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The potential of circulating microRNAs as novel diagnostic biomarkers of COVID-19: a systematic review and meta-analysis

Melaku Ashagrie Belete[1*], Denekew Tenaw Anley[2], Sintayehu Simie Tsega[3], Natnael Moges[4], Rahel Mulatie Anteneh[2], Melkamu Aderajew Zemene[2], Asaye Alamneh Gebeyehu[2], Anteneh Mengist Dessie[2], Natnael Kebede[5], Ermias Sisay Chanie[4] and Ermiyas Alemayehu[1]

Abstract

Introduction The COVID-19 pandemic has caused an unprecedented health threat globally, necessitating innovative and efficient diagnostic approaches for timely identification of infected individuals. Despite few emerging reports, the clinical utility of circulating microRNAs (miRNAs) in early and accurate diagnosis of COVID-19 is not well-evidenced. Hence, this meta-analysis aimed to explore the diagnostic potential of circulating miRNAs for COVID-19. The protocol for this study was officially recorded on PROSPERO under registration number CRD42023494959.

Methods Electronic databases including Embase, PubMed, Scopus, and other sources were exhaustively searched to recover studies published until 16th January, 2024. Pooled specificity, sensitivity, positive likelihood ratio (PLR), negative likelihood ratio (NLR), diagnostic ratio (DOR), positive predictive value (PPV), negative predictive value (NPV), and area under the curve (AUC) were computed from the metadata using Stata 14.0 software. Risk of bias appraisal of included articles was carried out using Review Manager (Rev-Man) 5.3 package through the modified QUADAS-2 tool. Subgroup, heterogeneity, meta-regression and sensitivity analyses were undertaken. Publication bias and clinical applicability were also evaluated via Deeks' funnel plot and Fagan nomogram (scattergram), respectively.

Result A total of 43 studies from 13 eligible articles, involving 5175 participants (3281 COVID-19 patients and 1894 healthy controls), were analyzed. Our results depicted that miRNAs exhibit enhanced pooled specificity 0.91 (95% CI: 0.88–0.94), sensitivity 0.94 (95% CI: 0.91–0.96), DOR of 159 (95% CI: 87–288), and AUC values of 0.97 (95% CI: 0.95–0.98) with high pooled PPV 96% (95% CI: 94–97%) and NPV 88% (95% CI: 86–90%) values. Additionally, highest diagnostic capacity was observed in studies involving larger sample size (greater than 100) and those involving the African population, demonstrating consistent diagnostic effectiveness across various specimen types. Notably, a total of 12 distinct miRNAs were identified as suitable for both exclusion and confirmation of COVID-19 cases, denoting their potential clinical applicability.

Conclusion Our study depicted that miRNAs show significantly high diagnostic accuracy in differentiating COVID-19 patients from healthy counterparts, suggesting their possible use as viable biomarkers. Nonetheless, thorough

*Correspondence:
Melaku Ashagrie Belete
melakuashagrie@gmail.com

Full list of author information is available at the end of the article

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and wide-ranging longitudinal researches are necessary to confirm the clinical applicability of miRNAs in diagnosing COVID-19.

Keywords Circulating miRNAs, Diagnostic biomarker, COVID-19, Systematic review, Meta-analysis

Introduction

The virus known as Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) causes Coronavirus Disease 2019 (COVID-19) and leads to a variety of clinical presentations, ranging from mild respiratory symptoms to pneumonia, and in severe cases, multiple organ failure [1]. The involvement of multiple body systems in COVID-19 may be attributed to an imbalanced immune response, contributing to the disease's progression [2]. As of February 2024, COVID-19 has affected over 774,469,939 individuals globally and resulted in over 7,026,465 deaths [3]. Treatment options for COVID-19 are not specific, and disease management relies on empirical methods [4].

Diagnosing COVID-19 involves detecting SARS-CoV-2 ribonucleic acids (RNA) from upper respiratory tract specimens using polymerase chain reaction (PCR) [5, 6]. However, PCR has faced criticism due to its invasiveness and increased risk of cross-infection [7]. Moreover, it requires high purity samples, expensive equipment, specialist training, and long reaction times [8]. RT-PCR, though pivotal in detecting active COVID-19 infections [9], has several limitations, including variable sensitivity [10], resource-intensive processes, and delays in results [9]. Its invasive nature also makes it unsuitable for screening asymptomatic individuals [11]. Additionally, RT-PCR is prone to diagnostic challenges, such as difficulty distinguishing new infections from reinfections, issues with detecting viral mutations, and cases of false positives [12–14] and false negatives [15, 16], raising questions about its status as the "gold-standard" diagnostic method [17, 18]. Other molecular methods like clustered regularly interspaced short palindromic repeats (CRISPR) and gene sequencing share similar drawbacks [19]. Chest computed tomography (CT) is also commonly used for diagnosis, but it cannot identify specific viruses and is unavailable in many settings [20, 21]. Additionally, serological diagnostic tests identify specific antibodies against SARS-CoV-2 (IgG or IgM) but have low sensitivity early in the disease, high false-negative rates, and limited validation [22]. Consequently, finding effective diagnostic biomarkers and severity predictors is crucial for accurate and targeted therapy [23].

Numerous biomarkers, aside from cytokines, exhibit alterations in COVID-19 and are linked to diagnosis, disease outcomes, and prognosis [24]. Certain biomarkers offer straightforward predictions of disease severity, intensive care unit (ICU) admission, hospitalization, and mortality. However, other modalities, such as proteomic and metabolomic analyses, remain primarily investigational and pose challenges in translation to clinical application despite their prognostic capacity [25, 26]. Due to the scarcity of accessible data on biomarkers, there is still an imperative to discover novel non-invasive biomarkers capable of diagnosing COVID-19 and distinguishing between various disease stages. Achieving this necessitates a deeper comprehension of the interplay among the virus, host cells, viral pathogenesis, and cellular injury [27].

MicroRNAs (miRNAs), small non-coding RNAs comprising 17 to 22 nucleotides, regulate post-transcriptional gene expression by inhibiting translation [28]. These molecules play significant roles in various biological processes, including inflammation, apoptosis, cell proliferation, and the immune response to viral infections [29, 30]. Since their discovery, miRNAs have been proposed as biomarkers for disease severity, treatment response, and predictors of disease outcomes [31]. Their potential as disease indicators, especially for identifying viral infections, is attributed to their high sensitivity and specificity [32]. In COVID-19, dysregulated miRNAs have been linked to virus replication, cell proliferation, immune response, and inflammation [33, 34]. In the landscape of COVID-19 biomarkers, circulating miRNAs present distinct advantages and complementarity compared to other biomarkers. While several biomarkers, including viral RNA, antibodies, cytokines, and traditional clinical parameters like C-reactive protein (CRP) and D-dimer, have been extensively studied for COVID-19 diagnosis and prognosis, miRNAs offer unique characteristics that enhance their utility in clinical practice [35]. These unique advantages include early indication of diseases, stability, non-invasive sampling, and potential use in personalized medicine [36–38]. Moreover, miRNAs are best candidates for diagnostic applications mainly attributable to their regulatory roles in gene expression, post-transcriptional gene silencing, modulation of diverse biological processes, resistance to enzymatic degradation, and their stability in diverse body fluids such as serum, plasma, urine, and saliva [39–41]. Studies have also compared miRNA expression between COVID-19 patients and healthy individuals to identify potential diagnostic biomarkers [42–45]. Furthermore, analyzing miRNA expression in COVID-19 patients with varying severities aids in diagnosis and prediction [46]. However, the expression patterns of miRNAs in individuals with COVID-19 have yielded inconsistent findings. Given this variability in insights, conducting an all-inclusive analysis becomes essential to judge the suitability of such

biomarkers as diagnostic aids for COVID-19. Hence, this systematic review and meta-analysis were undertaken to evaluate the diagnostic capacity of miRNAs in COVID-19 diagnosis.

Methods

Study design and protocol

This study is carried out and reported in compliance with the recommendations delineated in the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) standard [47] as shown in Supplementary file 1. Furthermore, the protocol of this study was formally recorded in the International Prospective Register of Systematic Reviews (PROSPERO) under a documentation ID: CRD42023494959.

Article search and study selection

As delineated in Supplementary file 2, electronic bibliographic databases, including PubMed, Scopus, and Embase, were systematically searched for relevant articles until January 16, 2024. Additionally, a manual search was conducted on Google, and the bibliography items of identified articles were scrutinized to discover pertinent studies inadvertently overlooked in the initial search. The list of Medical Subject Heading (MeSH) items and key words applied in the overall search included "microRNAs", "miRNAs", "miRNA", "microRNA", "miR", "diagnos*", "COVID-19", "coronavirus", "novel coronavirus 2019", "SARS-CoV-2", "2019-nCoV", and "2019 nCoV". Besides, Boolean operators ("AND" and "OR") were strategically employed in the advanced search.

