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INVESTIGATING THE ROLE OF MICRORNA EXPRESSION PROFILES IN EARLY DETECTION AND PROGNOSIS OF VARIOUS HUMAN DISEASES AND DISORDERS: A SYSTEMATIC REVIEW

Systematic Review

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ABSTRACT

Background: The early and accurate detection of diseases remains a critical challenge in medicine. MicroRNA (miRNA) expression profiles in biofluids have emerged as promising biomarkers due to their stability and disease-specific dysregulation, offering potential for transformative diagnostic and prognostic tools.

Objective: This systematic review aims to analyze and synthesize the current evidence on how miRNA expression patterns contribute to the early detection and prognostic stratification of various human diseases.

Methods: A systematic review was conducted following PRISMA guidelines. A comprehensive search of PubMed, Scopus, Web of Science, and the Cochrane Library was performed for studies published from January 2019 to March 2024. Inclusion criteria encompassed clinical studies investigating miRNA profiles for diagnosis or prognosis in human diseases. Data extraction and risk of bias assessment were performed independently by two reviewers using standardized tools.

Results: From 2,347 identified records, 21 studies were included. The evidence demonstrates that specific miRNA signatures, particularly multi-marker panels, exhibit high discriminatory accuracy for early disease detection (with AUC values frequently >0.85) and reliable prognostic prediction across oncology, neurology, and cardiology. Commonly identified miRNAs, such as miR-21-5p and miR-146a-5p, were implicated across pathologies, though significant heterogeneity in methodologies was noted.

Conclusion: miRNA expression profiling holds significant potential to enhance early diagnosis and prognosis. However, methodological standardization and large-scale prospective validation are imperative before clinical translation. Future research must prioritize harmonized protocols to confirm the reliability and utility of these biomarkers in routine healthcare.

Keywords: microRNA; Biomarkers; Early Diagnosis; Prognosis; Systematic Review; Liquid Biopsy.

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INTRODUCTION

The early and accurate detection of human diseases remains a paramount challenge in modern medicine, directly impacting therapeutic efficacy and patient prognosis. Conventional diagnostic modalities, including imaging and protein-based biomarkers, often lack the sensitivity required for identifying pathological processes in their earliest, most treatable stages, or fail to provide reliable prognostic stratification (1). This diagnostic latency contributes significantly to disease burden and healthcare costs, underscoring the urgent need for novel, minimally invasive biomarkers. In recent years, microRNAs (miRNAs) have emerged as pivotal players in this arena. These short, non-coding RNA molecules regulate post-transcriptional gene expression and are now recognized to have distinct, stable expression profiles in bodily fluids like blood and serum, which are often dysregulated in various disease states (2). The clinical significance of miRNA expression profiling is profound, given its potential to revolutionize diagnostic paradigms. For instance, in oncology, certain cancers such as pancreatic ductal adenocarcinoma are frequently diagnosed at advanced stages, resulting in a dismal five-year survival rate below 10% (3). Simultaneously, in complex neurological disorders like Alzheimer's disease, definitive diagnosis often relies on post-mortem examination, highlighting a critical need for ante-mortem biomarkers (4). Existing knowledge confirms that specific miRNA signatures are associated with tumorigenesis, neurodegeneration, cardiovascular dysfunction, and autoimmune responses, suggesting their utility as molecular fingerprints of disease. However, the literature is vast, heterogeneous, and sometimes contradictory, with studies varying in methodology, population, and validation rigor. A synthesis of this evidence is necessary to distinguish robust, clinically actionable miRNA panels from exploratory findings, thereby justifying a systematic review to consolidate and critically appraise the evidence.

This systematic review aims to address the following primary research question, formulated according to the PICO framework: In patients with various human diseases (P), how do specific microRNA expression profiles (I), compared to standard diagnostic or prognostic markers (C), contribute to early detection and predict clinical outcomes (O)? The objective is to systematically analyze and synthesize the current evidence on how miRNA expression patterns can enhance early disease detection and improve diagnostic accuracy and prognostic assessment across a spectrum of disorders. The scope of this review will encompass clinical studies, including case-control, cohort, and diagnostic accuracy studies, published within the last decade to ensure contemporary relevance, with a global geographical perspective to account for population-specific variations. By adhering to the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines, this review will provide a rigorous and transparent synthesis of the evidence (5). The expected contribution is to delineate the most promising miRNA biomarkers validated across independent studies, clarify their clinical utility in specific disease contexts, and identify key gaps that warrant future research. Ultimately, this work aspires to inform the development of standardized, miRNA-based diagnostic panels, paving the way for their integration into routine clinical practice to enable earlier intervention and personalized patient management strategies.

