UDC 577.21:616.61-036.12-07 **doi:** https://doi.org/10.15407/ubj98.04.065

ESTIMATION OF CIRCULATING MICRORNAS AS BIOMARKERS IN EARLY STAGES OF CHRONIC KIDNEY DISEASE

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Received: 29 April 2026; **Revised:** 24 June 2026; **Accepted:** 27 July 2026

Background. *Chronic kidney disease (CKD) is a progressive disorder that affects 10–13% of the worldwide population. The conventional biomarkers, like serum creatinine and estimated glomerular filtration rate (eGFR), have poor sensitivity in detecting early stage CKD, especially as it relates to Type 2 Diabetes Mellitus (T2DM). Recent studies suggest that circulating microRNAs (miRNAs) are sensitive molecular markers of renal pathology.* ***Objectives.*** *This study quantified the serum levels of miR-21, miR-155 and miR-192 in early-stage CKD patients with or without co-existing T2DM and determined the diagnostic performance of these microRNAs compared to cystatin C and eGFR.* ***Methods.*** *A case-control study was conducted with a total of 100 participants classified into three groups; healthy controls (n = 30), CKD patients with T2DM (n = 35) and CKD patients without T2DM (n = 35). The expression of serum miRNA was measured with real-time quantitative PCR using the $2^{-\Delta\Delta Ct}$ method, U6 snRNA as internal reference. Cystatin C was determined by particle-enhanced immunonephelometry; eGFR was calculated using the CKD-EPI equation. Statistical analyses were performed using Kruskal-Wallis test, Spearman partial correlations, receiver operating characteristic (ROC) analysis, and multivariate logistic regression.* ***Results.*** *Significant upregulation of all three miRNAs in both CKD groups ($P < 0.001$) with a differing magnitude of fold-change compared with controls and the highest amplification for miR-192 was detected. ROC analysis identified miR-192 to be the best performing single biomarker (AUC = 0.937; 95% CI: 0.880–0.978). Spearman partial correlation networks showed strong positive relations of miRNAs with cystatin C (ρ = 0.74–0.80) and stronger negative associations with eGFR ($\rho = -0.69$ to -0.78).* ***Conclusions.*** *Circulating miR-21, miR-155 and miR-192 are promising minimally invasive biomarkers better than conventional markers for early CKD detection. Cystatin C was co-measured with it, yielding a highly accurate diagnostic panel especially in the diabetic nephropathy subphenotype. Clinical applicability requires validation in large multicentre cohorts.*

K e y w o r d s: microRNA, chronic kidney disease, biochemical markers, cystatin C, eGFR, ROC analysis.

Chronic kidney disease (CKD) is one of the leading global health challenges of the twenty-first century. Globally, the estimated number of CKD in people is 697.5 million and a prevalence of 9.1%, by pooled analyses of population-based studies [1]. Despite this major epidemiological burden, CKD is still commonly under-diagnosed in its earlier, potentially reversible stages primarily since the so-called gold standard diagnostic triumvirate serum creatinine combined with urinary albumin-to-creatinine ratio (UACR) and estimated glomerular filtration rate (eGFR) has well-known limitations of sensitivity and specificity specifically when confounded by comorbidities like type 2 diabetes mellitus (T2DM) [2].

Diabetes mellitus is the leading cause of CKD in both developed and developing countries, and accounts for around 30–40% of new end-stage renal disease (ESRD) cases [3]. Pathophysiology of DN includes haemodynamic, metabolic and inflammatory mechanisms that work together to induce podocyte loss, mesangial expansion and progressive tubulointerstitial fibrosis. Importantly, substantial structural injury may accumulate before serum creatinine rises above reference interval as creatinine production is exquisitely sensitive to muscle mass

and dietary protein intake [4]. Cystatin C a cysteine protease inhibitor that is produced at a constant rate by all nucleated cells and freely filtered across the glomerulus has thus emerged as a more reliable surrogate of GFR, particularly in those with lower muscle mass or early-stage CKD [5]. However, neither cystatin C nor eGFR provides information linking phenotypes of renal injury with action mechanism(s) at the molecular level [6].