After the article search, retrieved articles were brought into Endnote 20.0 software (Clarivate analysis, Philadelphia) and underwent screening to identify eligible studies. After duplicate removal, two reviewers (MAB and EA) carried out an initial screening based on titles and abstracts, to identify eligible studies. Full texts of included studies were independently reviewed according to the pre-stated inclusion and exclusion standards. Any inconsistencies were assessed by a third researcher (DTA), and consensus resolved any differences.

Inclusion and exclusion criteria

In this study, we took in observational studies (cohort, cross-sectional, case-control studies) involving human, exploring the value of miRNAs as diagnostic markers for distinguishing COVID-19 patients from healthy individuals. Eligible studies were necessarily stipulated to report essential data, including sample sizes for both groups (COVID-19 patients and healthy controls), specificity, and sensitivity, facilitating calculation of core diagnostic measurements (TP, TN, FP, FN). Exclusions encompassed various article types (reviews, editorials, conference proceedings, case reports, and author replies), non-peer-reviewed articles, and those missing critical inputs for computing TP, TN, FP, and FN.

Metadata extraction

Two reviewers (MAB and EA) meticulously extracted important information from included studies into an Excel worksheet, with discrepancies being unravelled through in-depth dialogues and the intruding of a third researcher (NM). In addition to computed values such as sensitivity, area under the curve (AUC) and specificity, the extracted data encompassed details including author, country, year of publication, type of miRNA or miRNA panel, miRNA regulation mode, reference controls, type of specimen, number of COVID-19 patient and healthy participants, cut-off points, and detection techniques.

Quality appraisal

The Quality Assessment of Diagnostic Accuracy Studies-2 (QUADAS-2) tool, encompassing criteria for selection of patients, efficiency of detection techniques, test flow, and adequacy of reference standard, was deployed to evaluate the risk of bias pertained by the included articles using Review Manager (RevMan) 5.3 software [48]. Accordingly, the appraisal outputs characterized the risk of bias and applicability concerns as unclear, low, or high.

Data analysis and synthesis

Extracted metadata were statistically analyzed using Stata software version 14.0 (Stata Corp LP, TX, USA). In the diagnostic accuracy meta-analysis, we employed specificity, sensitivity, negative likelihood ratios (NLR), positive likelihood ratios (PLR), and diagnostic odds ratios (DOR), along with their accompanying 95% confidence intervals to assess the diagnostic efficacy of miRNAs. Furthermore, pooled positive predictive value (PPV) and negative predictive value (NPV) were computed to summarize the diagnostic accuracy of miRNAs across studies, enhancing the generalizability, precision, and clinical relevance of the findings. Quantitative evaluation of diagnostic accuracy involved determining the area under the curve (AUC) computed from summary receiver operating characteristic curve (SROC). Additionally, the Hierarchical Summary Receiver Operating Characteristic (HSROC) model was used to account for study variability and differing thresholds, offering a summary of test performance. The model produces a summary curve combining sensitivity and specificity for an overall assessment of diagnostic accuracy, and interpreted using key parameters including the β estimate, indicating trends in accuracy (a negative value suggests reduced performance), and the lambda estimate, which reflects variability in diagnostic odds ratios across studies [49]. Heterogeneity tests utilized the Q test and I^2 statistics through application of a random-effects model, with an I^2 value above

50% and a p-value less than 0.05 indicating statistically substantial heterogeneity among the eligible studies. Evaluation of heterogeneity resulted from the threshold effect employed the ROC plane and Spearman correlation coefficient, with bivariate boxplots and Galbraith Star charts estimating the level of heterogeneity. Additionally, subgroup analysis, meta-regression, and sensitivity analysis were performed to evaluate and understand sources of heterogeneity and ensure result stability. Potential publication bias was appraised through the use of Deeks' funnel plot, in which a p-value > 0.1 suggests no publication bias. Likelihood ratio scattergram and Fagan's nomogram were utilized to judge the clinical value of miRNAs as a diagnostic biomarker of COVID-19.

Results

Search results and selection of studies

The initial literature search process yielded a total of 848 records (PubMed = 301, Embase = 187, Scopus = 344, and other sources = 16), of which 294 duplicates were removed. Subsequently, the remaining 554 articles underwent evaluations of their title and abstract, and 503 were excluded. Finally, full text of 51 articles were assessed based on the eligibility criteria, and 13 studies [50–62] were identified and included in the quantitative analysis (Fig. 1).

Study characteristics

A total of 43 individual studies from the eligible 13 articles, involving 5175 participants (3281 COVID-19

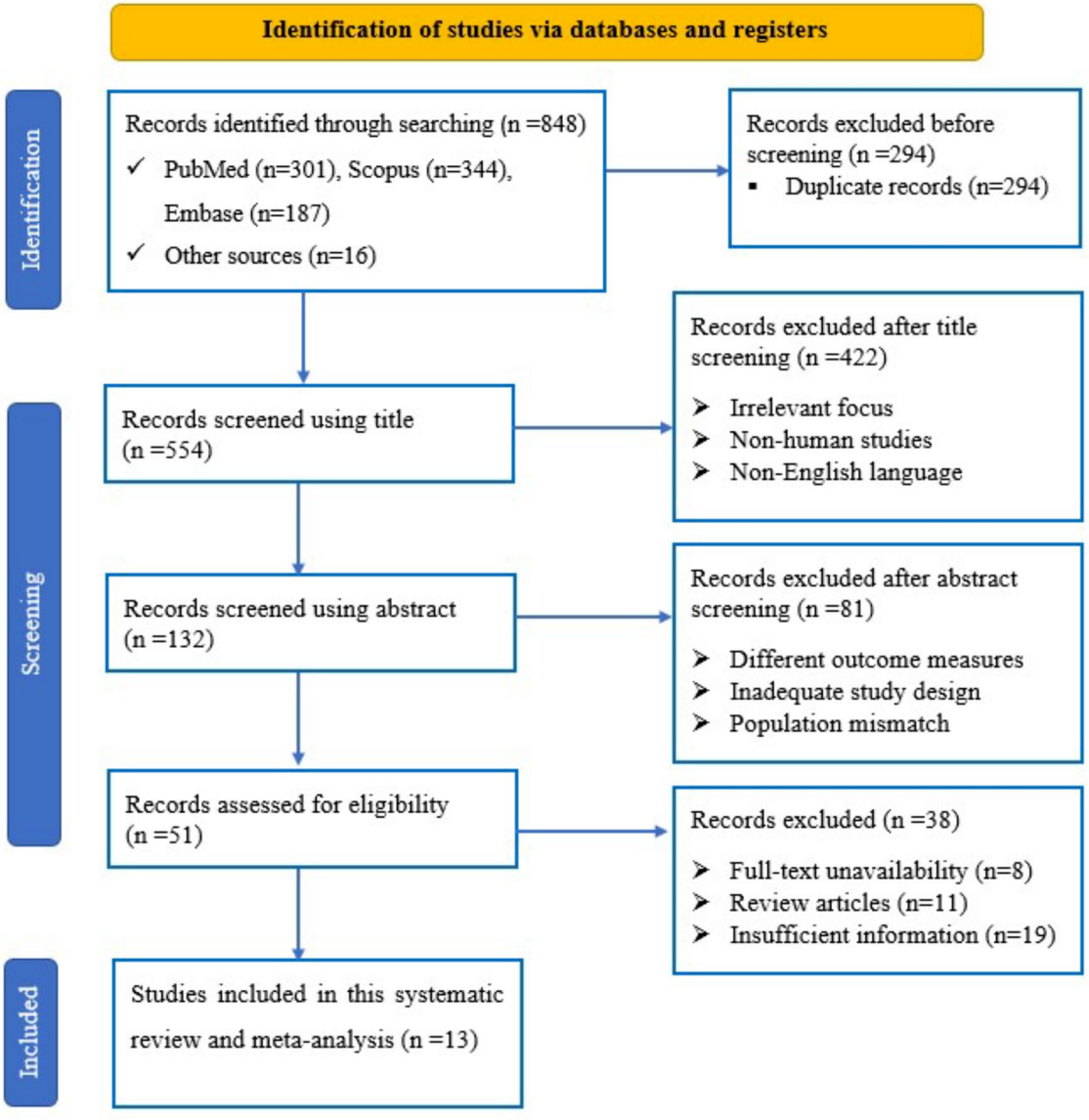

Fig. 1 PRISMA flow chart of article selection process for the systematic review and meta-analysis

patients and 1894 healthy controls), were thoroughly analyzed. All the included studies were published between 2021 and 2023. The location of studies spans three continents, with the majority of the studies conducted in Africa (6 studies from Egypt), followed by Asia (4 studies from Lebanon, Iran, China and Iraq), and Europe (3 studies from Italy, Spain and Romania). Quantitative real-time PCR (qRT-PCR) was the commonly employed technique for assessing miRNA expression. Different specimen types were utilized, encompassing serum samples in seven studies, plasma samples in five studies, and peripheral blood mononuclear cells (PBMCs) in one study. Only 7 studies deployed U6 as internal reference, while 2 studies used cel-miR-39-3p. From all eligible studies, a total of 41 distinct miRNAs were analyzed, demonstrating that the majority of these miRNAs exhibited upregulation in COVID-19 patients. In-depth features of the eligible studies are tabulated in Table 1.