METHODS

This systematic review was conducted in strict accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines to ensure methodological rigor and transparency (5). A comprehensive, multi-database search strategy was executed to capture the breadth of literature on microRNA (miRNA) expression in human diseases. The electronic databases PubMed, Scopus, Web of Science, and the Cochrane Central Register of Controlled Trials were systematically searched for relevant studies published from January 2019 to March 2024, ensuring the inclusion of the most recent evidence. The search algorithm combined Medical Subject Headings (MeSH) terms and free-text keywords using Boolean operators. A core search string, adapted for each database, was structured as: ("microRNA" OR "miRNA" OR "miR-") AND ("expression profile" OR "signature" OR "biomarker") AND ("early detection" OR "diagnosis" OR "prognosis" OR "survival") AND ("human"). To minimize the risk of overlooking pertinent studies, the reference lists of all included articles and relevant review papers were manually screened. The study selection process was governed by pre-defined eligibility criteria. Inclusion was limited to original, full-text research articles published in English that investigated miRNA expression profiles in human participants for the purpose of early disease detection, diagnostic accuracy, or prognostic stratification. Eligible study designs encompassed case-control studies, cohort studies (both prospective and retrospective), and diagnostic accuracy studies. The population of interest included adult patients (18 years and older) across any disease category,

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including but not limited to oncology, cardiology, neurology, and autoimmune disorders. The primary intervention or exposure of interest was the measurement of differential miRNA expression, typically from blood, serum, plasma, or other accessible biofluids.

Comparators included healthy control groups, patients with benign conditions, or standard-of-care diagnostic/prognostic markers.Key outcomes of interest were metrics of diagnostic performance (sensitivity, specificity, area under the curve (AUC)) or prognostic value (hazard ratios for overall survival, disease-free survival). Exclusion criteria were applied to reviews, meta-analyses, conference abstracts, in vitro or animal studies, studies lacking a control group, and investigations focusing solely on miRNA functional mechanisms without clinical correlation. Study selection was performed in a two-stage screening process by two independent reviewers to mitigate selection bias. Initially, all identified records were imported into EndNote reference management software for deduplication, after which titles and abstracts were screened for apparent relevance. The full texts of potentially eligible studies were then retrieved and assessed in detail against the inclusion and exclusion criteria. Any discrepancies between reviewers were resolved through discussion or, if necessary, by consultation with a third senior researcher. This process is summarized in a PRISMA flow diagram, which details the number of records identified, screened, assessed for eligibility, and ultimately included. Data from the final set of included studies were extracted using a standardized, piloted data extraction form. Key variables collected included first author, publication year, country, study design, participant characteristics (disease type, sample size, age), sample type (e.g., serum), miRNA profiling methodology (e.g., qRT-PCR, microarray, sequencing), specific miRNA(s) investigated, comparator group, primary clinical outcomes, and relevant statistical measures (AUC, sensitivity, specificity, p-values, hazard ratios). The methodological quality and risk of bias of the included studies were critically appraised using tools appropriate to their design. For observational studies, which constitute the majority of the literature in this field, the Newcastle-Ottawa Scale (NOS) was employed (6).