MicroRNAs (miRNAs) are small (~22 nucleotide), endogenously expressed, single-stranded non-coding RNA molecules that post-transcriptionally regulate gene expression by binding to the 3′-untranslated region (3′-UTR) of target messenger RNAs (mRNAs), thereby mediating their translational repression or degradation [7]. MiRNAs circulating as exosomes, Argonaute complexes or associated with high-density lipoproteins are notably stable in blood and urine, making them promising candidates for liquid biopsy-based diagnostics [8].

In particular, miR-21, miR-155 and miR-192 have attracted significant investigational attention among the renal-expressed miRNAs. For example, miR-21 is a kidney-enriched and the most highly expressed miRNA which targets phosphatase and tensin homolog (PTEN) and programmed cell death protein 4 (PDCD4) to promote fibrogenic TGF-β/Smad signalling while suppressing apoptosis of tubular cells during hypoxic stress [9]. miR-155 is the other key microRNA that regulates the NF-κB inflammatory cascade and it has been associated with macrophage-induced renal inflammation in cases of both immune-mediated and metabolic nephropathy [10]. Internalization of excess levels of miR-192, a TGF-β-responsive miRNA that is abundantly expressed in the renal cortex [11], represents one such key alteration consistently upregulated in DN and directly repressing transcription factors ZEB1/ZEB2 to drive epithelial-to-mesenchymal transition (EMT) and collagen deposition [12].

Although a growing body of literature exists regarding the association of such miRNAs in vitro and in animal models, clinical evidence for an association with early CKD - particularly CKD stages G1–G3 as defined by the Kidney Disease: Improving Global Outcomes (KDIGO) 2022 guidelines - is limited and methodologically heterogeneous [13]. Published study often overlook the diabetic vs. non-diabetic nephropathy distinction, use various normalisation approaches and have relatively small sample numbers, making comparison across studies difficult [14]. Moreover, to the best of our knowledge, previous studies did not assess at once partial-correlation network structure within these three miRNAs and classical renal functional markers in a stratified diabetic/non-diabetic CKD population [15].

Accordingly, the current study was aimed to formulated and involve of the quantifying serum miR-21, miR-155, and miR-192 in well-characterised early CKD patients stratified by comorbidity with T2DM, compare diagnostic performance of each against cystatin C and eGFR-CKD-EPI, create a partial-correlation biomarker network to delineate molecular–biochemical interdependencies, and assess composite panel diagnostic accuracy through multivariate logistic regression followed by ROC analysis.

Materials and Methods

Study design and ethical approval. This was a prospective case-control study carried out from January 2024 to December 2025 at the Nephrology Unit in Merjan Teaching Hospital and Department of biology, College of science, Al-Qasim Green university. The study protocol was reviewed and approved by Al-Qasim Green, Institutional Review Board (IRB-2025-Med-0047). All participants provided written informed consent in accordance with the Declaration of Helsinki (2013 revision). Patient data were de-identified and stored on a secured institutional server.

Groups and participants selection. A total of 100 participants were enrolled and randomly assigned to three groups. Group I – healthy controls (n = 30) age and sex matched healthy volunteers with no history of renal disease, diabetes, hypertension or any systemic illness. Inclusion: eGFR ≥ 90 ml/min/1.73 m^2, UACR 40 kg/m^2; severe anaemia (haemoglobin < 8 g/dl); and blood transfusion or major surgery within 30 days. Group II – patients with chronic kidney disease and type 2 diabetes mellitus (CKD+T2DM) (n = 35). The diagnosis of hyperotensive patients, denoted by higher diastolic blood pressure levels was established from the Nephrology Unit. CKD was defined as per KDIGO 2022 guidelines based on the presence of evidence of kidney damage and/or decreased renal function lasting >3 months. An electronic search for relevant trial articles was conducted across multiple observational studies from 2011 to October 2023; inclusion criteria consisted of age ≥18 years, established diagnosis of T2DM at least one year prior and CKD stages G1–