Quality appraisal of eligible studies

Based on the QUADAS-2 tool quality assessment findings, overall validated and enhanced methodological standards were pertained by the eligible studies, demonstrating low risk of bias and applicability concerns in the majority of the quality appraisal criteria (Fig. 2).

Diagnostic accuracy of miRNAs as biomarkers of COVID-19 diagnosis

According to our meta-analysis utilizing the random effect model, the pooled sensitivity, specificity, PLR, NLR, and DOR of miRNAs in distinguishing between COVID-19 patients and healthy controls were found to be 0.94 (95% CI: 0.91–0.96), 0.91 (95% CI: 0.88–0.94), 10.96 (95% CI: 8.02–14.98), 0.07 (95% CI: 0.05–0.10), and 159 (95% CI: 87–288), respectively (Fig. 3, Supplementary file 3 A). The pooled PPV of the miRNA based diagnostic test across the included studies was 96% (95% CI: 94–97%) whereas the pooled NPV was found to be 88% (95% CI: 86–90%). This indicates that out of all patients who tested positive, 96% were correctly diagnosed with the condition, while only 4% were false positives (Supplementary file 3B) Additionally, SROC curve analysis depicted an impressive AUC value of 0.97 (95% CI: 0.95–0.98), signifying a high level of diagnostic accuracy of miRNAs as potential diagnostic biomarkers for COVID-19 (Fig. 4A). These SROC curve analysis findings were further confirmed through the application of the Hierarchical SROC (HSROC) tool, depicting a β estimate of -0.33 (95% CI: -0.81−0.14, $P=0.176$) and lambda estimate of 3.19 (95% CI: 2.72–3.67) (Fig. 4B).

Heterogeneity and threshold effect

The significant contribution of both threshold and non-threshold effects to the observed heterogeneity in diagnostic tests is noteworthy. Our study revealed substantial heterogeneity in the combined sensitivity, specificity, PLR, NLR and DOR with values ($I^2=81.81\%$, $P<0.001$), ($I^2=71.22\%$, $P<0.001$), ($I^2=70.58\%$, $P<0.001$), ($I^2=78.36\%$, $P<0.001$), and ($I^2=100.00\%$, $P<0.001$), respectively. An I^2 value of >50% indicated non-threshold effect heterogeneity among the eligible studies. In both the Galbraith star chart and the bivariate box plot, 13 out of 43 studies and 12 out of 43 studies, respectively, situated out of the lower and upper limits of the 95% CI (see Supplementary file 4 A and 4B).

Furthermore, an evaluation was carried out to assess the impact of the threshold effect on observed heterogeneity, employing the ROC plane along with Spearman correlation coefficient. The existence of a robust negative correlation ($p<0.05$) and a distinct shoulder-arm appearance on the ROC plane serves as an indication of the threshold effect. In our study, the Spearman correlation coefficient was 0.361 with a p-value of 0.067 ($p>0.05$), and an atypical shoulder-arm structure was noticed in the ROC plane, signifying an absence of heterogeneity attributed to the threshold effect (Supplementary file 4 C).

Subgroup analyses and meta-regression

In the subgroup analysis, the pooled diagnostic accuracy of miRNA was slightly higher in studies involving the African population (AUC 0.99, 95% CI: 0.97−0.99) in contrast to those conducted in European (AUC 0.95, 95% CI: 0.93−0.97) and Asian populations (AUC 0.94, 95% CI: 0.91−0.95). Furthermore, when examining various specimen types, it was found that miRNAs showed comparable diagnostic accuracy in diagnosing COVID-19, with an AUC of 0.98 (95% CI: 0.96−0.99) in studies utilizing PBMCs samples and an AUC of 0.97 (95% CI: 0.95−0.98) in studies using serum and plasma samples. Similarly, in the subgroup analysis based on regulation mode, analogous diagnostic accuracy with AUC of 0.97 (95% CI: 0.95−0.98) was observed for both upregulated and downregulated miRNAs.

In terms of internal reference control, it was noted that studies utilizing U6 as the internal reference control showcased enhanced diagnostic ability for miRNAs in differentiating COVID-19 patients from healthy counterparts (AUC 0.99, 95% CI: 0.97−0.99), in comparison to those using other reference controls. Furthermore, miRNAs showcased peak diagnostic accuracy when the sample size of the included studies was above 100, with an AUC of 0.98 (95% CI: 0.96−0.99) contrasting with studies involving participant numbers ≤ 100 (AUC 0.92, 95% CI: 0.89−0.94) (Table 2).

As illustrated in Fig. 5, the findings of meta-regression analysis showed that covariates including specimen type, country, expression mode, sample size, and cut-off point

Table 1 Overall characteristics of studies included in the meta-analysis

Authors	Year	Country	Specimen	Method	Reference	Participants		miRNA	Expression	Cut-off	Sen (%)	Spec (%)	AUC
						CP	HC						
Kazan et al.	2021	Lebanon	Plasma	qRT-PCR	MiR-502-5p	33	10	miR-19a-3p	Up	0.834	88.0	85.0	0.815
Kazan et al.	2021	Lebanon	Plasma	qRT-PCR	MiR-502-5p	33	10	miR-19b-3p	Up	0.237	89.0	86.0	0.875
Kazan et al.	2021	Lebanon	Plasma	qRT-PCR	MiR-502-5p	33	10	miR-92a-3p	Up	0.47	90.0	87.0	0.850
Kazan et al.	2021	Lebanon	Plasma	qRT-PCR	MiR-502-5p	33	10	miR-19a-3p, miR-19b-3p, miR-92a-3p	Up	0.52	92.0	89.0	0.917
Agwa et al.	2021	Egypt	Serum	qRT-PCR	U6	100	100	IL11RA mRNA	Up	1.15	100.0	83.0	0.985
Agwa et al.	2021	Egypt	Serum	qRT-PCR	U6	100	100	HSA-MIR-4257	Down	2.07	88.0	81.0	0.911
Donyavi et al.	2021	Iran	PBMCs	qRT-PCR	SNORD47 RNA	18	15	miR-155-5p	Up	NR	83.3	100.0	0.900
Donyavi et al.	2021	Iran	PBMCs	qRT-PCR	SNORD47 RNA	18	15	miR-let-7b-3p	Up	NR	83.3	93.3	0.930
Donyavi et al.	2021	Iran	PBMCs	qRT-PCR	SNORD47 RNA	18	15	miR-29a-3p	Up	NR	83.3	100.0	1.00
Donyavi et al.	2021	Iran	PBMCs	qRT-PCR	SNORD47 RNA	18	15	miR-146a-3p	Up	NR	100.0	93.3	0.980
Li et al.	2022	China	Serum	qRT-PCR	U6	16	16	miR-125b-5p	Up	NR	100.0	93.7	1.00
Li et al.	2022	China	Serum	qRT-PCR	U6	16	16	miR-155-5p	Up	NR	100.0	93.7	1.00
Giannella et al.	2022	Italy	Serum	NGS	GRCh38	89	45	miR-320b	Up	509.3	94.4	95.6	0.970
Giannella et al.	2022	Italy	Serum	NGS	GRCh38	89	45	miR-320c	Up	1154	89.9	95.6	0.970
Giannella et al.	2022	Italy	Serum	NGS	GRCh38	89	45	miR-320d	Up	398.5	91.1	92.1	0.970
Giannella et al.	2022	Italy	Serum	NGS	GRCh38	89	45	miR-483-5p	Up	1317	91.0	91.1	0.940
Giannella et al.	2022	Italy	Serum	NGS	GRCh38	89	45	miR-320a-3p	Up	1164	80.9	88.9	0.910
Giannella et al.	2022	Italy	Serum	NGS	GRCh38	89	45	miR-30d-5p	Down	6822	94.4	91.1	0.980
Giannella et al.	2022	Italy	Serum	NGS	GRCh38	89	45	miR-25-3p	Down	5160	96.6	91.1	0.980
Giannella et al.	2022	Italy	Serum	NGS	GRCh38	89	45	miR-93-5p	Down	5523	94.4	93.3	0.970
Giannella et al.	2022	Italy	Serum	NGS	GRCh38	89	45	miR-16-5p	Down	1405	92.1	95.6	0.970
Giannella et al.	2022	Italy	Serum	NGS	GRCh38	89	45	miR-101-3p	Down	3572	88.8	86.7	0.960
Giannella et al.	2022	Italy	Serum	NGS	GRCh38	89	45	miR-185-5p	Down	1094	86.8	88.9	0.940
Giannella et al.	2022	Italy	Serum	NGS	GRCh38	89	45	miR-425-5p	Down	2609	83.2	95.6	0.920
Haroun et al.	2022	Egypt	Plasma	qRT-PCR	U6	150	50	miR-155	Up	1.81	90.0	100.0	0.986
Dominguez et al.	2022	Spain	Serum	qRT-PCR	cel-miR-39-3p	17	16	hsa-miR-98-3p	Up	NR	72.7	63.6	0.826
Dominguez et al.	2022	Spain	Serum	qRT-PCR	cel-miR-39-3p	17	16	hsa-miR-423-3p	Up	NR	75.0	73.3	0.787
Dominguez et al.	2022	Spain	Serum	qRT-PCR	cel-miR-39-3p	17	16	hsa-miR-1246	Up	NR	82.4	87.5	0.875
Dominguez et al.	2022	Spain	Serum	qRT-PCR	cel-miR-39-3p	17	16	hsa-miR-98-3p, hsa-miR-423-3p, hsa-miR-1246	Up	NR	63.6	69.1	0.663
Shaker et al.	2023	Egypt	Serum	qRT-PCR	SNORD 68	98	30	miR-200c-3p	Up	5.59	94.9	99.0	NR
Ayeldeen et al.	2023	Egypt	Serum	qRT-PCR	U6	200	80	miRNA-200	Up	6.97	100	89.2	NR
Moatar et al.	2023	Romania	Plasma	qRT-PCR	cel-miR-39-3p	89	89	miR-195	Down	NR	85.2	96.5	0.922
Hassan et al.	2023	Egypt	Plasma	qRT-PCR	U6	70	10	miRNA-618	Up	1	75.0	44.0	0.625
Hassan et al.	2023	Egypt	Plasma	qRT-PCR	U6	70	10	miRNA-16–2-3p	Up	3.5	84.0	61.0	0.743
Abed et al.	2023	Iraq	Plasma	qRT-PCR	U6	145	145	miRNA-20a	Down	14.55	100.0	95.0	1.00

