disease and died during the course of the infection. We found 10 unique miRNAs that were only present in the deceased group, including: miR-145, miR-17-p, miR-208a, miR-24, miR-422a, miR-499, miR-8061, miR-885, miR-101-5p, and miR-132 3p. Thus, presence of these miRNAs may indicate a poor prognosis for patient survival. Of course, this would need further validation.

As mentioned in the introduction, the biogenesis of miRNAs results into two strands (arms), namely 5p and 3p. Although most of the studies observed concordant dysregulation of the targets by both arms, there is much evidence where both arms oppositely regulate their targets or result in distinct biological effects.[126–128] During the course of analysis performed for this study, some studies identified the 5p or 3p character of the miRNAs, while others did not that could have resulted in different effects on their targets. To avoid this conflict, we removed several frequently existing miRNAs (≥ five times) from many groups. While these miRNAs were not included in the downstream analysis, they still have their significance in disease progression and include, miR-126, miR-150, miR-155, miR-17, miR-21, miR-223, miR-29c, miR-3180, miR-320a, and miR-98. The expression of miR-3180, miR-3180-3p, miR-3180-5p, miR-320a, miR-320a-3p, miR-98, and miR-98-3p was upregulated in the infected patients among different groups (Table S7); whereas, the expression of miR-223-3p, miR-29c-5p, and miR-98-5p was down-regulated upon SARS-CoV-2 infection. The expression of miR-150-5p and miR-155-5p was down-regulated in the Inf *vs* C group. However, contradictory results were observed among other disease sub-stages. The expression of different transcripts of the other miRNAs remained inconsistent among disease groups.

We also found some studies where researchers reported significant alteration of some miRNAs in SARS-CoV-2-infected patients during the presence of other co-morbidities like influenza, community-acquired pneumonia, diabetes, pregnancy, cerebrovascular disease, common cold, or bone fractures. We compared these results from other studies and identified five unique miRNAs (miR-362-3p, miR-376a-3p, miR-382-5p, miR-451ab, and miR-99b-5p) that could differentiate community-acquired pneumonia from SARS-CoV-2-infected patients and one miRNA (miR-2392) that could distinguish common cold patients from SARS-CoV-2-infected patients. Furthermore, we identified 19 miRNAs that were uniquely dysregulated in infected patients due to bone fractures and 10 miRNAs unique to pregnant women only (Table 4). Thus, these identified miRNAs may serve as promising candidates to differentiate between different infections and diseased conditions or states. Furthermore, the molecular mechanisms and pathways associated with these miRNAs could be helpful in better understanding disease pathophysiology as well as for the development of specific treatments.

4.1 | Current status of already proposed biomarkers miRNAs in COVID-19

The published studies included in this review proposed multiple miRNAs as possible biomarkers and most of them were validated in the secondary cohorts of the same study. We found that most of the miRNAs detected in one study were either present in other studies or had an opposite expression within the specific group. For example, Akula et al, 2022 observed significant dysregulation of eight miRNAs in infected patients; however, out of these, only miR-150-5p was reported by others with identical expression trends. Therefore, miRNAs reported once or not expressed in similar manners were removed from further analysis. Table 5 signifies miRNAs that were reported in at least two different studies and showed a consistent expression profile across the studies. It also shows the tissue sample used for extraction of the RNA and reports results (up or down) between different groups, including Inf *vs* C, S *vs* C, S *vs* patients from other disease stages.