G3 (eGFR ≥30 ml/min/1.73 m^2) with stable clinical condition during the previous three months. Graduation conditions included intense renal harm, type 1 diabetes more established than the age (age) of study with serious determination illness, discharge treatment cases in less one calendar month into recruiting days per individual frame on ground ward/ICU. Group III – CKD patients without T2DM (CKD-only) (n = 35). This group included patients with chronic kidney disease but no history or laboratory evidence of diabetes mellitus. The diagnostic criteria and CKD stage classification of participants were met by those in Group II (KDIGO 2022, stages G1–G3). Eligibility base age: ≥ 18 years and stable renal disease for at least three months. Exclusion CriteriaIndividuals with diabetes mellitus, acute kidney injury, active inflammatory or infectious diseases, malignancy and pregnancy or recent major surgery were excluded. The CKD-only group was chosen because it allowed for the assessment of renal dysfunction independently from metabolic derangements related to diabetes.

Clinical and anthropometric assessment. At enrolment, a structured medical history was obtained. Systolic and diastolic blood pressure were measured in triplicate with a portable aneroid sphygmomanometer after five minutes of seated rest. BMI was defined as weight (kg)/height (m)^2. The disease duration (CKD and T2DM) was extracted from medical records and confirmed with the subject.

Fasting venous samples (10 ml) were obtained between 07:00 and 09:00 h to minimise the effects of diurnal variation. Blood for serum biochemistry was collected into serum-separating tube (SST) and allowed to clot at room temperature for 30 min before centrifugation (3,000 × g for 10 min at 4°C), while RNA extraction was carried out from the remaining blood by collecting 4 mL into a PAXgene Blood RNA tube (PreAnalytiX, Qiagen) and storing it at −80°C within 4 hours of collection.

Biochemical analyses. Serum creatinine was determined using the kinetic Jaffe method (Roche Cobas c501 analyser; inter-assay CV < 2.5%). Elderly patients were defined as those aged over 65 years, and eGFR was calculated using the CKD-EPI Creatinine-Cystatin C 2021 equation [11]. Serum cystatin C was measured using particle-enhanced immunonephelometry (N Latex Cystatin C, Siemens Healthineers; analytical range 0.195–7.330 mg/l; inter-assay CV <3.1%) Haemoglobin was measured by high-performance liquid chromatography (Bio-Rad Variant II Turbo) and glycated haemoglobin (HbA1c) calculated. Fasting glucose, blood urea nitrogen (BUN), uric acid, total cholesterol, triglycerides and high-density lipoprotein cholesterol (HDL-C) were performed using a Beckman Coulter AU680 analyser (Beckman Coulter Inc., Brea, CA, USA) with manufacturer-validated reagents. Urine albumin was quantified using immunoturbidimetry; UACR was calculated from the first-morning urine sample.

MicroRNA extraction and quantification. Twenty-five milliliters of serum was used for extraction of total RNA including small RNA using the miRNeasy Serum/Plasma Advanced Kit (Qiagen, Germany) according to manufacturer's instructions. For normalisation of extraction efficiency, a cel-miR-39-3p spike-in (Qiagen) was added at the lysis step. RNA concentration and purity (A260/A280 and A260/A230) were determined using a NanoDrop OneC spectrophotometer (ThermoFisher Scientific). Samples with an A260/A280 ≥ 1.8 were processed for further analysis.