Table 1 (continued)

Authors	Year	Country	Specimen	Method	Reference	Participants		miRNA	Expression	Cut-off	Sen (%)	Spec (%)	AUC
						CP	HC						
Abed et al.	2023	Iraq	Plasma	qRT-PCR	U6	145	145	miRNA-320	Up	24.05	100.0	91.0	1.00
Soltane et al.	2023	Egypt	Serum	qRT-PCR	U6	106	57	MiR-21	Up	NR	93.3	85.7	0.836
Soltane et al.	2023	Egypt	Serum	qRT-PCR	U6	106	57	MiR-155	Up	NR	86.6	75.0	0.929
Soltane et al.	2023	Egypt	Serum	qRT-PCR	U6	106	57	MiR-146a	Up	NR	97.1	100.0	0.775
Soltane et al.	2023	Egypt	Serum	qRT-PCR	U6	106	57	MiR-146b	Up	NR	100.0	97.3	0.757
Soltane et al.	2023	Egypt	Serum	qRT-PCR	U6	106	57	MiR-let-7	Up	NR	100.0	96.9	0.972
Soltane et al.	2023	Egypt	Serum	qRT-PCR	U6	106	57	MiR-223	Up	NR	100.0	87.5	0.984
Soltane et al.	2023	Egypt	Serum	qRT-PCR	U6	106	57	MiR-342	Up	NR	91.0	100.0	0.863

Note CP: COVID-19 patients; HC: healthy control; NR: not reported; NGS: next generation sequence; PBMCs: peripheral blood mononuclear cells; qRT-PCR: quantitative real-time polymerase chain reaction; Sen: sensitivity; Spec: specificity; AUC: area under curve

were delineated as significant contributors to the heterogeneity in both specificity and sensitivity.

Applicability of miRNAs in clinical use for diagnosing COVID-19

According to the Fagan's nomogram, our finding indicated that a positive diagnostic test result would elevate the post-test probability of an individual having COVID-19 to approximately 73%, given a pre-test probability of 20%. This means that if a patient is tested positive for COVID-19 using miRNA biomarkers, the likelihood that they truly have the disease increases to 73%. Conversely, a negative test result would lower the post-test probability to around 2%, suggesting that a negative miRNA test effectively rules out the disease with high certainty. This implies that miRNAs exhibit potential as indicator for the diagnosis of COVID-19 (see Fig. 6A).

In Fig. 6B, we present a scattergram to illustrate the diagnostic performance of miRNAs across various studies. The scattergram plots the PLR against the NLR for individual studies. Studies positioned in the left upper quadrant (PLR > 10 and NLR < 0.1) indicate strong diagnostic markers for both confirming and excluding COVID-19. Specifically, five studies from Giannella et al., two from Abed et al., two from Soltane et al., and individual studies from Donyavi et al., Li et al., and Shaker et al. positioned in the left upper quadrant (PLR > 10 and NLR < 0.1), signifying the robustness of the biomarkers in both confirming and ruling out COVID-19. This result is further supported by a summary (overall) effect situated in the left upper quadrant signifying the overall applicability of miRNAs for both exclusion and confirmation of COVID-19. This summary effect consolidates the findings from all included studies, providing a comprehensive overview of the diagnostic efficacy of miRNAs. Notably, our analysis identified a total of 12 distinct miRNAs, including miR-146a-3p, miR-155-5p, miR-320b, miR-30d-5p, miR-25-3p, miR-93-5p, miR-16-5p, miR-200c-3p, miRNA-20a, miRNA-320, MiR-146a, MiR-146b as being particularly effective for both confirming and excluding COVID-19. These miRNAs exhibit consistent diagnostic performance across various studies and specimen types, denoting their potential clinical applicability in diagnosing COVID-19 (Fig. 6B).

Sensitivity analysis

The trustworthiness and robustness of this meta-analysis model was relatively affirmed through bivariate normality and goodness-of-fit analysis, as showcased in Fig. 7a and b. Furthermore, the outlier detection segment of the sensitivity analysis pinpointed three studies as potential sources of heterogeneity (Fig. 7d). However, even after excluding these outlier studies, no substantial alterations were detected in the pooled sensitivity (0.94 vs. 0.93),