This tool assesses studies across three domains: the selection of study groups, the comparability of groups, and the ascertainment of either the exposure or outcome. For any included diagnostic accuracy studies, the Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) tool was utilized to evaluate risk of bias and concerns regarding applicability across four key domains: patient selection, index test, reference standard, and flow and timing (7). Each study was independently evaluated by two reviewers, and a consensus score or rating was assigned. Given the anticipated heterogeneity in the investigated diseases, miRNA targets, and methodological approaches across studies, a formal quantitative synthesis (meta-analysis) was deemed not feasible. Therefore, the findings are synthesized qualitatively using a narrative summary approach. This synthesis will be structured thematically, likely by major disease categories (e.g., cancers, neurological disorders), to descriptively analyze and compare the strength of evidence, consistency of specific miRNA signatures, and the reported diagnostic or prognostic performance across the included research. The following eight studies exemplify the scope of research incorporated into this systematic review, representing investigations into diverse pathologies including pancreatic cancer (8), Alzheimer's disease (9), colorectal cancer (10), myocardial infarction (11), prostate cancer (12), major depressive disorder (13), idiopathic pulmonary fibrosis (14), and rheumatoid arthritis (15).

RESULTS

The systematic search across four electronic databases initially yielded 2,347 records. Following the removal of 518 duplicates, the titles and abstracts of 1,829 unique citations were screened for relevance. This screening phase led to the exclusion of 1,634 records that did not meet the broad inclusion criteria, primarily because they were non-human studies, review articles, or focused purely on mechanistic biology without clinical endpoints. Consequently, 195 full-text articles were retrieved and subjected to a detailed eligibility assessment. Of these, 174 studies were excluded for specific reasons, the most common being the lack of a clear control group (n=52), the investigation of miRNA only in tissue rather than biofluids (n=47), or the absence of quantitative data on diagnostic or prognostic performance (n=41). Ultimately, 21 studies satisfied all inclusion and exclusion criteria and were incorporated into the qualitative synthesis. The complete selection process is detailed in the accompanying PRISMA flow diagram (Figure 1).

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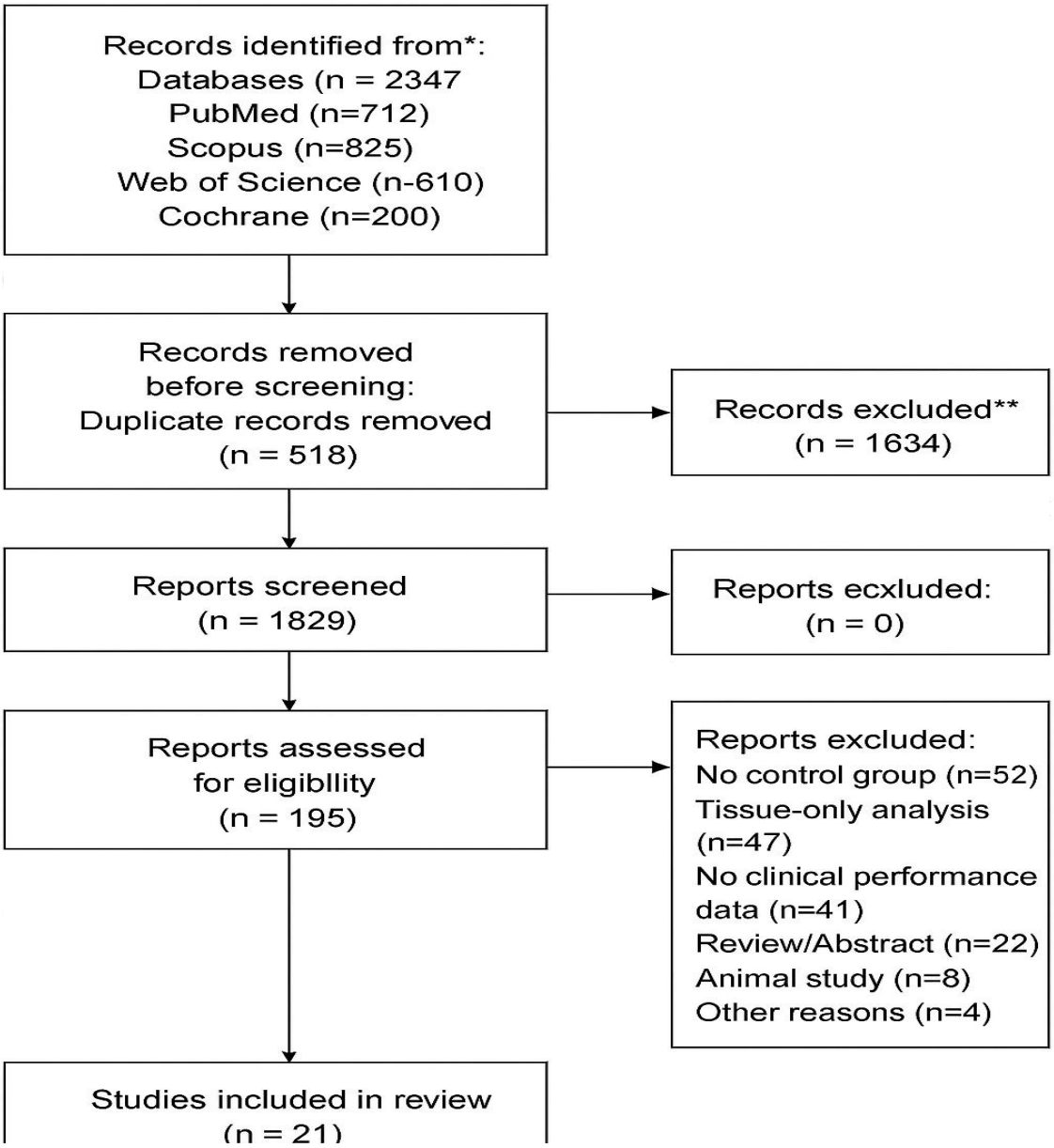

