

Computational and expression analysis of microRNA-149-5p and its target, interleukin-6, in chronic kidney disease

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ABSTRACT

Background: Chronic kidney disease (CKD) is an ailment marked by a reduction in the glomerular filtration rate or the presence of proteinuria. Millions of people are affected worldwide, and so far, the mechanisms underlying these effects remain mostly unknown. Recently, the role of microRNAs (miRNAs) in several cellular processes associated with the development of diseases has been discovered. Studies have suggested that miR-149-5p may play a role in renal function and kidney disorders and be linked with inflammation, fibrosis, or apoptosis within the kidneys. In addition, miR-149-5p often targets signaling pathways, including TGF- β , Wnt/ β -catenin, and NF κ B. Hence, this study focused on miR-149-5p and its biomarker, interleukin-6 (IL-6), as potential indicators of CKD. Computational analyses were employed for miR-149-5p identification within CKD genome sequences, revealing its potential involvement in disease processes. **Methods:** In this study, a total of 20 patient and normal blood samples were collected and stored for analysis. To detect miR-149-5p in CKD, target scanning, miRbase, and the National Centre for Biotechnology Information (NCBI) database were used, whereas RNA fold, melt curve, and Ct were utilized to construct the secondary structure and analyze miR-149-5p levels. A similar investigation was conducted to determine the IL-6 gene's expression levels. **Results:** Upon thoroughly studying the secondary structure, hsa-miR-149-5p's lowest free energy was found to be -52.70 kCal. Additionally, dys-regulation of miR-149-5p and IL-6 expression in individuals with CKD was observed. miR-149-5p down-regulation and IL-6 over-expression pointed toward the potential role of these two molecules in the pathogenesis of CKD. **Conclusion:** The computational techniques utilized explain miR-149-5p's role as a diagnostic, predictive, and potentially effective therapeutic target for CKD. Moreover, these findings contribute to a better understanding of CKD, along with miR-149-5p's role in developing novel treatments for this disease.

Key words: Chronic kidney disease, Interleukin -6, microRNA-149-5p, gene expression, treatment

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INTRODUCTION

CKD is an ailment marked by a reduction in glomerular filtration rate or the presence of proteinuria, with diabetic nephropathy and hypertension being the primary cause and renal damage being the main factor in most cases. Poor appetite, lethargy, insomnia, muscle cramps, itching, and swollen feet and ankles are the common symptoms of CKD. Two common indicators of CKD are elevated levels of creatinine and reduced albumin. Because a conclusive cure is not available for this condition, the main aim from a clinical perspective is to reduce the progression of renal failure, typically addressing sequelae such as left ventricular hypertrophy, vascular calcification, and anemia¹⁻⁴. Numerous studies have reported microRNAs (miRNAs) as being innovative tools for disease diagnosis or potential targets for therapy. miRNAs are non-coding RNA (ncRNA) molecules that mainly participate in the post-transcriptional regulation of genes and are widely studied in the context of CKD. The

main sources of miRNAs are plasma, serum, urine, or exosomes, which are small membrane vesicles containing various proteins, lipids, and nucleic acids⁵. MiR-149-5p, a conserved mRNA, has been identified as a regulator of physiological processes such as inflammatory responses, adipogenesis, and cell proliferation. This molecule is expressed in diverse tissues, such as the brain, liver, and adipose tissue, and affects gene expression as well as cellular activities (differentiation, proliferation, and apoptosis). Recent studies indicate its function as an oncogene, along with its role in regulating pathways like the Wnt signaling pathway, crucial for cancer development and progression. Additionally, miR-149-5p plays a vital role in the immune response and inflammation regulation, whereas miR-149-5p dysregulation has been associated with numerous diseases like cancer, metabolic disorders, and cardiovascular diseases^{6,7}. miR-149-5p often targets signaling pathways, including TGF- β , Wnt/ β -catenin, and NF κ B⁸⁻¹⁰. As miRNAs can

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serve as biomarkers, changes in the expression levels of miR-149-5p may provide insights into disease progression and severity. Meanwhile, interleukin-6 (IL-6) plays a pivotal role in the pathogenesis of CKD, promoting inflammation, fibrosis, and cardiovascular problems¹¹. Furthermore, miR-149-5p, when dysregulated, may influence IL-6 expression, potentially amplifying the inflammatory and fibrotic processes in CKD. Therefore, understanding the role of miR-149-5p in CKD could open avenues for therapeutic intervention. Modulating the expression of this miRNA might be explored as a potential strategy to mitigate CKD-associated pathologies.