Based on this analysis, we highlighted 10 possible miRNAs as COVID-19 biomarkers, showing consistent expression profile among several studies (Table 6). Four of these were up-regulated (miR-193a-5p, miR-320b, miR-423-5p, miR-6721-5p) and five were down-regulated (miR-150-5p, miR-342-3p, miR-144-3p, miR-144-5p, miR-29b-3p) during disease progression, whereas the expression of one miRNA (miR-15a-5p) was down-regulated during the severe stage. Most of these miRNAs were associated with host-immune response after infection or the virus itself. MiR-193a-5p has been found to regulate TOMM70 receptor and it is possible that this miRNA may be associated with SARS-CoV-2 life cycle during its pathogenesis.[108] Mir-320 family has been well studied in COVID-19 pathogenesis and found to be associated with TGF-β signalling pathway that may further regulate pro-inflammatory and thromboembolic processes in infected patients.[77,129] Another up-regulated miRNA after infection was miR-423-5p. This miRNA regulates MALAT1 expression and has been found to induce survival and decrease metastases in mice model by inhibiting MALTA1-mediated proliferation, tumour growth and metastasis.[130] As this miRNA also induces apoptosis and autophagy in cancer cells,[131] it is possible that induced expression of miR-423-5p after SARS-CoV-2 infection may help host to clear infected cells. We also observed consistent up-regulation of miR-6721-5p in SARS-CoV-2 infected patients. Although the exact role of this miRNA is not known, putative target prediction showed that it may regulate cellular transport. MiR-150-5p inhibits the viral structural protein Nsp10 expression and this suggests that decline in miR-150-5p may increase COVID-19 disease severity by allowing SARS-CoV-2 infection.[69]

TABLE 5 Potential miRNA biomarkers detected consistently in at least two studies.

Study	Tissue sample	Disease stages	MiRNAs detected	
			Up-regulated	Down-regulated
MiRNAs detected in infected patients compared to healthy controls				
Eichmeier et al, 2022[71]	Nasal Swab	Inf *vs* C (n = 1)	miR-21	-
Farr et al, 2022[72]	Plasma	Inf *vs* C (n = 13)	miR-103a-3p, miR-1290, miR-148a-3p, miR-193a-5p, miR-19a-3p, miR-320a-3p, miR-320b, miR-320c, miR-423-5p, miR-6721-5p	miR-1275, miR-145-3p, miR-150-5p
Fayyad-Kazan et al, 2021[57]	Plasma	Inf *vs* C (n = 4)	miR-140-3p, miR-15a-5p, miR-194-5p, miR-19a-3p	-
Fernandez-Pato et al, 2022[73]	Plasma	Inf *vs* C (n = 30)	let-7a-3p, miR-10399-3p, miR-103a-3p, miR-1246, miR-125b-5p, miR-1290, miR-15a-5p, miR-193a-5p, miR-194-5p, miR-22-3p, miR-27b-3p, miR-320a-3p, miR-320b, miR-320c, miR-320d, miR-423-5p, miR-4443, miR-4659a-3p, miR-4685-3p, miR-505-5p, miR-574-3p, miR-6511a-3p, miR-6721-5p, miR-6873-3p	miR-1275, miR-146b-5p, miR-150-5p, miR-328-3p, miR-342-3p, miR-487b-3p
de Gonzalo-Calvo et al, 2021[55]	Plasma	Inf *vs* C (n = 1)	miR-27b-3p	-
Gutmann et al, 2022[78]	Plasma	Inf *vs* C (n = 15)	let-7a-3p, miR-1246, miR-1290, miR-148a-3p, miR-15a-5p, miR-22-3p, miR-27b-3p, miR-574-3p	miR-10a-5p, miR-145-3p, miR-150-5p, miR-183-5p, miR-342-3p, miR-4433b-5p, miR-487b-3p
Keikha et al, 2021[68]	Serum	Inf *vs* C (n = 1)	-	miR-126-3p
Li et al, 2020[85]	Serum	Inf *vs* C (n = 7)	miR-10399-3p, miR-125b-5p, miR-4659a-3p, miR-4685-3p, miR-505-5p	miR-146b-5p, miR-183-5p
Li et al, 2022[81]	Whole blood	Inf *vs* C (n = 1)	-	miR-150-5p
McDonald et al, 2021[63]	Plasma andNasal Swab	Inf *vs* C (n = 1)	-	miR-10a-5p
Nicoletti et al, 2022[82]	Plasma	Inf *vs* C (n = 9)	miR-6873-3p, miR-320b, miR-7111-3p, miR-320c, miR-6511a-3p, miR-320d	miR-126-3p, miR-150-5p, miR-4433b-5p
Saulle et al, 2021[66]	Plasma/ Placenta	Inf *vs* C (n = 7)	miR-21	-
Wu et al, 2022[83]	Nasal Swab	Inf *vs* C (n = 7)	miR-140-3p, miR-186-5p, miR-320b, miR-320c, miR-4443, miR-7111-3p	miR-328-3p
miRNAs detected in patients with severe disease compared to healthy controls				
Akula et al, 2022[69]	Plasma	S *vs* C (n = 2)	-	miR-150-5p, miR-375
Chen et al, 2020[84]	Whole blood	S *vs* C (n = 29)	miR-1226-3p, miR-1273h-5p, miR-1292-5p, miR-1307-3p, miR-1538, miR-185-3p, miR-210-5p, miR-3177-3p, miR-423-3p, miR-671-3p, miR-6721-5p, miR-937-3p	miR-106b-5p, miR-150-5p, miR-375, miR-144-3p, miR-144-5p, miR-15a-5p, miR-18a-5p, miR-16-2-3p, miR-16-5p, miR-192-5p, miR-20b-5p, miR-29b-3p, miR-31-5p miR-32-5p, miR-624-5p, miR-7-5p, miR-95-3p