Reverse transcription was performed using the TaqMan™ Advanced miRNA cDNA Synthesis Kit (Applied Biosystems) – 5 µl of total RNA was added to each reaction. RT-qPCR was performed using TaqMan™ Advanced miRNA Assays for hsa-miR-21-5p (Assay ID: 477975_mir), hsa-miR-155-5p (Assay ID: 477910_mir), and hsa-miR-192-5p (Assay ID: 477893_mir) in a QuantStudio 7 Flex Real-Time PCR System (Applied Biosystems); for targeting U6 snRNA, the endogenous control RNU6B was used. All reactions were carried out in technical triplicates. Relative quantification was performed using the $2^{-\Delta\Delta Ct}$ (delta-delta Ct) method [16], with U6 snRNA as the internal reference and the mean Ct of healthy controls as a reference calibrator.

Statistical analyses. The Shapiro-Wilk test was used to evaluate data normality. Continuous variables with normal distribution are expressed as mean ± standard deviation (SD); those that do not meet normality, as median [interquartile range (IQR)]. For between-group comparisons, one-way ANOVA (normal data) or Kruskal-Wallis test with Dunn-Bonferroni correction (non-normal data). Pearson's chi-square test was used to compare categorical variables. Pairwise correlations were calculated with Spearman rank partial correlation, while controlling for age, sex and BMI; the resulting matrix was visualised as a weighted, undirected network graph using a custom Python NetworkX (v3. 2) script and drawn with edges for significant correlation (P < 0.05 after

Benjamini-Hochberg FDR). ROC analysis was used to assess the diagnostic performance of individual and composite biomarker panels; optimal cut-off values were defined by Youden J index. To identify independent predictors of early CKD, multivariate binary logistic regression was employed. Sample sizes were calculated based on target power 80% for a minimum fold-change of ≥2.5 (α = 0.05) yielding a minimum of 28 participants per group (G*Power 3.1). Analyses were conducted in SPSS v28 0 and Python 3.11 (SciPy 1.11, pandas 2.1). A two-tailed $P < 0.05$ was considered statistically significant.

Results

Demographic and clinical characteristics. Details of the baseline characteristics for all three study cohorts are described in Table 1. There were no significant differences in age, sex distribution, or BMI between the three groups ($P > 0.05$ for all), thus adequate matching was confirmed. As anticipated, patients with CKD+T2DM had significantly elevated HbA1c (8.4 ± 1.1%) and fasting glucose (9.2 ± 2.3 mmol/l) relative to the control and CKD-only groups ($P < 0.001$). In the CKD+T2DM group, disease duration was 8.4 ± 3.2 years for T2DM and 4.1 ± 1.8 years for CKD. The prevalence of hypertension was the highest in CKD-only group, (71.4%) and decrease to second-lowest rate of proportion in CKD+DM (60.0%), are both significantly higher than that for control (0%). Systolic blood pressure was significantly higher for both CKD groups compared with the controls ($P < 0.001$).

MicroRNA expression levels. Serum miR-21, miR-155, and miR-192 were significantly upregulated in both CKD groups relative to healthy controls (all $P < 0.001$; Kruskal-Wallis with Dunn-Bonferroni correction). The CKD+T2DM group exhibited the highest expression for all three miRNAs, followed by the CKD-only group (Fig. 1). Detailed descriptive statistics are presented in Table 2.

Diagnostic performance of individual and composite biomarkers. ROC analysis was conducted to determine the optimal diagnostic cut-off for each biomarker in distinguishing early CKD from healthy controls. Among individual miRNAs, miR-192 demonstrated the highest diagnostic accuracy (AUC = 0.937), followed by miR-21 (AUC = 0.914) and miR-155 (AUC = 0.905). These values exceeded those of the conventional markers cystatin C (AUC = 0.889) and eGFR (AUC = 0.871) (Fig. 2; Table 3). A stepwise multivariate logistic regression identified miR-192, cystatin C, and eGFR as independent predictors of early CKD (all $P < 0.01$). A composite three-biomarker panel achieved AUC = 0.971 (95% CI: 0.932–0.993), sensitivity = 91.4%, and specificity = 93.3%. AUC values with 95% confidence intervals are provided in Table 3.