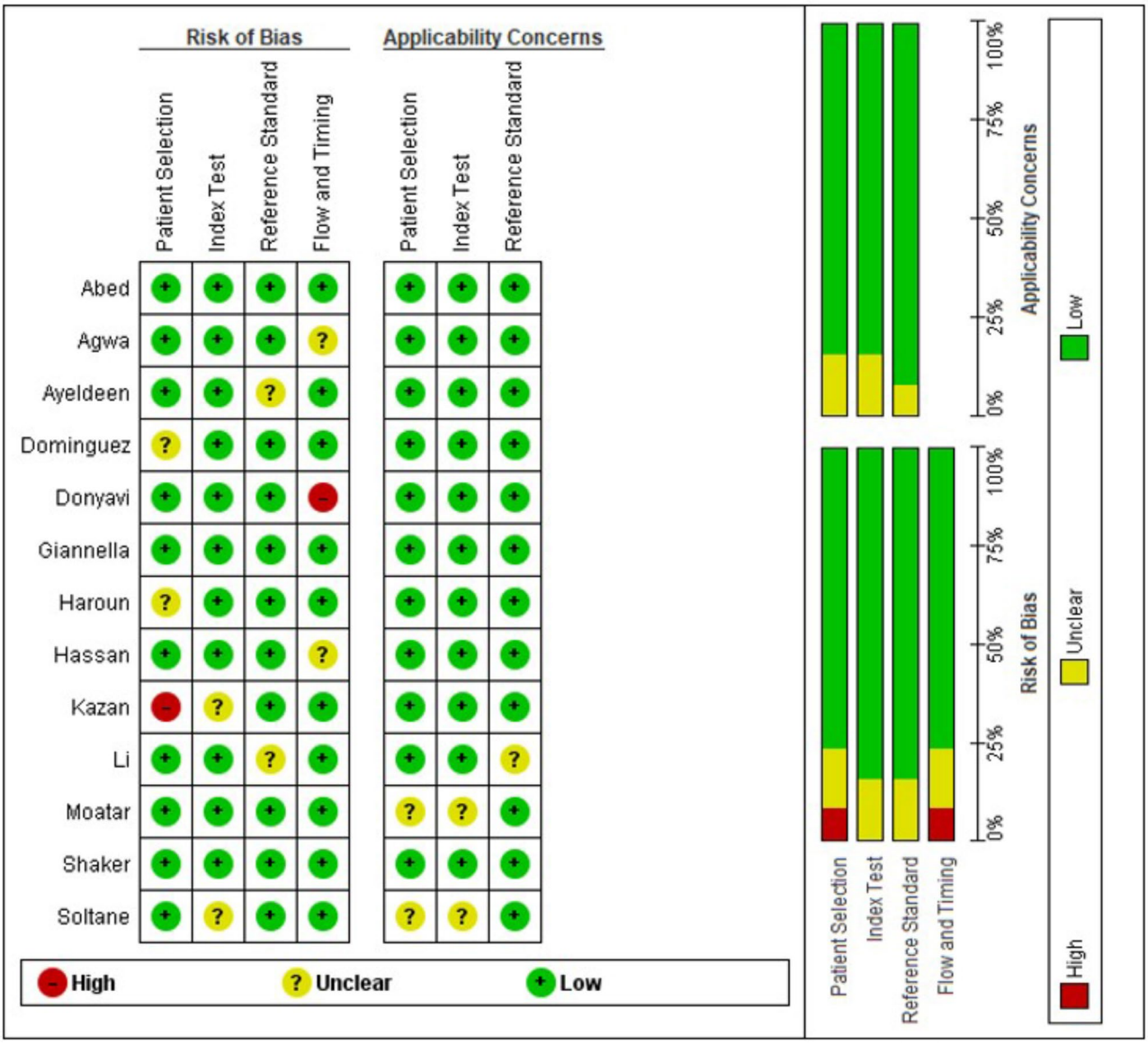

Fig. 2 Quality appraisal (risk of bias and applicability concern assessment) of eligible studies using QUADAS-2

specificity (0.91 vs. 0.92), PLR (10.98 vs. 11.4), NLR (0.07 vs. 0.08), and AUC (0.97 vs. 0.97), indicating an overall low sensitivity among the included studies.

Publication bias

The Deek's funnel plot revealed apparently asymmetrical distribution of studies along both sides of the regression line, with a p-value of <0.001 illustrating the presence of significant publication bias in the metadata (Fig. 8).

Discussion

This study, comprising primary data of 41 distinct miRNAs and two miRNA panels across 13 studies, revealed that miRNAs demonstrated strong diagnostic accuracy and proved their possible use as biomarkers in COVID-19 diagnosis, with a higher combined sensitivity 0.94 (95% CI: 0.91–0.96) and specificity 0.91 (95% CI: 0.88–0.94), along with PLR, NLR, DOR, and AUC values of 10.96 (95% CI: 8.02–14.98), 0.07 (95% CI: 0.05–0.10), 159.0 (95% CI: 87–288), and 0.97 (95% CI: 0.95–0.98), respectively. Such illustrated high levels of sensitivity and specificity underscore the accuracy of miRNAs to efficiently distinguishing COVID-19 patients from healthy individuals. This finding is also supported by systematic review reports illustrating the possible use of miRNAs as potential biomarkers of COVID-19 [31] and other infectious diseases including tuberculosis, sepsis, and viral hepatitis [37]. Similar findings depicting the potential of miRNAs as diagnostic biomarkers were also reported beyond COVID-19, including other pathologies such as,

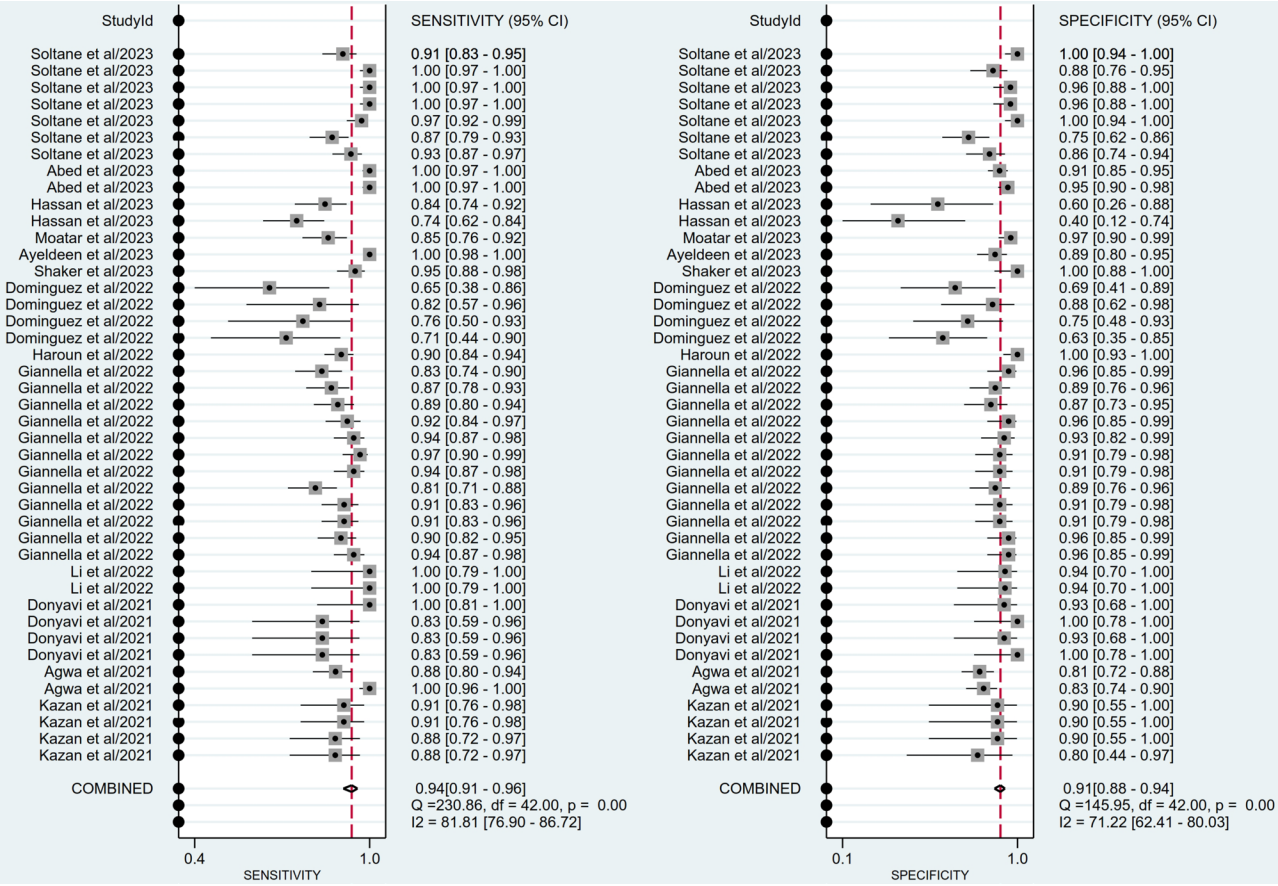

Fig. 3 Forest plot showing overall specificity and sensitivity of miRNAs in the diagnosis of COVID-19

cervical cancer [63], hepatocellular carcinoma [64], and oncogenic viral infections such as Human T-lymphotropic virus, Human papilloma virus, Merkel cell polyoma virus, Human herpes virus-8, and Epstein–Barr virus-associated cancers [65, 66].

The integration of microRNA-based diagnostics with existing tests such as RT-PCR, imaging, and immunological assays used to monitor COVID-19 offers complementary diagnostic information beyond pathogen detection, including early detection of asymptomatic cases, differentiating between new infection and reinfections, enhances multimodal monitoring strategies through holistic surveillance of viral dynamics, host immune responses and treatment responses, and enables point-of-care applications [67]. By leveraging the synergies between microRNA profiling and conventional diagnostics, healthcare providers can enhance the accuracy, timeliness, and comprehensiveness of COVID-19 surveillance and management through facilitating early detection of disease recurrence, emergence of vaccine escape variants, and complications associated with long COVID, ultimately improving patient outcomes [68].

Despite such tremendous significance and compelling features to be used as efficient biomarkers, the implication of miRNAs is not adequately explored for COVID-19 diagnosis [31, 69]. Apart from few systematic review reports [31, 67, 68, 70–72], till date, there are no all-inclusive quantitative studies providing thorough statistical summary of the diagnostic potential of miRNAs in COVID-19. Hence, this study is the first meta-analysis to assess the diagnostic potential of miRNAs in diagnosing COVID-19.