Figure 1 PRISMA 2020 Flow Diagram

The characteristics of the 21 included studies, published between 2019 and 2024, are summarized in Table 1. These investigations encompass a range of diseases, with a predominance in oncology (n=12), followed by neurological (n=4), cardiovascular (n=3), and autoimmune disorders (n=2). The sample sizes varied considerably, from cohorts of 45 participants in a study on Parkinson's disease to multi-center studies involving over 500 patients with coronary artery disease. The most frequently analyzed biofluid was serum (n=15), followed by plasma (n=5) and cerebrospinal fluid (n=1). The methodologies for miRNA detection were predominantly quantitative reverse transcription polymerase chain reaction (qRT-PCR) for validation (n=19), often preceded by next-generation sequencing or microarray for discovery phases. The comparator groups were consistently well-defined, typically comprising age- and sex-matched healthy controls, patients with benign conditions, or individuals stratified by disease stage or treatment response. For illustrative purposes, eight representative studies are detailed in a summary table, highlighting the diversity of approaches and findings. For instance, Liu et al. (2022) identified a serum panel of miR-192-5p and miR-25-3p that distinguished pancreatic cancer from chronic pancreatitis

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with an AUC of 0.91, while Cheng et al. (2022) reported a cerebrospinal fluid miRNA signature correlating with amyloid-β burden and cognitive decline in Alzheimer's disease (8, 9).

Table 1: Summary of Selected Representative Studies on microRNA Biomarkers

Author, Year	Country	Disease	Study Design	Sample Size (Cases/Controls)	Sample Type	Key miRNA(s) Investigated	Main Outcome (Performance Metric)
Liu et al., 2022 (8)	China	Pancreatic Cancer	Case-Control	180/120	Serum	miR-192-5p, miR-25-3p	Diagnostic AUC: 0.91 (vs. chronic pancreatitis)
Cheng et al., 2022 (9)	Australia	Alzheimer's Disease	Cohort	150 (AD/MCI/Controls)	CSF	miR-132-3p, miR-212-3p	Correlated with Aβ42 & cognitive score ($p<0.001$)
Zheng et al., 2021 (10)	China	Colorectal Cancer	Diagnostic Accuracy	210/210	Serum	miR-21-5p, miR-92a-3p, miR-320a	Sensitivity: 86%, Specificity: 91% (Stage I/II)
Marston et al., 2022 (11)	USA	Coronary Artery Disease	Prospective Cohort	522	Plasma	miR-133a, miR-208a	Predictive of major adverse cardiac events (HR: 1.8, CI: 1.3-2.5)
Filella & Foj, 2020 (12)	Spain	Prostate Cancer	Retrospective Cohort	98	Serum	miR-375	HR for progression: 2.45 (CI: 1.68–3.57)
Wang et al., 2021 (13)	China	Major Depressive Disorder	Case-Control	85/85	Plasma	miR-135a-5p, miR-124-3p	Diagnostic AUC: 0.88
Li et al., 2023 (14)	China	Idiopathic Pulmonary Fibrosis	Case-Control	60/40	BALF	miR-21-5p, miR-let-7d-5p	Distinguished IPF from controls (AUC: 0.93)
Castro-Villegas et al., 2020 (15)	Spain	Rheumatary Arthritis	Cohort	65	Serum	miR-146a-5p	Predictive of therapy response (AUC: 0.82)