Spearman partial correlation network among biomarkers. To describe the interdependence structure between circulating miRNAs and classical renal biomarkers, Spearman partial correlations were calculated for each pairwise combination of all eight analytes after adjustment for age, sex and BMI. Correlations were visualised als a weighted undirected

Table 1. Baseline demographic and clinical characteristics of study groups

Parameter	Controls (n = 30)	CKD + T2DM (n = 35)	CKD-only (n = 35)
Age (years)	41.3 ± 9.8	48.7 ± 11.2	46.1 ± 10.5
Sex (M/F)	17/13	20/15	19/16
BMI (kg/m^2)	25.2 ± 3.1	28.9 ± 4.2	27.1 ± 3.8
SBP (mmHg)	118 ± 9	146 ± 14***	142 ± 13***
HbA1c (%)	5.2 ± 0.3	8.4 ± 1.1***	5.4 ± 0.4
Serum creatinine (mg/dl)	0.85 ± 0.15	3.80 ± 1.10***	2.90 ± 0.90***
Cystatin C (mg/l)	0.82 ± 0.12	2.95 ± 0.75***	2.20 ± 0.65***
eGFR (mlmin/1.73 m^2)	96.4 ± 9.8	27.8 ± 7.9***	42.3 ± 11.0***
BUN (mg/dl)	14.2 ± 3.1	42.8 ± 12.5***	31.4 ± 9.8***
UACR (mg/g)	18.4 ± 5.2	285.6 ± 98.4***	194.3 ± 74.1***

Note. Data are mean ± SD unless stated. ***$P < 0.001$ vs. controls (Tukey post-hoc). BMI – body mass index; SBP – systolic blood pressure; UACR – urinary albumin-to-creatinine ratio