(Continues)

TABLE 5 (Continued)

Study	Tissue sample	Disease stages	MiRNAs detected	
			Up-regulated	Down-regulated
Tang et al, 2020[86]	Whole blood	S *vs* C (n = 27)	miR-1226-3p, miR-1273h-5p, miR-1292-5p, miR-1307-3p, miR-1538, miR-185-3p, miR-210-5p, miR-3177-3p, miR-423-3p, miR-671-3p, miR-6721-5p, miR-937-3p	miR-106b-5p, miR-144-3p, miR-144-5p, miR-15a-5p, miR-16-2-3p, miR-16-5p, miR-18a-5p, miR-192-5p, miR-20b-5p, miR-29b-3p, miR-31-5p, miR-32-5p, miR-624-5p, miR-7-5p, miR-95-3p
miRNAs detected in severe stage of COVID-19 compared to other disease stages				
Garcia-Giralt et al, 2022[75]	Serum	S *vs* MI (n = 7)	miR-193a-5p, miR-320b	miR-144-3p, miR-144-5p, miR-29b-3p, miR-342-3p, miR-451a, miR-96-5p
Grehl et al, 2021[60]	Plasma	S *vs* MI (n = 6)	miR-320b	miR-144-5p, miR-29b-3p, miR-363-3p, miR-451a
Gutmann et al, 2022[78]	Plasma	S *vs* MI (n = 1)	miR-193a-5p	-
Nicoletti et al, 2022[82]	Plasma	S *vs* MI (n = 6)	-	miR-144-3p, miR-144-5p, miR-342-3p miR-363-3p, miR-451a, miR-96-5p
Fernandez-Pato et al, 2022[73]	Plasma	S *vs* MO (n = 4)	miR-185-5p, miR-423-5p, miR-584-5p, miR-629-5p	-
Gutmann et al, 2022[78]	Plasma	S *vs* MO (n = 1)	miR-182-5p	-
Tang et al, 2020[86]	Whole blood	S *vs* MO (n = 5)	miR-182-5p, miR-185-5p, miR-423-5p, miR-584-5p, miR-629-5p	

Abbreviations: Inf *vs* C, Infected versus Control; S *vs* C, Severe versus Control; S *vs* MI, Severe versus Mild; S *vs* MO, Severe versus Moderate.

TABLE 6 MiRNAs with consistent expression profiles in different disease stages of SARS-CoV-2 infection.

MiRNA	Potential target	Pathway	Expression				
			Inf *vs* C	MO *vs* C	S *vs* C	S *vs* MI	S *vs* MO
miR-193a-5p	TOMM70 receptor	Transportation	Up			Up	Up
miR-320b	TGFβ	Immune response	Up			Up	Up
miR-423-5p	MALAT1 expression	Tumour suppressor	Up		Up		Up
miR-6721-5p	Cellular transport	Immune response	Up		Up		
miR-15a-5p	mTOR signalling		Up		Down		
miR-150-5p	Nsp 10	Viral replication, apoptosis	Down		Down		
miR-342-3p	Nucleocapsid	Viral replication; immune response	Down		Down	Down	Down
miR-144-3p	Cytokines	Immune response	Down	Down	Down	Down	
miR-144-5p	Cytokines	Immune response	Down	Down	Down	Down	
miR-29b-3p	Inflammation, IL-8	Immune response	Down	Down	Down	Down	

Abbreviations: Inf *vs* C, Infected versus Control; MO *vs* C, Moderate versus Control; S *vs* C, Severe versus Control; S *vs* MI, Severe versus Mild; S *vs* MO, Severe versus Moderate.

