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Original Research

miRNAs as Molecular Signatures in Coronary Artery Disease: A Case–Control Study

Vinodhini Subramanian[1], Jeevan Kumar[2], T R Muralidharan[3], Vettriselvi Venkatesan[1,*]

ABSTRACT

Background: Coronary artery disease (CAD) remains a leading cause of global mortality, driven by complex interplay among chronic vascular inflammation, endothelial dysfunction, and myocardial injury. Epigenetic regulators, particularly microRNAs (miRNAs), play crucial roles in modulating atherogenesis. This study aimed to quantify the expression levels of three candidate miRNAs—miR-146a, miR-196a2, and miR-149—in peripheral blood mononuclear cells (PBMCs) of patients with CAD compared with healthy controls, evaluate their diagnostic utility, and predict downstream target pathways in silico. **Methods**: This case–control study enrolled 250 participants (125 patients with angiographically confirmed CAD and 125 healthy controls). Total RNA was extracted from isolated PBMCs, and miRNA expression was quantified by TaqMan Advanced quantitative reverse transcription-polymerase chain reaction (qRT-PCR) on an Applied Biosystems QuantStudio 5 Real-Time PCR System using RNU44 as the endogenous reference control. Relative fold changes were calculated using the $2^{-\Delta\Delta Ct}$ method. Inter-group comparisons were evaluated using adjusted p-values, and diagnostic accuracy was assessed using receiver operating characteristic (ROC) curve analysis with area under the curve (AUC) calculation. Bonferroni correction was applied to control for multiple comparisons, with $p < 0.05$ considered statistically significant. Putative target genes were predicted in silico using TargetScan 8.0, miRDB v6.0, and miRTarBase v10.0. **Results:** qRT-PCR analysis demonstrated distinct differential expression profiles of the candidate miRNAs between CAD patients and healthy controls. PBMC miR-146a was significantly upregulated in the CAD group (fold change = 2.37, adjusted $p = 0.008$) with moderate diagnostic accuracy (AUC = 0.728). Conversely, miR-149 was markedly downregulated (fold change = 0.11, adjusted $p < 0.005$) with high diagnostic discriminatory capacity (AUC = 0.917). miR-196a2 exhibited a non-significant upward expression trend (fold change = 1.72, adjusted $p = 0.098$; AUC = 0.741). miRNA expression levels did not differ significantly across angiographic disease severity subgroups. In silico target prediction identified 11, 7, and 10 consensus target genes for miR-146a, miR-149, and miR-196a2, respectively, which were enriched in biological pathways governing vascular inflammation, apoptosis, oxidative stress, and endothelial homeostasis. **Conclusions**: PBMC-derived miR-146a and miR-149 are significantly dysregulated in CAD patients. In particular, miR-149 demonstrates strong potential as an adjunct diagnostic biomarker. These findings highlight the involvement of these miRNAs in CAD-associated molecular mechanisms and provide a rationale for further mechanistic and clinical validation.

Key words: Coronary artery disease, microRNA, miR-146a, miR-149, miR-196a2, peripheral blood mononuclear cells, gene expression, in silico target prediction, biomarker

[1]*Department of Human Genetics, Sri Ramachandra Institute of Higher Education and Research (DU), Chennai, India*

[2]*Department of Biomedical Sciences, The Apollo University, Chittoor, India*

[3]*Institute of Cardiac Sciences, SRM Global Hospitals, Chennai, India*

Correspondence

Dr. Vettriselvi Venkatesan, PhD, Sri Ramachandra Institute of Higher Education and Research (DU), Porur, Chennai - 600116, India

Email: vettriselviv@sriramachandra.edu.in

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INTRODUCTION

Cardiovascular diseases (CVDs) remain the leading cause of mortality worldwide, accounting for an estimated 19.8 million deaths in 2022, with more than three-quarters occurring in low- and middle-income countries[1]. In India, the burden of CVDs, particularly coronary artery disease (CAD), has increased substantially alongside rapid epidemiological transitions and the escalating prevalence of cardiometabolic risk factors[2]. CAD primarily stems from atherosclerosis, a multifaceted pathological process involving subendothelial lipid accumulation, endothelial dysfunction, and chronic vascular inflammation[3]. In addition to conventional cardiovascular risk factors such as diabetes mellitus and hypertension, epigenetic regulators—particularly microRNAs (miRNAs)—critically influence the initiation, development, and progression of atherosclerotic lesions[4].

miRNAs are small, endogenous, non-coding RNA molecules (approximately 22 nucleotides in length) that post-transcriptionally regulate gene expression. By binding complementarily to target sequences within the 3′ untranslated region (3′UTR) of messenger RNAs (mRNAs), miRNAs promote mRNA

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degradation or suppress protein translation[5]. First discovered in Caenorhabditis elegans by Lee et al. in 1993[6], miRNAs have since been implicated in diverse physiological and pathophysiological processes, including embryonic development, immune responses, apoptosis, and vascular biology[7]. In cardiovascular pathology, their critical regulatory roles have been increasingly recognized, and stable miRNAs detectable in cellular fractions and biofluids demonstrate promising utility as disease biomarkers and therapeutic targets[8].