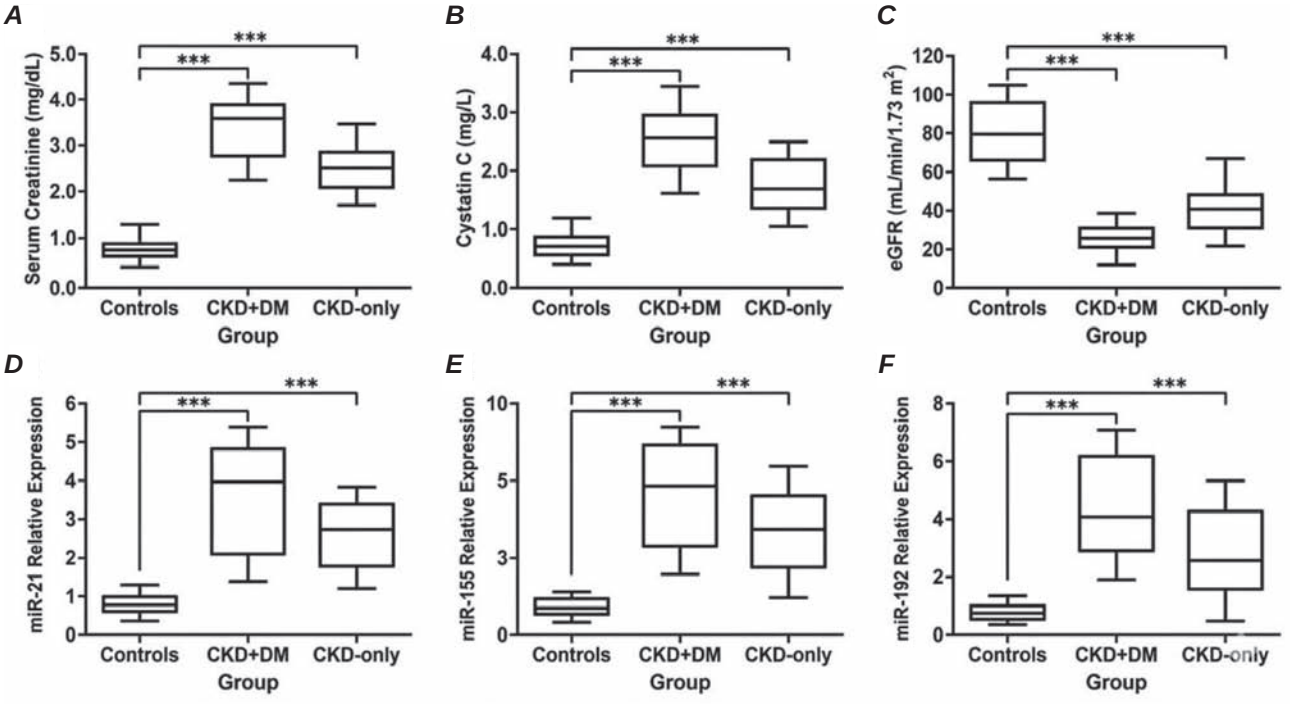

Fig. 1. Distribution of serum biochemical and miRNA biomarkers across the three study groups depicted as box-and-whisker plots. Panels ***A*****–*****C:*** *conventional renal function markers (creatinine, cystatin C, eGFR). Panels* ***D*****–*****F****: miRNA relative expression values. ***P < 0.001 vs. controls. Relative expression (fold change ± SEM) of miR-21, miR-155, and miR-192 in control, CKD+T2DM, and CKD-only groups. U6 snRNA was used as internal reference. **P < 0.01, ***P < 0.001 vs. controls*

Table 2. Relative expression levels of miR-21, miR-155, and miR-192 across study groups

miRNA	Controls	CKD+T2DM	CKD-only	*P*-value
miR-21	1.00 ± 0.25	4.85 ± 1.10†‡	3.40 ± 0.90†	< 0.001
miR-155	1.00 ± 0.20	5.20 ± 1.25†‡	3.70 ± 0.95†	< 0.001
miR-192	1.00 ± 0.22	6.10 ± 1.50†‡	4.20 ± 1.10†	< 0.001

Note. †$P < 0.001$ vs. controls; ‡$P < 0.01$ vs. CKD-only (Dunn-Bonferroni). Values are mean ± SD relative expression (fold change) by $2^{-\Delta\Delta ct}$ method

Table 3. Diagnostic performance of individual biomarkers and composite panel for early CKD detection

Biomarker	AUC	95% CI	Cut-off	Sensitivity, %	Specificity, %	Youden J
miR-192	0.937	0.880–0.978	≥2.8 FC	88.6	90.0	0.786
miR-21	0.914	0.852–0.958	≥2.2 FC	85.7	86.7	0.724
miR-155	0.905	0.840–0.951	≥2.4 FC	84.3	86.7	0.710
Cystatin C	0.889	0.820–0.938	≥1.35 mg/l	82.9	86.7	0.696
eGFR	0.871	0.798–0.925	≤60 ml/min	80.0	83.3	0.633
Composite panel[a]	0.971	0.932–0.993	Model score	91.4	93.3	0.847

Note. [a]Composite panel – miR-192 + Cystatin C + eGFR (multivariate logistic regression score). FC – fold change; AUC – area under ROC curve; CI – confidence interval