The high PLR and low NLR further affirm the diagnostic prowess of miRNAs [73, 74]. Notably, the PLR value of 10.96 in our study shows approximately 11 times higher probability of detecting positive miRNA results in COVID-19 patients in contract with healthy controls. On the other hand, the NLR value of 0.07 infers there is only a 7% probability of developing COVID-19 among those tested negative. Additionally, a DOR>1 and AUC approaching 1 are indicative of best distinguishing capability and overall efficacy of a test [75–77], and evidently, we obtained a DOR of 159 and AUC value of 0.97 which affirm the extraordinary ability of miRNA markers to effectively differentiate COVID-19 patients from healthy individuals. These findings collectively prove the potential of miRNAs as reliable and accurate diagnostic markers for COVID-19, offering a promising avenue for the

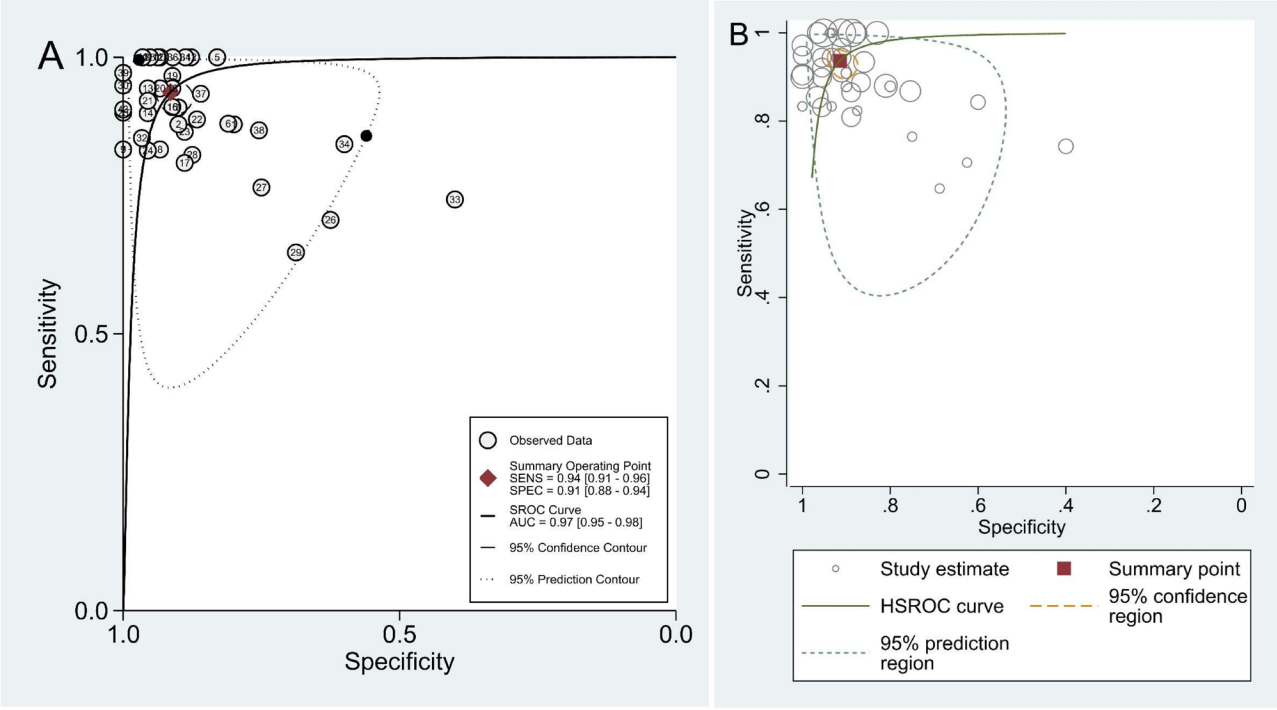

Fig. 4 SROC curve analysis of miRNAs for diagnosing COVID-19; (**A**) the SROC curve with the 95% confidence contour and 95% prediction contour and (**B**) the HSROC model with the 95% confidence region and 95% confidence prediction region

Table 2 Subgroup analysis of the diagnostic accuracy of miRNA in COVID-19

Subgroup	No of studies	Sen (95% CI)	Spe (95% CI)	PLR (95% CI)	NLR (95% CI)	DOR (95% CI)	AUC (95% CI)
Country							
Europe	17	0.88 (0.84 – 0.91)	0.91 (0.87 – 0.93)	9.4 (6.5 – 13.7)	0.13 (0.10 – 0.18)	72 (39 – 136)	0.95 (0.93 – 0.97)
Africa	14	0.97 (0.92 – 0.99)	0.93 (0.83 – 0.97)	13.6 (5.4 – 34.5)	0.03 (0.01 – 0.09)	457 (87 – 2398)	0.99 (0.97 – 0.99)
Asia	12	0.96 (0.89 – 0.99)	0.93 (0.89 – 0.96)	14.2 (8.6 – 23.5)	0.04 (0.01 – 0.12)	359 (101 – 1279)	0.94 (0.91 – 0.95)
Specimen							
Serum	29	0.94 (0.91 – 0.96)	0.91 (0.88 – 0.94)	10.6 (7.6 – 14.7)	0.06 (0.04 – 0.10)	166 (82 – 335)	0.97 (0.95 – 0.98)
Plasma	10	0.93 (0.84 – 0.97)	0.90 (0.78 – 0.96)	9.2 (4.0 – 21.0)	0.08 (0.03 – 0.18)	119 (28 – 504)	0.97 (0.95 – 0.98)
PMBCs	4	0.88 (0.77 – 0.94)	0.97 (0.87 – 0.99)	27.1 (6.1 – 120.6)	0.13 (0.07 – 0.25)	213 (38 – 1197)	0.98 (0.96 – 0.99)
Regulation mode							
Upregulated	33	0.94 (0.90 – 0.97)	0.91 (0.87 – 0.94)	10.9 (7.2 – 16.5)	0.06 (0.04 – 0.11)	170 (77 – 372)	0.97 (0.95 – 0.98)
Downregulated	10	0.93 (0.88 – 0.96)	0.92 (0.88 – 0.95)	11.7 (7.8 – 17.6)	0.08 (0.05 – 0.14)	145 (67 – 314)	0.97 (0.95 – 0.98)
Reference							
U6	17	0.99 (0.95 – 1.00)	0.92 (0.85 – 0.96)	11.9 (6.4 – 22.2)	0.01 (0.00 – 0.06)	1041 (156 – 6967)	0.99 (0.97 – 0.99)
GRCh38	12	0.91 (0.88 – 0.93)	0.92 (0.89 – 0.94)	11.4 (8.5 – 15.4)	0.10 (0.08 – 0.13)	114 (72 – 181)	0.95 (0.93 – 0.96)
cel-miR-39-3p	5	0.77 (0.66 – 0.85)	0.83 (0.65 – 0.93)	4.6 (1.9 – 11.0)	0.28 (0.17 – 0.46)	16 (4 – 62)	0.84 (0.80 – 0.87)
SNORD47 RNA	4	0.88 (0.77 – 0.94)	0.97 (0.87 – 0.99)	27.1 (6.1 – 120.6)	0.13 (0.07 – 0.25)	213 (38 – 1197)	0.98 (0.96 – 0.99)
MiR-502-5p	4	0.89 (0.83 – 0.94)	0.87 (0.73 – 0.95)	7.2 (3.1 – 16.3)	0.12 (0.07 – 0.20)	59 (20 – 175)	0.95 (0.92 – 0.96)
Sample size							
> 100	27	0.96 (0.93 – 0.98)	0.93 (0.90 – 0.95)	13.8 (10.0 – 19.2)	0.04 (0.03 – 0.08)	310 (156 – 617)	0.98 (0.96 – 0.99)
≤ 100	16	0.87 (0.81 – 0.90)	0.87 (0.77 – 0.92)	6.4 (3.6 – 11.5)	0.15 (0.11 – 0.23)	41 (17 – 100)	0.92 (0.89 – 0.94)
Cut-off value							
Given	25	0.93 (0.90 – 0.96)	0.91 (0.87 – 0.93)	10.0 (7.2 – 13.8)	0.07 (0.05 – 0.11)	139 (74 – 260)	0.97 (0.95 – 0.98)
Not given	18	0.94 (0.88 – 0.97)	0.93 (0.87 – 0.96)	13.4 (7.1 – 25.2)	0.06 (0.03 – 0.14)	213 (63 – 716)	0.98 (0.96 – 0.99)

Note AUC: area under curve; DOR: diagnostic odds ratio; NLR: negative likelihood ratio; PLR: positive likelihood ratio; Sen: sensitivity; Spec: specificity