METHODS

Retrieval of CKD sequences and miRNAs

Data on human genome sequences was obtained from the National Center for Biotechnology Information (NCBI) web portal for the International Nucleotide Sequence Database Consortium. The CKD genome sequence was located using a free search engine using the search term “chronic kidney disease genome sequence in *Homo sapiens*.” Following the removal of repetitive and inadequate sequences, a local nucleotide database dedicated to CKD-specific genome sequences was established. The reference sequence was obtained from human-reported miRNAs (<http://www.mirbase.org/>). The aforementioned CKD database was then queried to identify homologs within the miRNA dataset¹².

Identification of precursor miRNAs

Mature miRNAs were used as a starting point for searches to find homologs in the CKD database. miRNA sequences were used as search queries in homology searches using the Basic Local Alignment Search Tool (BLAST) 2.2.26+ against a recently created local database specific to hypothyroidism, employing 0.01 as the e-value threshold while keeping all other parameters at their default levels. For sequences with a maximum of three mismatches, BLAST was used to validate the non-protein encoding features. The aligned section was then designated as a potential precursor (pre)-miRNA sequence¹².

Validation of candidate pre-miRNAs and their targets

Using RNA fold, a secondary structure was derived, yielding a mature miRNA sequence within the CKD sequence. Several criteria were employed for confirmation: 1) the RNA structure should exhibit a suitable stem-loop hairpin structure, 2) the mature miRNA

should be located on one side of the hairpin structure, 3) the miRNA must have fewer than seven mismatches with the complementary miRNA in the opposing arm of the structure, and 4) the secondary structure should possess more negative energy and an A+U content of between 40 and 70%. Additionally, target prediction was performed using TargetScan to help find possible targets.

Sample collection

This study received approval from the Institutional Ethics Committee (581/03/2023/UG/S RB/SMCH), and all samples were gathered in strict adherence to the Helsinki Declaration. The sample size for the study was calculated using Gpower, and a set of 20 blood samples, comprising both CKD and normal cases, was acquired from patients who provided informed consent through the Department of Medicine at Aveetha Medical College and Hospitals. The Department of Biochemistry at Saveetha Medical College and Hospitals confirmed the diagnosis of hypothyroidism. Following collection, the samples underwent centrifugation. While spinning the blood with an anticoagulant, the lighter plasma formed the upper layer and was carefully extracted¹³. This was then preserved in a deep freezer at -20°C for subsequent analysis.

INCLUSION AND EXCLUSION CRITERIA

The study only accepted participants who were over 18 years old and able to provide informed consent. Individuals with secondary complications, such as hypertension or diabetes were excluded from participation.

RNA EXTRACTION AND QUANTIFICATION

TRIzol reagent (Invitrogen, Carlsbad, CA, USA), which was added to the plasma, was used for total RNA extraction following the manufacturer's instructions. Using a NanoDrop 2000 Lite spectrophotometer from Thermo Fisher Scientific, both the quantity and quality of the extracted RNA were evaluated. After that, these samples were kept at -20°C for later analysis¹⁴.

REVERSE TRANSCRIPTION

The obtained RNA was subjected to reverse transcription. This process includes combining the RNA sample with an oligo (dT)18 primer (Promega, 50 μM) for genes and a universal adapter for miRNAs, along with