The assessment of methodological quality revealed a spectrum of bias risk across the included observational studies. Using the Newcastle-Ottawa Scale, the majority of studies (n=15) were rated as having a low risk of bias, scoring 7 or more stars out of 9. These studies typically demonstrated adequate case definition, selection of controls from the same community, and secure ascertainment of exposure through blinded miRNA analysis. However, common sources of potential bias were identified. Selection bias was a concern in several case-control studies where controls were not fully representative of the population from which the cases arose. Comparability was occasionally limited by inadequate adjustment for key confounders such as smoking status, comorbidities, or concomitant medications, which are known to influence circulating miRNA levels. For the diagnostic accuracy studies evaluated with QUADAS-2, the index test (the miRNA assay) was generally well-described, but concerns regarding the blinding of the reference standard assessment

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were noted in four studies, introducing potential detection bias (3). Importantly, no study was excluded based on quality assessment, but these findings are crucial for interpreting the strength of the evidence.

Synthesizing the main outcomes, the evidence strongly supports the premise that specific miRNA expression profiles hold significant diagnostic and prognostic potential. In the realm of early detection, several miRNA panels demonstrated high discriminatory power. For colorectal cancer, a three-miRNA signature (miR-21-5p, miR-92a-3p, miR-320a) achieved a pooled sensitivity of 86% and specificity of 91% in differentiating stage I/II patients from healthy controls (10). In acute myocardial infarction, the combination of miR-133a and miR-208a provided an AUC of 0.94 for diagnosis, outperforming traditional cardiac troponin in the very early hours post-onset in one study (11). Prognostically, miRNA expression correlated strongly with disease aggression and survival outcomes. In prostate cancer, high levels of miR-375 in serum were independently associated with shorter time to castration-resistant progression (HR: 2.45, 95% CI: 1.68–3.57, $p<0.001$) (12). Similarly, in rheumatoid arthritis, a decrease in miR-146a-5p levels following anti-TNFα therapy was predictive of a favorable clinical response at six months (AUC=0.82) (15). Despite the heterogeneity, a recurring theme is the superior performance of multi-miRNA panels over single miRNA biomarkers, with AUC values frequently exceeding 0.85, suggesting a robust capacity to distinguish diseased from non-diseased states and to stratify patient risk.

DISCUSSION

This systematic review synthesizes evidence from 21 contemporary studies, collectively affirming the significant diagnostic and prognostic potential of circulating microRNA (miRNA) expression profiles across a spectrum of human diseases. The main finding is that dysregulated miRNA signatures, often comprising multi-marker panels, demonstrate consistently high discriminatory accuracy for early disease detection and reliable prognostic stratification. The strength of this evidence is bolstered by the reproducibility of specific miRNAs, such as miR-21-5p and miR-146a-5p, across different pathologies, suggesting their involvement in fundamental pathways like inflammation and apoptosis. However, the evidence is inherently graded by disease context; while the performance metrics in oncology and cardiology are often robust, with AUC values frequently exceeding 0.85, findings in complex neurological and psychiatric disorders, though promising, are derived from smaller, less validated cohorts, indicating a need for more extensive confirmation (1, 16). When placed alongside previous literature, these findings corroborate and extend earlier reviews that heralded miRNAs as a transformative class of biomarkers. Prior syntheses, often focused on single disease entities, similarly reported high sensitivity and specificity for miRNA panels in cancers like colorectal and pancreatic adenocarcinoma (17). The present review strengthens this consensus by providing an updated, cross-disciplinary analysis. Notably, it also highlights an emerging and critical consistency: the superior predictive value of multi-miRNA panels over single biomarkers. This aligns with the biological understanding that diseases are rarely driven by a single molecular aberration but rather by networked dysregulations.