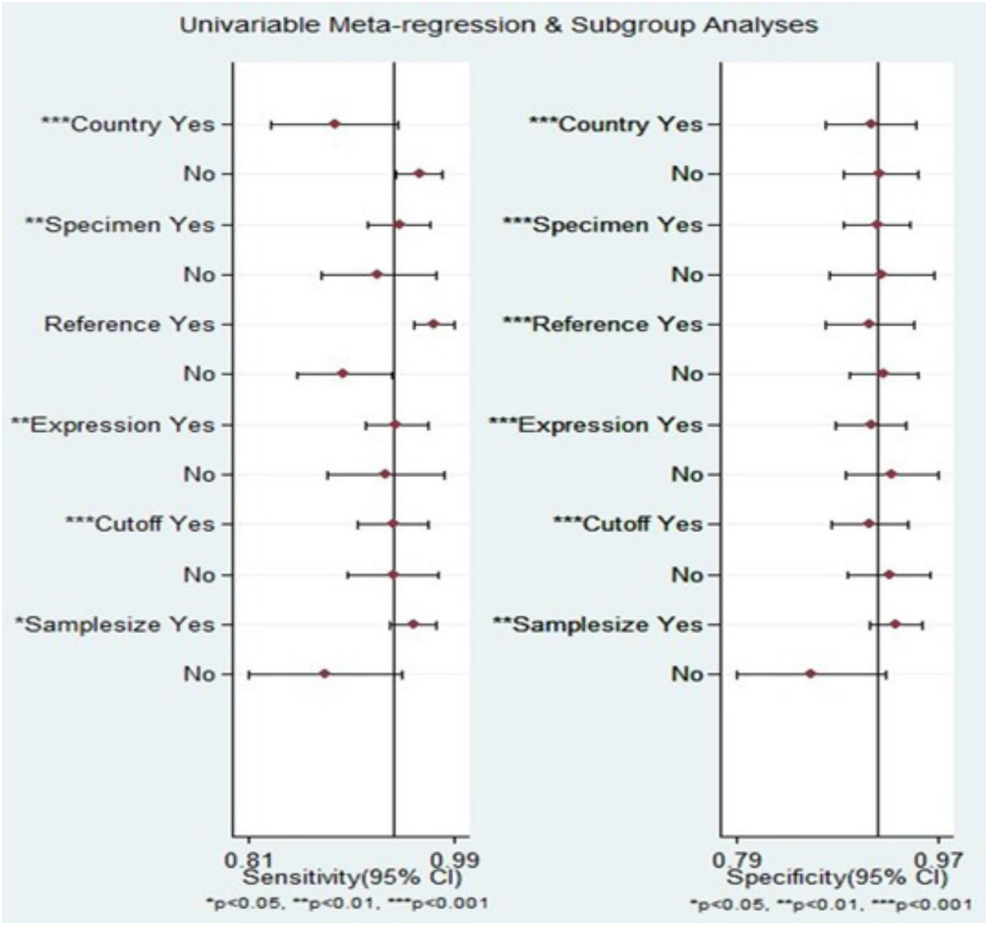

Fig. 5 Meta-regression analysis to assess sources of heterogeneity in sensitivity and specificity of miRNAs

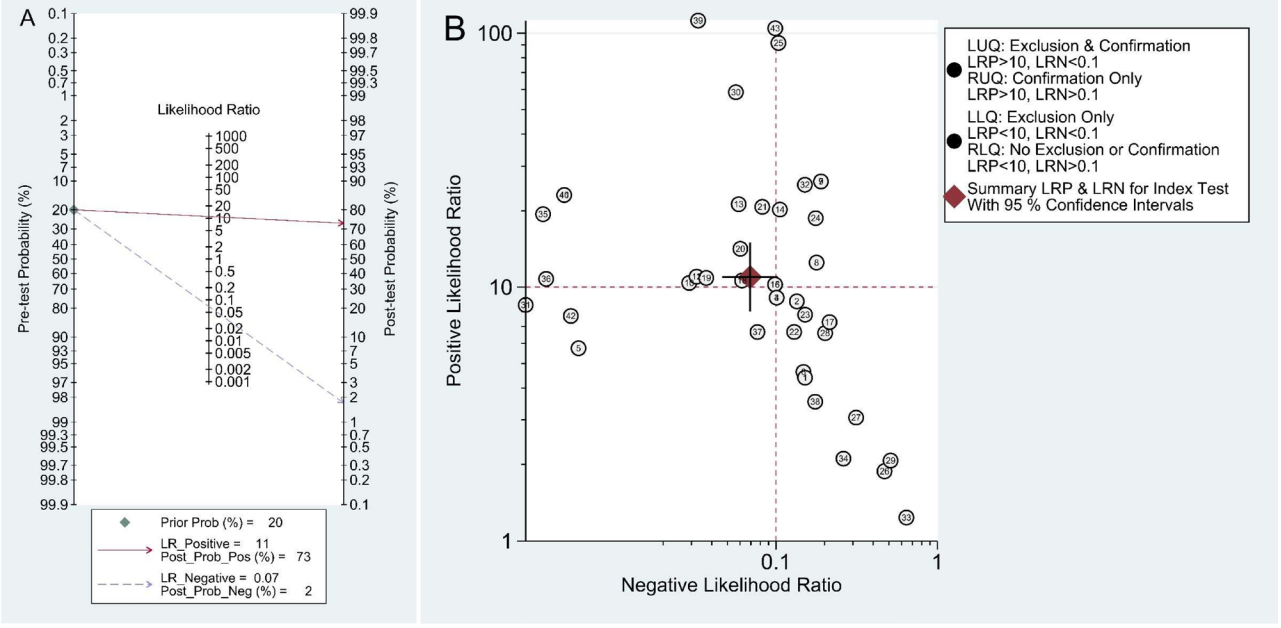

Fig. 6 Assessment of the clinical applicability of miRNAs in diagnosing COVID-19, including (**A**) Fagan's nomogram revealing post-test probabilities under a pre-test probability set at 20% and (**B**) Likelihood ratio scattergram depicting the likelihood ratios as indicators of clinical applicability

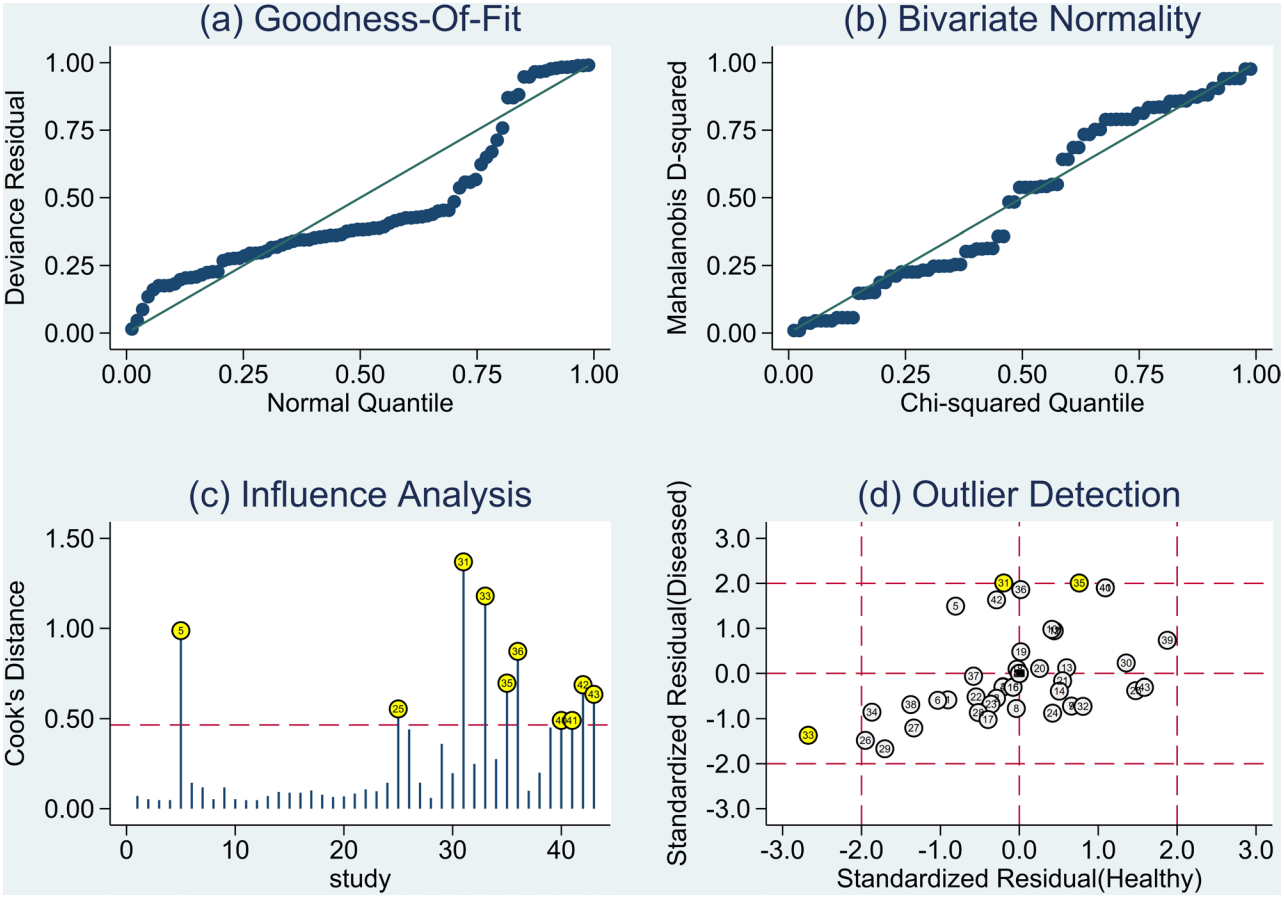

Fig. 7 Sensitivity analysis of included studies evaluating the reliability and validity of the meta-analysis results and outlier detection

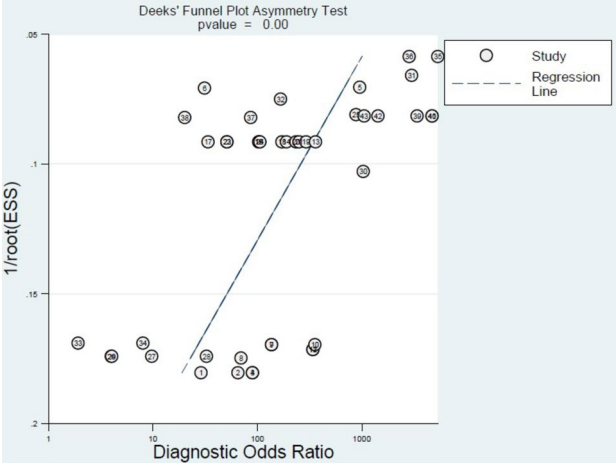

Fig. 8 Deek's funnel plot asymmetry test for evaluating publication bias in the included studies

development of sensitive and specific diagnostic tools in the ongoing battle against the pandemic.