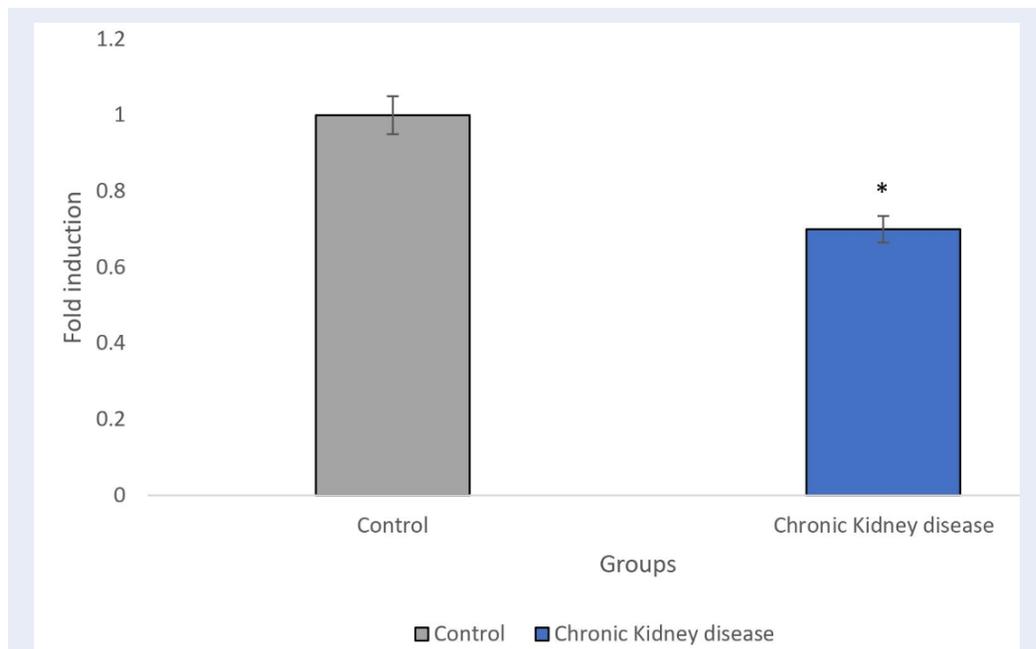


Figure 2: Figure represents the expression Levels of miR-149-5p in CKD and control sample $P < 0.05$ is considered statistically significant.

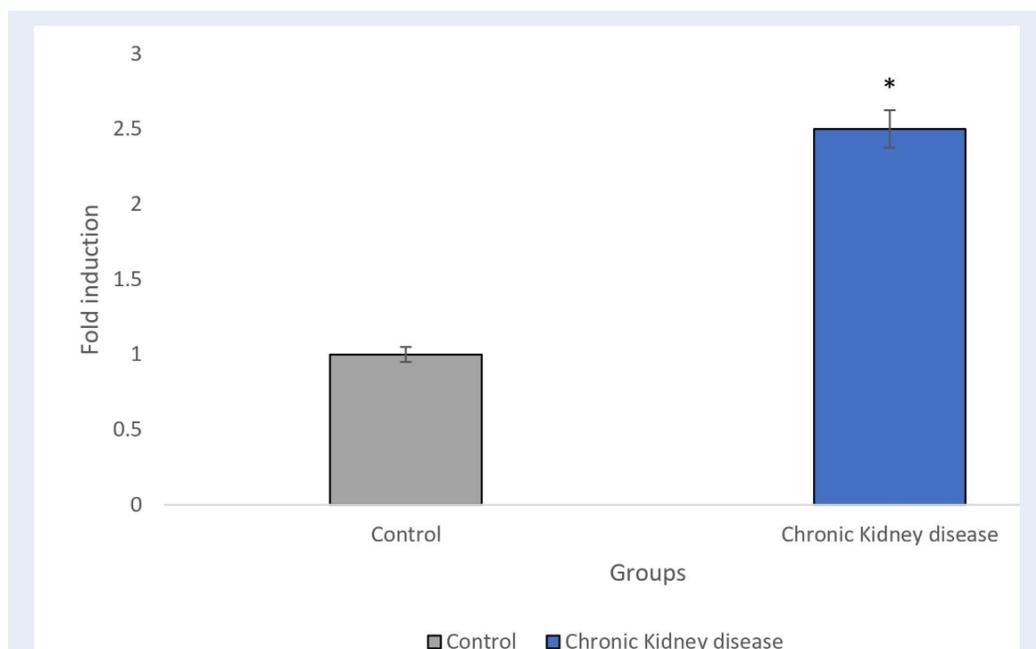


Figure 3: Figure represents the expression levels of IL6 in CKD vs normal patients. The IL6 levels were significantly elevated in CKD when compared to normal. (* $P < 0.05$, Control vs CKD).