Several miRNAs have been directly implicated in the molecular mechanisms underlying CAD. miR-146a functions as a key negative regulator of the nuclear factor-kappa B (NF-κB) signaling pathway by directly targeting tumor necrosis factor receptor-associated factor 6 (TRAF6) and interleukin-1 receptor-associated kinase 1 (IRAK1), thereby suppressing downstream inflammatory cascades[9,10]. Aberrant miR-146a expression has been documented in various cardiovascular and inflammatory disorders[11]. miR-196a2, located within the HOXC gene cluster[12], plays an essential role in modulating vascular inflammation and endothelial integrity; notably, the functional rs11614913 polymorphism within miR-196a2 has been linked with heightened susceptibility to CAD across several genetic models[13]. miR-149 is critically involved in endothelial homeostasis through the negative regulation of pro-inflammatory mediators associated with endothelial activation; downregulation of miR-149 has been linked to tumor necrosis factor-alpha (TNF-α)-induced endothelial dysfunction, whereas restoration of miR-149 attenuates the expression of matrix metalloproteinase-9 (MMP-9), inducible nitric oxide synthase (iNOS), and interleukin-6 (IL-6)[14].

Among the dysregulated miRNAs in cardiovascular pathology, these three candidates were selected based on their established biological relevance, consistency across preclinical and clinical literature, and direct participation in pathways central to CAD pathogenesis. The present study investigated the expression profiles of miR-146a, miR-196a2, and miR-149 in peripheral blood mononuclear cells (PBMCs) from a clinically characterized cohort of CAD patients and healthy controls, evaluated their diagnostic utility, and utilized multi-database in silico target prediction to delineate their putative molecular target networks.

MATERIALS AND METHODS

Study Population and Sample Collection

This case–control study enrolled 250 participants, comprising 125 patients with angiographically confirmed CAD and 125 control individuals with no clinical, electrocardiographic, or diagnostic evidence of CAD. The sample size was calculated assuming a type I error (α) of 5% with a statistical power (1 - β) of 80%. All participants were recruited from the Department of Cardiology, Sri Ramachandra Medical Centre (SRMC), Chennai, India. Ethical approval was obtained from the Institutional Ethics Committee of Sri Ramachandra Institute of Higher Education and Research (SRIHER) under reference number IEC-NI/22/JAN/81/24. Written informed consent was obtained from all participants prior to study enrollment.

Eligible participants were adults aged 18 years or older. Case subjects were included based on clinical evaluation and angiographic confirmation of CAD. Individuals with a history of malignancy, active infectious disease, or major systemic inflammatory conditions were excluded. Control participants were confirmed to have no prior history or clinical evidence of CAD and satisfied all other general eligibility criteria.

PBMC Isolation, RNA Extraction, and cDNA Synthesis

Peripheral blood mononuclear cells (PBMCs) were isolated from whole blood samples using Ficoll-Paque density gradient centrifugation. Total RNA was subsequently extracted from the isolated PBMC fraction using RNAiso Plus reagent (Takara Bio Inc., Kusatsu, Japan), according to the manufacturer's protocol. Briefly, cells were lysed in TRIzol-based reagent, followed by chloroform addition for phase separation. RNA in the aqueous phase was precipitated with isopropanol, washed with 75% ethanol, air-dried, and resuspended in RNase-free water. RNA concentration and purity were assessed using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA); only samples exhibiting an A260/A280 ratio between 1.8 and 2.0 and intact RNA profiles were used for downstream applications.

For cDNA synthesis, total RNA from each sample was reverse transcribed using the TaqMan™ Advanced miRNA cDNA Synthesis Kit (Applied Biosystems, Foster City, CA, USA) following the manufacturer's instructions. The protocol involved four sequential enzymatic reactions: (1) 3′ polyadenylation

of mature miRNAs by poly(A) polymerase; (2) ligation of a 5′ adaptor to the polyadenylated miRNAs; (3) reverse transcription using a universal RT primer to synthesize cDNA from the modified miRNAs; and (4) miR-Amp amplification using universal primers to uniformly increase cDNA yield. All reactions were conducted in a thermal cycler under optimized enzymatic reaction parameters. The resulting cDNA product was diluted and stored at -20 ℃ until quantitative real-time PCR analysis.

Quantitative Real-Time PCR (qRT-PCR) miRNA Expression Analysis

Quantification of miRNA expression was carried out using specific TaqMan™ Advanced miRNA Assays for hsa-miR-146a-5p, hsa-miR-196a-5p, and hsa-miR-149-5p. All reactions were performed in triplicate on 96-well plates using the Applied Biosystems™ QuantStudio 5 Real-Time PCR System, with RNU44 serving as the endogenous reference control for normalization. The thermal cycling conditions were: initial enzyme activation at 95 ℃ for 20 seconds, followed by 40 cycles of denaturation at 95 ℃ for 1 second and combined annealing/extension at 60 ℃ for 20 seconds. Relative expression fold changes between CAD patients and healthy controls were calculated using the comparative threshold cycle ($2^{-\Delta\Delta Ct}$) method.