According to our finding, the application of the HSROC model revealed a β estimate of -0.33 (95% CI: -0.81−0.14), indicating a trend in the reduction of diagnostic accuracy. However, the corresponding P value of 0.176 suggests that this reduction is not statistically significant, emphasizing the need for cautious interpretation. The lambda estimate, a crucial parameter in the HSROC model, was determined to be 3.19 (95% CI: 2.72–3.67), reflecting the variability in the diagnostic odds ratio among the studies, with a higher lambda value indicative of increased heterogeneity [78]. The observed lambda estimate of 3.19 underscores a notable degree of variability in the diagnostic performance of the microRNAs across the included studies.

In the subgroup analysis, a slightly elevated diagnostic accuracy was observed in studies involving the African population (AUC 0.99, 95% CI: 0.97−0.99) in contrast with those conducted in European (AUC 0.95, 95% CI 0.93−0.97) and Asian population (AUC 0.94, 95% CI: 0.91−0.95) highlighting the potential influence of population-specific factors on miRNA efficacy as COVID-19 diagnostic biomarkers. These observed differences underscore the need to consider demographic and geographical variations in future investigations and the development of diagnostic strategies, enhancing the accuracy and applicability of miRNA-based diagnostics for diverse populations affected by the pandemic.

Our subgroup analysis results also illustrate no significant difference in diagnostic accuracy when considering different specimen types, including serum, plasma and PMBCs with a consistent AUC of 0.97, 0.97, and 0.98, respectively. This consistent diagnostic performance across diverse specimen types indicates the robustness of miRNA-based diagnostics for COVID-19, offering flexibility in specimen selection for clinical applicability without compromising accuracy. Additionally, this study depicted heightened diagnostic capacity with AUC value of 0.98 (95% CI: 0.96–0.99) in studies comprising > 100 sample size compared to studies with ≤ 100 study participants (AUC: 0.92, 95% CI: 0.89–0.94). Such variation might be attributable to sample size induced bias and statistical power [79].

According to the Fagan's nomogram analysis [80, 81] depicting an overall PLR of 0.73 and NLR of 0.02, the positive test results for miRNAs are indicative of about 73% likelihood of acquiring COVID-19. On the other hand, the likelihood declines to about 2% in cases where samples yielded negative results for miRNAs. Such findings proved the promising diagnostic ability of miRNAs in differentiating individuals with SARS-CoV-2 from healthy counterparts, with strong evidence of possible use for exclusion and confirmation of COVID-19. This diagnostic ability of miRNAs is paramount and hopeful in overcoming the rising concerns of the currently available diagnostic methods for the frequently mutated SARS-CoV-2 pandemic to enable early and accurate detection.

This meta-analysis is the first of its kind to comprehensively investigate the potential of miRNAs in effectively diagnosing COVID-19. Despite it is a strength to analyze a more comprehensive list of miRNAs from included studies pertaining to high methodological quality, this study faces some limitations. Initially, the metadata were quite limited attributable to the diverse cutoff values of the miRNAs, potentially causing significant heterogeneity among the eligible studies. Secondly, the scarcity of similar miRNAs hindered the subgroup analysis, and we were not able to identify a single miRNA or a panel of miRNA biomarker with best diagnostic performance. Thirdly, the study may be influenced by reduced statistical power due to the small number of sample sizes in majority of the eligible articles. Fourthly, the absence of similarity in the use of internal references resulted in varying outcomes in miRNA quantitative analysis, and could impact the reliability and reproducibility of miRNA expression profiles, potentially confounding the interpretation of diagnostic performance metrics. Fifthly, variations in the techniques used for quantifications of miRNAs, such as qRT-PCR and NGS, may lead to potential inconsistencies in the reported miRNA levels, impact the comparability of miRNA measurements, and could contribute to variability in the reported diagnostic accuracy of circulating miRNAs for COVID-19. Sixthly, the HSROC model may be less sensitive to subtle differences in diagnostic accuracy between individual studies, and the model assumes homogeneity in study populations, which may not fully capture the complexity of diverse clinical settings. Finally, the limited number of available studies restricted our ability to gather sufficient data on the role of miRNAs as indicators of disease severity or predictors of COVID-19. Although the abovementioned limitations may have influenced the results, we believe that it will lay a solid groundwork for future research endeavors. As such, it is essential to approach and infer our findings with caution. We strongly urge upcoming scholars to corroborate and substantiate our conclusions by conducting wide-ranging studies pertaining consistent research methodologies and larger sample sizes. Future studies should also consider alternative models or combine HSROC with other methods, such as bivariate models, to provide a more nuanced analysis of diagnostic accuracy.

In conclusion, this study underscores the high diagnostic accuracy of miRNAs in distinguishing COVID-19 patients from healthy counterparts, suggesting their promise as valuable biomarkers for detecting SARS-CoV-2. Remarkably, miRNAs among studies with substantial sample sizes and those focusing on the African population exhibit heightened diagnostic capacity, indicating consistent diagnostic effectiveness across different specimen types. Nonetheless, thorough and wide-ranging researches with superb methodological qualities involving both individual miRNA and miRNA panel assessments need to be conducted to confirm the clinical applicability of miRNAs in diagnosing COVID-19.

Policymakers could consider supporting the integration of miRNA-based diagnostics into public health strategies, particularly for early detection and monitoring of viral outbreaks. In clinical practice, miRNAs could complement existing diagnostic methods like RT-PCR, offering a non-invasive, rapid, and accurate alternative, particularly in resource-limited settings. Future research should aim to standardize miRNA detection techniques and validate these findings across diverse populations and settings. Additionally, the development of point-of-care miRNA diagnostic tools could revolutionize pandemic preparedness and response.

Abbreviations

AUC	area under the curve
DOR	diagnostic odds ratio
ELISA	enzyme linked immunosorbent assay
FN	false negative
FP	false positive
HSROC	Hierarchical Summary Receiver Operating Characteristic
IgG	Immunoglobulin G
IgM	Immunoglobulin M

LFIA	lateral flow immunoassay
miRNAs	microRNAs
NGS	next generation sequencing
NLR	negative likelihood ratio
PBMCs	peripheral blood mononuclear cells
PLR	positive likelihood ratio
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-analysis
PROSPERO	Prospective Register of Systematic Reviews
qRT-PCR	quantitative real-time polymerase chain reaction
QUADAS-2	Quality Assessment of Diagnostic Accuracy Studies-2
RT-PCR	real-time polymerase chain reaction
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
SROCs	summary receiver operating characteristic curves
TN	true negative
TP	true positive

Supplementary Information

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Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Supplementary Material 4

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Not applicable.

Author contributions
MAB conceived and designed the study, carried out article searches, selected studies, extracted metadata, and assessed the quality of the included articles. MAB also performed statistical analyses and wrote the original manuscript. DTA, SST, NM, RMA, MAZ, AAG, AMD, NK, ESC and EA contributed in article searches, study selection and extraction, and provided support in analysis, reviewing, and editing of the manuscript. All authors reviewed and approved the final draft of the manuscript before submission for publication.

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Data availability
Data is provided within the manuscript or supplementary information files.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
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Competing interests
The authors declare no competing interests.

Author details
[1]Department of Medical Laboratory Sciences, College of Medicine and Health Sciences, Wollo University, Dessie, Ethiopia
[2]Department of Public Health, College of Health Science, Debre Tabor University, Debre Tabor, Ethiopia
[3]Department of Medical Nursing, School of Nursing, College of Medicine and Health Science, University of Gondar, Gondar, Ethiopia
[4]Department of Pediatrics and Child Health Nursing, College of Health Sciences, Debre Tabor University, Debre Tabor, Ethiopia
[5]Department of Health Promotion, School of Public Health College of Medicine Health Sciences, Wollo University, Dessie, Ethiopia

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