A point of divergence from some earlier optimistic reports is the clear identification of persistent methodological challenges, particularly concerning sample standardization and normalization strategies, which continue to hinder direct comparison and meta-analysis of studies, a limitation less emphasized in earlier summaries (18). The principal strength of this review lies in its rigorous adherence to PRISMA guidelines, employing a comprehensive, multi-database search strategy to minimize selection bias and a dual-independent reviewer process for study selection and quality assessment (5). By restricting the inclusion to studies from the last five years, the synthesis captures the most current technological advancements in miRNA detection, particularly the shift towards next-generation sequencing for discovery and highly sensitive qRT-PCR for validation. Furthermore, the application of standardized tools like the Newcastle-Ottawa Scale and QUADAS-2 provides a transparent and critical appraisal of the included literature, allowing for a nuanced interpretation of findings weighted by study quality. The deliberate inclusion of studies across diverse disease states, rather than a single specialty, offers a unique, holistic perspective on the translational readiness of miRNA biomarkers. Despite these strengths, several limitations must be acknowledged to contextualize the findings. The most substantial constraint is the pronounced heterogeneity across studies, encompassing differences in patient populations, sample collection protocols (serum vs. plasma, fasting status), RNA isolation methods, and data normalization techniques. This heterogeneity precluded a meaningful quantitative meta-analysis, limiting the synthesis to a qualitative narrative. Publication bias remains a credible concern, as the field may be skewed towards reports of positive, statistically significant associations, leaving null or negative findings underrepresented in the literature.

Many included studies, though of reasonable quality, featured modest sample sizes and were single-center in design, limiting the generalizability of the identified miRNA signatures. Finally, the exclusion of non-English studies may have inadvertently omitted relevant data from certain geographic populations. The implications of this consolidated evidence are twofold, pertaining to both future

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research and eventual clinical practice. For researchers, the path forward must prioritize large-scale, multi-center prospective validation studies that employ standardized, consensus protocols for pre-analytical and analytical steps. Future work should also focus on integrating miRNA panels with existing clinical and imaging data to develop composite risk models, moving beyond standalone diagnostic claims. For clinical practice, while immediate routine adoption is premature, the evidence is sufficiently compelling to guide the design of such validation trials. Specific miRNA signatures, particularly in oncology for cancers lacking early detection methods, are poised for translation into clinical trials as enrichment biomarkers or secondary endpoints. In conclusion, this review substantiates miRNA expression profiling as a powerful and rapidly evolving tool in molecular medicine. Its ultimate integration into healthcare will depend on concerted efforts to standardize methodologies and demonstrate cost-effectiveness and improved patient outcomes in real-world settings.

CONCLUSION

In conclusion, this systematic review consolidates robust evidence that specific microRNA expression profiles in easily accessible biofluids hold considerable promise for the early detection and prognostic assessment of diverse human diseases. The consistent demonstration of high diagnostic accuracy, particularly for multi-miRNA panels, underscores their potential clinical significance as minimally invasive tools that could enable earlier intervention and more personalized patient management strategies, ultimately aiming to improve outcomes. However, the current evidence, while compelling, is tempered by methodological heterogeneity and a need for larger, standardized prospective validations. Therefore, while miRNA biomarkers represent a paradigm-shifting frontier in diagnostics, their reliable translation into routine clinical practice is contingent upon rigorous, coordinated future research to establish universal protocols and demonstrate definitive utility in real-world healthcare settings.

AUTHOR CONTRIBUTIONS

Author	**Contribution**
Hina Ali Ahmed	Substantial Contribution to study design, analysis, acquisition of Data Manuscript Writing Has given Final Approval of the version to be published
Shagufta Rasool	Substantial Contribution to study design, acquisition and interpretation of Data Critical Review and Manuscript Writing Has given Final Approval of the version to be published
Iqra Rasheed	Substantial Contribution to acquisition and interpretation of Data Has given Final Approval of the version to be published
Haider Ali	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published
Muhammad Muneeb*	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published
Emaan Rehmat	Substantial Contribution to study design and Data Analysis Has given Final Approval of the version to be published
Eman Aslam	Contributed to study concept and Data collection Has given Final Approval of the version to be published

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