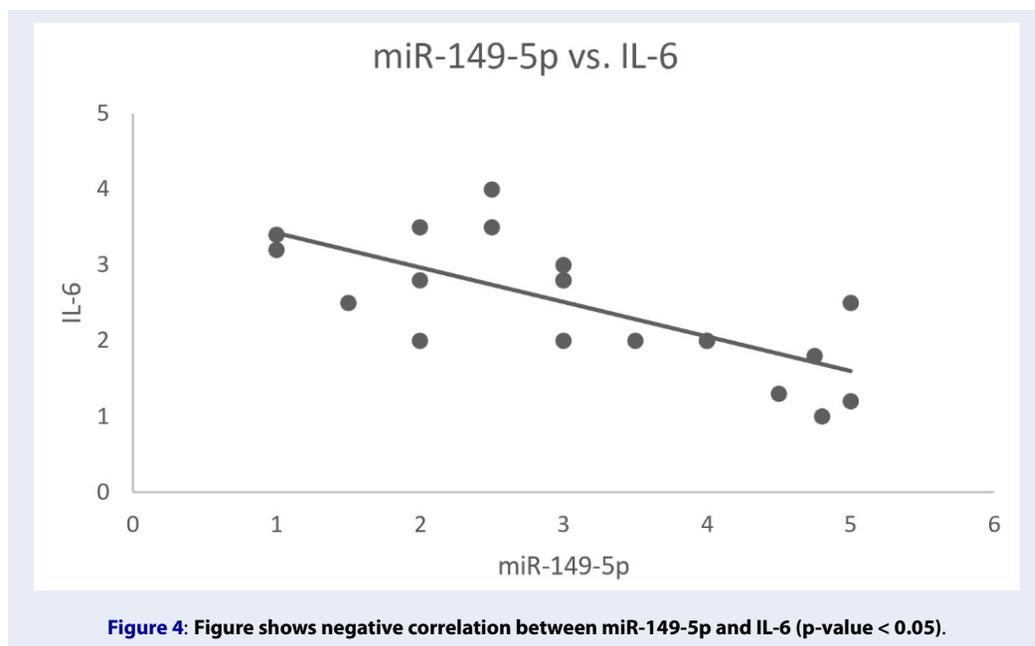


Table 1: The stemloop and mature sequence of miR-149a-5p

No	Structure	Sequence
1	Stem-loop	GCCGGCGCCGAGCUCUGGCUCGUGUCU UCACUCCGUGCUUGUCCGAGGAGGGAGGGAGGG ACGGGGGCUGUGCUGGGGCAGCUGGA
2	Mature miRNA	UCUGGCUCGUGUCUUCACUCCC

Table 2: The pre-miRNA length, minimum free energy, mature sequence, match extent, and A+U% content of has-miR-149-5p

Source miRNA	Source organism	Pre-miRNA length	Minimum Free Energy	Mature Sequence	Match Extent	Strand	A+U%
miR-149-5p	Homo sapiens	89	- 52.70 kcal	UCUGGCUCGUGU CUUCACUCCC	23/23	5p	26.9

Table 3: The target genes of miR-149-5p with their molecular function and biological process

No	Target Protein	Molecular function	Molecular function
1	Interleukin 6	Multifunctional cytokine	Cellular function
2	BCL2 binding component 3	Binding of BCL2	Cell apoptosis
3	Transmembrane protein 234	Signal transmission	Transfer of molecules across the membrane
4	Tetraspanin 14	Enzyme binding activity	Notch signalling
5	Zinc finger protein 74	transcription	Inhibits acetylation

ing the length of the pre-miRNA, minimum free energy, mature sequence, match extent, and A+U% content of hsa-miR-149-5p.

Identification of targets

TargetScan analysis pinpoints potential targets for the specific miRNA. Through this approach, several significant transcripts targeted by miR-149-5p, including IL-6, cyclin 1, BCL-2, cell division cycle 73, and transferrin receptor, among others, were identified. Table 3 provides an overview of these targets, along with their associated molecular functions and biological processes.