In Silico miRNA Target Gene Prediction

Putative target genes of miR-146a, miR-196a2, and miR-149 were predicted using three publicly available bioinformatics databases: TargetScan 8.0 (accessed July 2025), miRDB v6.0 (accessed June 2025), and miRTarBase v10.0 (accessed June 2025). Stringent filtering criteria were implemented: for TargetScan, predicted targets with a cumulative context++ score < -0.2 were selected; for miRDB, target predictions with a score ≥ 80 were included; and for miRTarBase, only experimentally validated miRNA-target interactions were collected. Overlapping targets across the three databases were determined using the Venny 2.1 tool. Only candidate genes supported by at least two independent databases were retained for consensus target network analysis.

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics for Windows, Version 26.0 (IBM Corp., Armonk, NY, USA). Continuous variables were expressed as mean ± standard deviation (SD) or median (interquartile range, IQR), as appropriate. Categorical variables were presented as frequencies and percentages. Inter-group differences in miRNA expression (ΔCt values) between CAD patients and healthy controls were evaluated using the Mann–Whitney U test. Differences across CAD angiographic severity subgroups were evaluated using the Kruskal–Wallis H test. Diagnostic performance of candidate miRNAs was evaluated using receiver operating characteristic (ROC) curve analysis, and the area under the ROC curve (AUC), sensitivity, and specificity were calculated. To reduce family-wise error and statistical bias from multiple comparisons, Bonferroni correction was applied. A two-tailed p-value < 0.05 was considered statistically significant. The reporting of this study follows the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) guidelines for case–control studies [15].

RESULTS

A total of 250 participants were enrolled in this case–control study, comprising 125 patients with angiographically confirmed CAD and 125 healthy controls. The mean age of the CAD group was 53.6 ± 12.3 years, while that of the control group was 49.5 ± 13.6 years. Among the 125 CAD patients, angiographic evaluation revealed single-vessel disease (SVD) in 34 patients (27.2%), double-vessel disease (DVD) in 39 patients (31.2%), triple-vessel disease (TVD) in 32 patients (25.6%), triple-vessel disease with left main coronary artery involvement (TVD+LM) in 16 patients (12.8%), and chronic total occlusion (CTO) in 4 patients (3.2%).

Expression Profiling and Diagnostic Evaluation of Candidate miRNAs

qRT-PCR analysis demonstrated distinct differential expression patterns of the three candidate miRNAs between CAD patients and healthy controls (Figure 1).

miR-146a was significantly upregulated in PBMCs of CAD patients compared with healthy controls, exhibiting a relative fold change of 2.37 (adjusted p = 0.008; Figure 1A). ROC curve analysis yielded an AUC of 0.728 (Figure 2A), indicating moderate diagnostic accuracy in distinguishing CAD patients from control individuals.

miR-149 was markedly downregulated in the CAD group, exhibiting a relative fold change of 0.11 (adjusted $p < 0.005$; Figure 1B). Among the three evaluated miRNAs, miR-149 demonstrated the highest

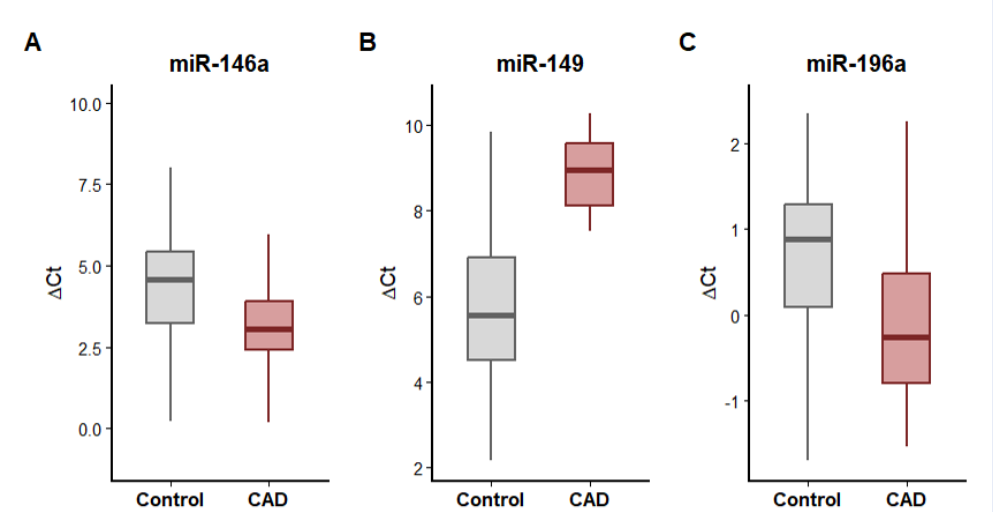

Figure 1: Relative Expression Levels (ΔCt) of Candidate microRNAs in Peripheral Blood Mononuclear Cells of CAD Patients versus Healthy Controls. PBMC-derived microRNA expression was measured by TaqMan Advanced quantitative real-time PCR (qRT-PCR) in patients with angiographically confirmed coronary artery disease (CAD; n = 125) and healthy controls (n = 125). RNU44 was used as the endogenous reference gene for normalization. (A) miR-146a expression is significantly upregulated in CAD patients compared to controls (fold change = 2.37, adjusted p = 0.008). (B) miR-149 expression is significantly downregulated in CAD patients (fold change = 0.11, adjusted p < 0.005). **(C)** miR-196a2 expression exhibits a non-significant upward trend in CAD patients (fold change = 1.72, adjusted p = 0.098). Box-and-whisker plots display the median (center line), interquartile range (box boundaries, 25th to 75th percentiles), and minimum-to-maximum range (whiskers). In the ΔCt metric (Ct,target - Ct,RNU44), lower ΔCt values correspond to higher relative transcript expression. Inter-group statistical comparisons were evaluated using the Mann–Whitney U test with Bonferroni correction for multiple testing (*p < 0.05). *Abbreviations:* CAD, coronary artery disease; Ct, threshold cycle; ΔCt, cycle threshold difference; PBMC, peripheral blood mononuclear cell; qRT-PCR, quantitative reverse transcription-polymerase chain reaction.