Interestingly, treatment with the anti-inflammatory drug "simvastatin" induced miR-150-5p levels and decreased disease severity in SARS-CoV-2 infected patients, supporting this hypothesis.[132]

We also observed a consistent down-regulation of miR-342-5p that is mainly involved in inflammatory stimulation of macrophages.[133] Targeting analysis showed that this miRNA might be able to target SARS-CoV-2 nucleocapsid, ORF1ab, and ORF3a domains and thus may be involved in regulating virus replication.[134] There is evidence that decreased miR-144 levels could indicate compromised immune response and could be used as a biomarker to predict COVID-19 disease severity and mortality.[104] Decreased expression of miR-29b-3p is associated with airway inflammation and regulate

inflammatory cytokine IL-8 expression.[135] Down-regulated expression of miR-29b-3p might be a biomarker of disease severity in SARS-CoV-2 infection.[105] We found miR-15a-5p as a suitable biomarker to distinguish severe stage from others during SARS-CoV-2 infection. Down-regulation of miR-15a-5p may be a sign of uncontrolled immune-thrombosis and/or thrombo-inflammation.[136] A current study by Wu et al suggests that down-regulated expression of miR-15a-5p could induce/activate interferon-1 signalling pathway to overcome SARS-CoV-2 severity.[105] Overall, if these miRNAs continue to show consistent expression in future studies, these could be considered as possible biomarkers in COVID-19 prognosis. Meanwhile, targeting these miRNAs may be helpful to create future therapies against SARS-CoV-2 infection.

5 | CONCLUSIONS

In this systematic review, we were able to identify miRNAs from published literature that not only distinguished infected patients from healthy controls, but also were able to discriminate stages of disease severity, poor disease prognosis, and even death. These miRNAs showed consistent expressions within groups and can potentially be used as possible biomarkers. Furthermore, we also identified unique miRNAs associated with patients with specific co-morbidities. Although any change in the expression of these miRNAs could be used as specific biomarkers of SARS-CoV-2 infection, COVID-19 disease progression, and mortality, further validation is needed. Considering that SARS-CoV-2 has become endemic in the human population and is here to stay, the emergence of new SARS-CoV-2 variants with pandemic potential exists. Thus, this study offers a valuable addition to the literature towards the identification of miRNA-based biomarkers that could eventually be used in the development of miRNA-based antivirals and therapeutics for COVID-19.

5.1 | Limitations and future perspectives

In this review, we included only those studies originating from human patients with the hope that our effort will help identify a list of miRNAs that could be used as potential biomarkers in SARS-CoV-2 infected patients as prognostic markers. The results extracted from these studies needs proper validation as we found vast differences in miRNA expression profiles within same groups between studies. It is not surprising that validated biomarkers in one study might not be the same as those in another study conducted elsewhere since ethnicity, gender, age, presence of co-morbidities, effect of medications taken for such co-morbidities, types of COVID-19 treatments and vaccines taken, and other environmental factors (such as the strain of SARS-CoV-2) may influence miRNAs expression profiles in COVID-19 patients. We anticipate that data gathered from other in vitro, in vivo, or in silico studies as well as future studies in humans could help confirm some of these miRNAs as biomarkers and/or clarify the mechanistic aspects of the function of the identified miRNAs.

AUTHOR CONTRIBUTIONS

Waqar Ahmad: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Software; Validation; Visualization; Writing-original draft; Writing-review & editing. **Bushra Gull**: Data curation; Formal analysis; Investigation. **Jasmin Baby**: Data curation; Formal analysis; Investigation. **Neena G. Panicker**: Investigation. **Thanumol A. Khader**: Investigation. **Shaima Akhlaq**: Investigation. **Tahir A. Rizvi**: Funding acquisition; Writing-review & editing. **Farah Mustafa**: Conceptualization; Funding acquisition; Investigation; Project administration; Resources; Supervision; Writing-review & editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

All the data included in this manuscript has been provided in the supplementary files.

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