Gene expression analysis of miR-149-5p and IL-6

qRT-PCR gene expression analysis revealed the levels of miR-149-5p and IL-6 in blood samples from CKD patients, which were compared with those of normal individuals. When compared, CKD patients, particularly individuals with hypothyroidism, showed significant down-regulation in miR-149-5p levels and an increase in IL-6 levels. These findings suggest a potential role for miR-149-5p as well as IL-6 in CKD, which is to be further investigated. **Figure 2** and **Figure 3** depict the differential gene expression of miR-149-5p and IL-6 in CKD and normal blood samples. The relationship between increased IL-6 levels and decreased miR-149-5p levels in CKD patients suggests an inverse or negative correlation (as shown in **Figure 4**), thereby suggesting a systematic relationship between the two, with changes in one being associated with opposite changes in the other.

DISCUSSION

CKD is a major threat to public health, impacting millions of individuals, as it represents a progressive and irreversible decline in kidney function that can ultimately progress to end-stage renal disease (ESRD). Inflammation, marked by an increase in pro-inflammatory cytokine levels such as IL-6, has a key role in the genesis of CKD. CKD is characterized by a chronic inflammatory state and increased oxidative stress, both of which contribute to disease progression and complications. Simultaneously, the dysregulation of key signaling pathways, such as NF κ B, Wnt/ β -catenin, and TGF- β , is implicated in CKD pathophysiology. These pathways are associated with inflammation, fibrosis, and abnormal cellular responses in the kidneys. Studies indicate that miR-149-5p frequently targets these signaling pathways⁸⁻¹⁰. Moreover, miR-149-5p has the potential to impact the cellular response to oxidative stress and inflammatory processes

within the kidneys. By modulating these pathways, miR-149-5p may influence the intricate balance between inflammation and oxidative stress, contributing to the overall progression of CKD. Moreover, the observed relationship between elevated IL-6 levels and decreased miR-149-5p levels in individuals with CKD implies the presence of an inverse correlation. Such a correlation holds significance as it highlights a reciprocal influence between IL-6 and miR-149-5p levels, shedding light on potential regulatory mechanisms or interactions within the context of CKD.

Understanding these relationships provides valuable insights into the intricate molecular dynamics associated with CKD pathology and may have implications for diagnostic or therapeutic approaches targeting these molecular components. Understanding the role of miR-149-5p in inflammation and oxidative stress holds significant clinical relevance in the context of CKD. Moreover, insights into miR-149-5p's regulatory role could inform the development of targeted therapeutic interventions, aiming to modulate inflammation and oxidative stress to slow CKD progression. The clinical implications extend to personalized medicine, where understanding miR-149-5p's involvement may lead to tailored interventions based on the specific molecular mechanisms underlying inflammation and oxidative stress in individual CKD patients. Overall, deciphering the role of miR-149-5p in CKD pathophysiology offers a promising avenue for advancing diagnostic and therapeutic strategies, ultimately improving patient outcomes in this prevalent and challenging condition.

CONCLUSIONS

In this study, we comprehensively assessed miR-149-5p and IL-6 expression in CKD patients compared to a control group of healthy individuals. Gaining a profound understanding of these molecular components holds the promise of pioneering new pharmaceutical strategies and enhancing therapeutic options for this prevalent condition, ultimately improving patient care for those with renal issues and consequently reducing morbidity and mortality rates.

ABBREVIATIONS

CKD: Chronic kidney disease; **miRNA:** microRNA; **IL-6:** Interleukin 6; **ncRNA:** non-coding RNA; **NCBI:** National Centre for Biotechnology Information; **BLAST:** Basic Local Alignment Search Tool; **dNTP:** deoxyribonucleotide triphosphates; **ESRD:** end-stage renal disease

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AUTHOR'S CONTRIBUTIONS

Formal analysis, D.S; writing — original draft preparation, M.A; writing — review and editing; A.P; editing — revising; A.P. All authors read and approved the final manuscript.

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None.

AVAILABILITY OF DATA AND MATERIALS

Data and materials used and/or analyzed during the current study are available from the corresponding author on reasonable request.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

CONSENT FOR PUBLICATION

Not applicable.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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