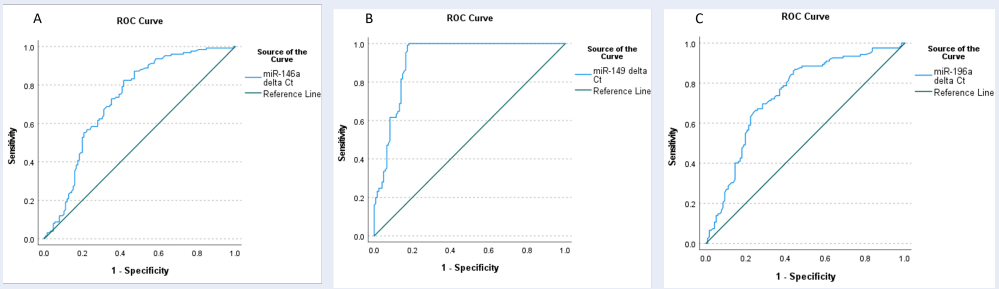

Figure 2: Receiver Operating Characteristic (ROC) Curves Assessing the Diagnostic Discriminatory Performance of Candidate microRNAs for Coronary Artery Disease. Diagnostic accuracy of PBMC-derived candidate microRNAs in distinguishing CAD patients (n = 125) from healthy control subjects (n = 125) was evaluated by ROC curve analysis. (A) miR-146a demonstrates moderate diagnostic accuracy with an area under the curve (AUC) of 0.728 (95% CI: 0.665–0.791). (B) miR-149 exhibits high diagnostic efficacy with an AUC of 0.917 (95% CI: 0.882–0.952). **(C)** miR-196a2 shows moderate diagnostic performance with an AUC of 0.741 (95% CI: 0.679–0.803). The solid curve illustrates sensitivity versus 1 - specificity across diagnostic cutoff thresholds; the diagonal dashed line denotes the reference line of chance (AUC = 0.50). Bonferroni correction was applied to adjust for multiple comparisons. Abbreviations: AUC, area under the ROC curve; CAD, coronary artery disease; CI, confidence interval; PBMC, peripheral blood mononuclear cell; ROC, receiver operating characteristic.

Table 1: Overview and Operational Characteristics of Bioinformatics Databases Employed for microRNA Target Prediction and Validation.

Database	Type	Data Source	Content & Output	Scoring Method / Metric	URL
TargetScan [16]	Predictive	3′UTR sequence conservation, seed region complementarity	Conserved and non-conserved miRNA target predictions based on 14 biochemical features	**Cumulative Context++ Score:** Integrates site type, 3′ UTR location, local AU content, target site abundance, and seed pairing stability	https://www.targetscan.org/
miRDB [17]	Predictive	High-throughput sequencing and CLIP-seq experimental datasets	Genome-wide miRNA target predictions generated using machine learning models	**Target Score (0–100):** SVM model trained on high-throughput experimental CLIP-seq data; scores ≥ 80 indicate high prediction confidence	https://mirdb.org/
miRTarBase [18]	Experimentally validated	Manual curation of peer-reviewed biomedical literature	Validated miRNA–target interactions supported by strong experimental evidence	**Categorical validation strength:** Classified by assay type (reporter assay, qPCR, Western blot, microarray, or high-throughput sequencing)	https://mirtarbase.cuhk.edu.cn/

Note: Table 1 outlines the analytical platforms utilized for the multi-database in silico target prediction framework. Candidates identified by TargetScan (cumulative context++ score < -0.2), miRDB (prediction score ≥ 80), and miRTarBase (experimentally verified) were cross-referenced, with consensus targets defined as those present in at least two independent databases. Abbreviations: 3′UTR, 3′ untranslated region; CLIP-seq, cross-linking immunoprecipitation followed by sequencing; miRNA, microRNA; NGS, next-generation sequencing; qPCR, quantitative polymerase chain reaction; SVM, support vector machine; URL, uniform resource locator.

diagnostic accuracy, with an AUC of 0.917 (Figure 2B), indicating excellent discriminatory power for identifying CAD.

miR-196a2 displayed an upward trend in expression in CAD patients with a fold change of 1.72; however, this increase did not reach statistical significance after adjustment for multiple testing (adjusted p = 0.098; Figure 1C). The ROC curve for miR-196a2 showed an AUC of 0.741 (Figure 2C).

To examine whether PBMC miRNA expression correlated with the anatomical extent of coronary atherosclerosis, ΔCt values were compared across CAD angiographic severity subgroups (SVD, DVD, TVD, TVD+LM, and CTO). Kruskal–Wallis analysis showed no statistically significant differences in ΔCt values across severity categories for miR-146a, miR-149, or miR-196a2. These findings indicate that while PBMC-derived miR-146a and miR-149 are differentially expressed in the presence of CAD, their expression levels do not parallel the anatomical burden of coronary stenoses.

In Silico Target Prediction and Functional Pathways

Integrative bioinformatics analysis was conducted to identify high-confidence, biologically relevant downstream target genes for miR-146a, miR-196a2, and miR-149 utilizing TargetScan 8.0, miRDB v6.0, and miRTarBase v10.0 (Table 1). TargetScan predictions were filtered by cumulative context++ score < -0.2, miRDB predictions were restricted to scores z 80, and miRTarBase queries were limited to experimentally validated targets.

Intersection analysis via Venny identified genes shared by at least two databases, yielding 11 consensus targets for miR-146a, 10 consensus targets for miR-196a2, and 7 consensus targets for miR-149 (Figure 3). Functional annotation indicated that these consensus target genes are involved in essential signaling pathways regulating vascular inflammation, endothelial integrity, apoptosis, and oxidative stress responses.

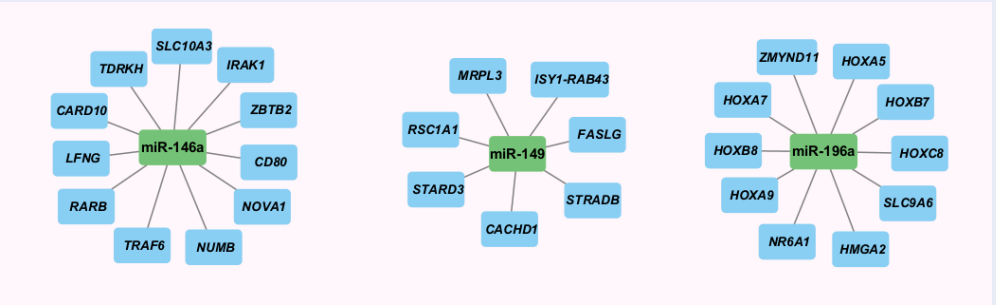

Figure 3: Interaction Network of CAD-Associated Candidate microRNAs and Their Predicted Consensus Downstream Target Genes. Bioinformatics network representation of predicted and validated downstream target genes for miR-146a, miR-149, and miR-196a2. Target gene lists were extracted from TargetScan 8.0 (cumulative context++ score < -0.2), miRDB v6.0 (prediction score ≥ 80), and miRTarBase v10.0 (experimentally validated). Candidate targets identified in at least two independent databases were retained to establish the high-confidence consensus network. The resulting interaction map comprises 11 consensus target genes for miR-146a, 7 consensus target genes for miR-149, and 10 consensus target genes for miR-196a2. In the network diagram, green circular nodes represent microRNAs, blue rectangular nodes indicate downstream target genes, and interconnecting edges denote regulatory interactions. The consensus target genes participate in biological pathways central to atherogenesis, including vascular endothelial homeostasis, vascular inflammation, apoptosis, and cellular oxidative stress. Abbreviations: CAD, coronary artery disease; miR / miRNA, microRNA; PBMC, peripheral blood mononuclear cell; 3′UTR, 3′ untranslated region.

DISCUSSION

The differential expression of PBMC-derived microRNAs observed in this study provides further evidence supporting the role of non-coding RNAs as circulating molecular indicators and contributors to CAD pathophysiology.

We observed significant upregulation of miR-146a in CAD patients, consistent with its established role as a negative feedback regulator of the NF-κB inflammatory signaling pathway. Mechanistically, miR-146a directly targets TRAF6 and IRAK1, thereby attenuating pro-inflammatory cytokine production in vascular and immune cells during atherogenesis[10,19]. Upregulation of miR-146a in PBMCs likely reflects a compensatory immune-regulatory response to chronic vascular inflammation in CAD. In clinical studies, altered miR-146a expression in PBMCs and plasma has been associated with CAD severity and genetic susceptibility. For instance, young CAD patients harboring the rs2910164 polymorphism exhibited genotype-dependent variations in PBMC miR-146a expression and altered IRAK1/TRAF6 protein levels[20]. Furthermore, circulating plasma miR-146a levels were positively correlated with Gensini scores in coronary heart disease patients with subclinical hypothyroidism[21]. Preclinical investigations corroborate these findings; miR-146a overexpression reduced atherosclerotic plaque progression and enhanced plaque stability in ApoE-/- mice by suppressing IRAK1 and TRAF6[22], and attenuated cardiac inflammation in lipopolysaccharide (LPS)-induced cardiomyocyte injury models by suppressing NF-κB signaling[23].

Conversely, miR-149 was significantly downregulated in CAD patients, demonstrating superior diagnostic accuracy (AUC = 0.917). This marked reduction aligns with experimental evidence indicating that downregulation of miR-149 promotes endothelial inflammatory activation and vascular dysfunction. Mechanistic studies have shown that miR-149 restoration suppresses the expression of key inflammatory mediators, including MMP-9, iNOS, and IL-6, in TNF-α-stimulated endothelial cells[14]. Furthermore, miR-149 mimic transfection preserved endothelial cell viability, decreased caspase-3 activity, and promoted protective autophagy via modulation of the Akt/mTOR signaling pathway in oxidized low-density lipoprotein (ox-LDL)-treated human umbilical vein endothelial cells (HUVECs)[24]. Although Lin et al.[25] reported that miR-149 aggravated cardiomyocyte pyroptosis via FoxO3 silencing in an ischemia-reperfusion model, these contrasting findings underscore the cell type- and microenvironment-dependent actions of miR-149 across vascular endothelial cells versus ischemic myocardial tissue.

miR-196a2 exhibited an upward expression trend in CAD patients (fold change = 1.72) and moder-

ate discriminatory ability (AUC = 0.741), although the elevation was not statistically significant after multiple-testing correction. Encoded within the *HOXC* gene cluster, miR-196a2 regulates vascular inflammation and angiogenesis by modulating targets such as annexin A1 (ANXA1)[26]. Previous studies by Saadatian et al.[27] demonstrated differential PBMC miR-196a expression between patients with significant coronary stenosis, non-significant stenosis, and healthy controls. Furthermore, the functional rs11614913 C>T polymorphism in miR-196a2 has been linked with an elevated risk of premature CAD in specific populations[28], in addition to its recognized oncological biomarker roles[29,30].

Subgroup analysis across CAD angiographic severity categories revealed no significant variation in ΔCt values for any of the three candidate miRNAs. This observation suggests that PBMC miRNA dysregulation reflects systemic immune-inflammatory and vascular regulatory alterations rather than anatomical stenosis severity per se. Because coronary angiography evaluates macrovascular luminal narrowing, whereas PBMC-derived miRNAs reflect cellular immune activation and epigenetic regulation, the absence of a linear severity gradient is biologically plausible. Hence, these candidate miRNAs may serve as molecular indicators of active CAD pathophysiology rather than structural lesion burden.

Several limitations of the present study should be acknowledged. First, the study cohort was recruited from a single tertiary-care medical center in South India; multi-center studies with independent validation cohorts across diverse ethnic backgrounds are required to confirm the generalizability and diagnostic robustness of these biomarkers. Second, while PBMCs provide a clinically accessible, minimally invasive compartment for biomarker assessment, complementary tissue-specific and circulating extracellular vesicle analyses would yield deeper insights into local vascular versus systemic miRNA dynamics. Third, while subgroup analyses across angiographic severity were conducted, interpretation was constrained by unequal subgroup distributions, particularly the small CTO subgroup. Finally, the in silico target prediction strategy yielded a high-confidence set of consensus genes that now warrant direct downstream functional validation in cellular and animal models of atherosclerosis.

CONCLUSIONS

This study demonstrates distinct expression alterations of PBMC-derived miRNAs in CAD patients, characterized by significant upregulation of miR-146a and pronounced downregulation of miR-149, alongside a modest, non-significant increase in miR-196a2. In silico target prediction and functional literature evidence indicate that these miRNAs converge on critical regulatory pathways governing vascular inflammation, apoptosis, oxidative stress, and endothelial homeostasis. In particular, miR-149 exhibits high diagnostic discriminatory accuracy for CAD. These findings underscore the potential utility of PBMC miRNAs as adjunct diagnostic biomarkers and provide a mechanistic foundation for future functional investigations in coronary artery disease.

ABBREVIATIONS

AUC (Area under the receiver operating characteristic curve), CAD (Coronary artery disease), cDNA (Complementary deoxyribonucleic acid), CI (Confidence interval), CTO (Chronic total occlusion), CVD (Cardiovascular disease), DVD (Double-vessel disease), HUVEC (Human umbilical vein endothelial cell), IL-6 (Interleukin-6), iNOS (Inducible nitric oxide synthase), IQR (Interquartile range), IRAK1 (Interleukin-1 receptor-associated kinase 1), miRNA / miR (MicroRNA), MMP-9 (Matrix metalloproteinase-9), mRNA (Messenger ribonucleic acid), NF-κB (Nuclear factor-kappa B), ox-LDL (Oxidized low-density lipoprotein), PBMC (Peripheral blood mononuclear cell), qRT-PCR / qPCR (Quantitative reverse transcription-polymerase chain reaction), ROC (Receiver operating characteristic), RNU44 (RNA, U44 small nucleolar), SD (Standard deviation), SRMC (Sri Ramachandra Medical Centre), SRIHER (Sri Ramachandra Institute of Higher Education and Research), STROBE (Strengthening the Reporting of Observational Studies in Epidemiology), SVD (Single-vessel disease), TNF-α (Tumor necrosis factor-alpha), TRAF6 (Tumor necrosis factor receptor-associated factor 6), TVD (Triple-vessel disease), TVD+LM (Triple-vessel disease with left main coronary artery involvement), and UTR (Untranslated region).

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AUTHOR'S CONTRIBUTIONS

- **VS:** Conceptualization, methodology, investigation, data curation, formal analysis, and writing – original draft preparation.
- **VV:** Conceptualization, project administration, supervision, methodology, and writing – review and editing.
- **JK:** Formal analysis, validation, supervision, and writing – review and editing.
- **TRM:** Investigation, clinical resources, supervision, and writing – review and editing. All authors critically revised the manuscript for important intellectual content and approved the final version for publication.

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AVAILABILITY OF DATA AND MATERIALS

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was approved by the Institutional Ethics Committee of Sri Ramachandra Institute of Higher Education and Research (SRIHER), Chennai, India (Reference Number: IEC-NI/22/JAN/81/24). All procedures involving human participants were conducted in accordance with the ethical standards of the institutional research committee and the 1964 Declaration of Helsinki and its subsequent amendments. Written informed consent was obtained from all individual participants prior to enrollment in the study.

CONSENT FOR PUBLICATION

Not applicable.

DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES IN THE WRITING PROCESS

None.

COMPETING INTERESTS

The authors declare that they have no competing interests.

REFERENCES

1. World Health Organization. Cardiovascular diseases (CVDs). Geneva: World Health Organization; 2025.
2. Prabhakaran D, Singh K, Roth GA, Banerjee A, Pagidipati NJ, Huffman MD. Cardiovascular Diseases in India Compared With the United States. Journal of the American College of Cardiology. 2018 Jul;72(1):79–95. PMID: 29957235. Available from: https://doi.org/10.1016/j.jacc.2018.04.042.
3. Libby P. Inflammation in atherosclerosis. Nature. 2002 Dec;420(6917):868–874. PMID: 12490960. Available from: https://doi.org/10.1038/nature01323.
4. Feinberg MW, Moore KJ. MicroRNA Regulation of Atherosclerosis. Circulation Research. 2016 Feb;118(4):703–720. PMID: 26892968. Available from: https://doi.org/10.1161/CIRCRESAHA.115.306300.
5. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell. 2004 Jan;116(2):281–297. PMID: 14744438. Available from: https://doi.org/10.1016/S0092-8674(04)00045-5.
6. Lee RC, Feinbaum RL, Ambros V. The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell. 1993 Dec;75(5):843–854. PMID: 8252621. Available from: https://doi.org/10.1016/0092-8674(93)90529-Y.
7. Small EM, Olson EN. Pervasive roles of microRNAs in cardiovascular biology. Nature. 2011 Jan;469(7330):336–342. PMID: 21248840. Available from: https://doi.org/10.1038/nature09783.
8. Chen X, Ba Y, Ma L, Cai X, Yin Y, Wang K, et al. Characterization of microRNAs in serum: a novel class of biomarkers for diagnosis of cancer and other diseases. Cell Research. 2008 Oct;18(10):997–1006. PMID: 18766170. Available from: https://doi.org/10.1038/cr.2008.282.
9. Saba R, Sorensen DL, Booth SA. MicroRNA-146a: A Dominant, Negative Regulator of the Innate Immune Response. Frontiers in Immunology. 2014 Nov;5:578. PMID: 25484882. Available from: https://doi.org/10.3389/fimmu.2014.00578.
10. Taganov KD, Boldin MP, Chang KJ, Baltimore D. NF-kappaB-dependent induction of microRNA miR-146, an inhibitor targeted to signaling proteins of innate immune responses. Proceedings of the National Academy of Sciences USA. 2006 Aug;103(33):12481–12486. PMID: 16885212. Available from: https://doi.org/10.1073/pnas.0605298103.
11. Mahdavi FS, Mardi S, Mohammadi S, Ansari S, Yaslianifard S, Fallah P, et al. MicroRNA-146: Biomarker and Mediator of Cardiovascular Disease. Disease Markers. 2022 Oct;2022:7767598. PMID: 39281713. Available from: https://doi.org/10.1155/2022/7767598.
12. Yekta S, Shih IH, Bartel DP. MicroRNA-directed cleavage of HOXB8 mRNA. Science. 2004 Apr;304(5670):594–596. PMID: 15105502. Available from: https://doi.org/10.1126/science.1097434.
13. Fragoso JM, Ramírez-Bello J, Martínez-Ríos MA, Peña-Duque MA, Posadas-Sánchez R, Delgadillo-Rodríguez H, et al. miR-196a2 (rs11614913) polymorphism is associated with coronary artery disease, but not with in-stent coronary restenosis. Inflammation Research. 2019 Mar;68(3):215–221. PMID: 30560371. Available from: https://doi.org/10.1007/s00011-018-1206-z.
14. Palmieri D, Capponi S, Geroldi A, Mura M, Mandich P, Palombo D. TNFα induces the expression of genes associated with endothelial dysfunction through p38MAPK-mediated down-regulation of miR-149. Biochemical and Biophysical Research Communications. 2014 Jan;443(1):246–251. PMID: 24299952. Available from: https://doi.org/10.1016/j.bbrc.2013.11.092.
15. von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP, et al. Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. BMJ. 2007 Oct;335(7624):806–808. PMID: 17947786. Available from: https://doi.org/10.1136/bmj.39335.541782.AD.

16. McGeary SE, Lin KS, Shi CY, Pham TM, Bisaria N, Kelley GM, et al. The biochemical basis of microRNA targeting efficacy. Science. 2019;366(6472):eaav1741. Available from: https://doi.org/10.1126/science.aav1741.
17. Chen Y, Wang X. miRDB: an online database for prediction of functional microRNA targets. Nucleic Acids Research. 2020 Jan;48(D1):D127–D131. PMID: 31504780. Available from: https://doi.org/10.1093/nar/gkz757.
18. Cui S, Yu S, Huang HY, Lin YC, Huang Y, Zhang B, et al. miRTarBase 2025: updates to the collection of experimentally validated microRNA-target interactions. Nucleic Acids Research. 2025 Jan;53(D1):D147–D156. PMID: 39578692. Available from: https://doi.org/10.1093/nar/gkae1072.
19. Takahashi Y, Satoh M, Minami Y, Tabuchi T, Itoh T, Nakamura M. Expression of miR-146a/b is associated with the Toll-like receptor 4 signal in coronary artery disease: effect of renin-angiotensin system blockade and statins on miRNA-146a/b and Toll-like receptor 4 levels. Clinical Science (London). 2010 Jul;119(9):395–405. PMID: 20524934. Available from: https://doi.org/10.1042/CS20100003.
20. Ramkaran P, Khan S, Phulukdaree A, Moodley D, Chuturgoon AA. miR-146a polymorphism influences levels of miR-146a, IRAK-1, and TRAF-6 in young patients with coronary artery disease. Cell Biochemistry and Biophysics. 2014 Mar;68(2):259–266. PMID: 23794009. Available from: https://doi.org/10.1007/s12013-013-9704-7.
21. Quan X, Ji Y, Zhang C, Guo X, Zhang Y, Jia S, et al. Circulating MiR-146a May be a Potential Biomarker of Coronary Heart Disease in Patients with Subclinical Hypothyroidism. Cell Physiology and Biochemistry. 2018;45(1):226–236. PMID: 29357324. Available from: https://doi.org/10.1159/000486769.
22. Chu T, Xu X, Ruan Z, Wu L, Zhou M, Zhu G. miR-146a contributes to atherosclerotic plaque stability by regulating the expression of TRAF6 and IRAK-1. Molecular Biology Reports. 2022 Jun;49(6):4205–4216. PMID: 35195809. Available from: https://doi.org/10.1007/s11033-022-07253-z.
23. An R, Feng J, Xi C, Xu J, Sun L. miR-146a Attenuates Sepsis-Induced Myocardial Dysfunction by Suppressing IRAK1 and TRAF6 via Targeting ErbB4 Expression. Oxidative Medicine and Cellular Longevity. 2018 Aug;2018(1):7163057. PMID: 30224945. Available from: https://doi.org/10.1155/2018/7163057.
24. Zhu Z, Li J, Tong R, Zhang X, Yu B. miR-149 Alleviates Ox-LDL-Induced Endothelial Cell Injury by Promoting Autophagy through Akt/mTOR Pathway. Cardiology Research and Practice. 2021 Aug;2021:9963258. PMID: 34484820. Available from: https://doi.org/10.1155/2021/9963258.
25. Lin J, Lin H, Ma C, Dong F, Hu Y, Li H. MiR-149 Aggravates Pyroptosis in Myocardial Ischemia-Reperfusion Damage via Silencing FoxO3. Medical Science Monitor. 2019 Nov;25:8733–8743. PMID: 31741467. Available from: https://doi.org/10.12659/MSM.918410.
26. Pin AL, Houle F, Fournier P, Guillonneau M, Paquet ÉR, Simard MJ, et al. Annexin-1-mediated endothelial cell migration and angiogenesis are regulated by vascular endothelial growth factor (VEGF)-induced inhibition of miR-196a expression. Journal of Biological Chemistry. 2012 Aug;287(36):30541–30551. PMID: 22773844. Available from: https://doi.org/10.1074/jbc.M112.393561.
27. Saadatian Z, Nariman-Saleh-Fam Z, Khaheshi I, Mansoori Y, Daraei A, Ghaderian SM, et al. Peripheral Blood Mononuclear Cells Expression Levels of miR-196a and miR-100 in Coronary Artery Disease Patients. Immunological Investigations. 2021 Nov;50(8):914–924. PMID: 32928012. Available from: https://doi.org/10.1080/08820139.2020.1791177.
28. Agiannitopoulos K, Samara P, Papadopoulou M, Efthymiadou A, Papadopoulou E, Tsaousis GN, et al. miRNA polymorphisms and risk of premature coronary artery disease. Hellenic Journal of Cardiology. 2021;62(4):278–284. PMID: 32092393. Available from: https://doi.org/10.1016/j.hjc.2020.01.005.
29. Xiong M, Wang P, Pan B, Nie J, Wang S, He B. The diagnostic and prognostic values of microRNA-196a in cancer. Bioscience Reports. 2021 Jan;41(1):BSR20203559. PMID: 33289788. Available from: https://doi.org/10.1042/BSR20203559.
30. Saito K, Inagaki K, Kamimoto T, Ito Y, Sugita T, Nakajo S, et al. MicroRNA-196a is a putative diagnostic biomarker and therapeutic target for laryngeal cancer. PLoS ONE. 2013 Aug;8(8):e71480. PMID: 23967217. Available from: https://doi.org/10.1371/journal.pone.0071480.