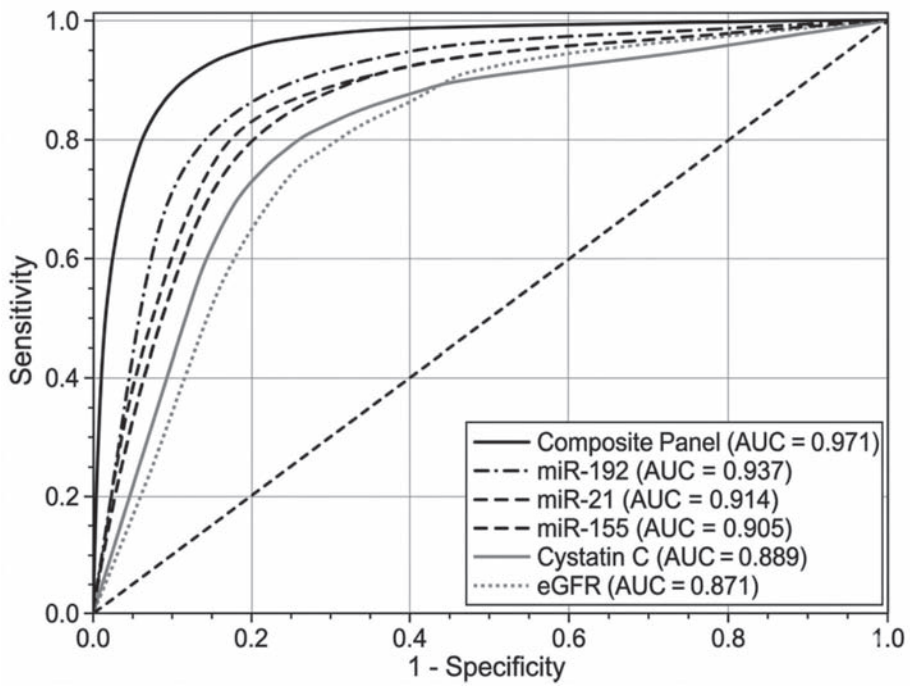

Fig. 2. Receiver operating characteristic (ROC) curves for individual biomarkers and the composite panel in early CKD detection

network graph (Fig. 3) for significant correlations (FDR-corrected $P < 0.05$). We demonstrated extensive connectivity and hub architecture, with miR-192 and cystatin C at the most connected nodes (6 and 5 significant connections, respectively).

All three miRNAs showed positive partial correlations with each other (miR-21–miR-155: $\rho = 0.68$; miR-21–miR-192: $\rho = 0.71$; miR-155–miR-192: $\rho = 0.65$), suggesting co-regulation consistent with common upstream TGF-β/Smad3 transcriptional activation. miR-192 had the highest positive correlation with cystatin C ($\rho = 0.80$; $P < 0.001$) and strongest inverse correlation with eGFR ($\rho = -0.78$; $P < 0.001$), which further confirmed its striking sensitivity to decline in glomerular filtration rate. Cystatin C and creatinine were strongly correlated ($\rho = 0.81$), while cystatin C and eGFR displayed the strongest inverse correlation in the network ($\rho = -0.85$). HbA1c had moderate positive correlations with miR-21 ($\rho = 0.55$) and miR-155 ($\rho = 0.60$), which indicated the effect of chronic hyperglycaemia on these circulating mediators reflected in their additional miRNA-dysregulating effect.

Discussion

The results of present study show that serum miR-21, miR-155 and miR-192 are strongly upregulated in the early stages of CKD and display diagnostic performance that exceeds serum creatinine measure and is commensurate with or better than cystatin C. The uniquely innovative aspects of this work include stratification according to diabetic versus non-diabetic CKD phenotype within the same cohort, construction of a partial-correlation biomarker network, and derivation of a composite diagnostic panel yielding near ideal AUC scores.

The observed upregulation of miR-192 in CKD, and especially T2DM, is concurrent with the early mechanistic study published by Motawea et al. [17] that serum miR-192 levels were significantly reduced in patients with diabetic nephropathy compared with diabetic patients without nephropathy and healthy controls, suggesting the possible protective role of miR-192 in early disease stages. In subsequent clinical studies, increased urinary miR-192 was confirmed in patients with diabetic nephropathy [18]. Our findings expand this to the analysis of circulating serum miR-192, demonstrate the highest fold-change noted in the CKD+T2DM cohort (6.10 ± 1.50 versus 4.20 ± 1.10 in CKD-only), and elucidate that the diabetic milieu augments TGF-β-dependent miR-192 transcription, likely based on advanced glycation end-product-induced Smad3 phosphorylation [19].

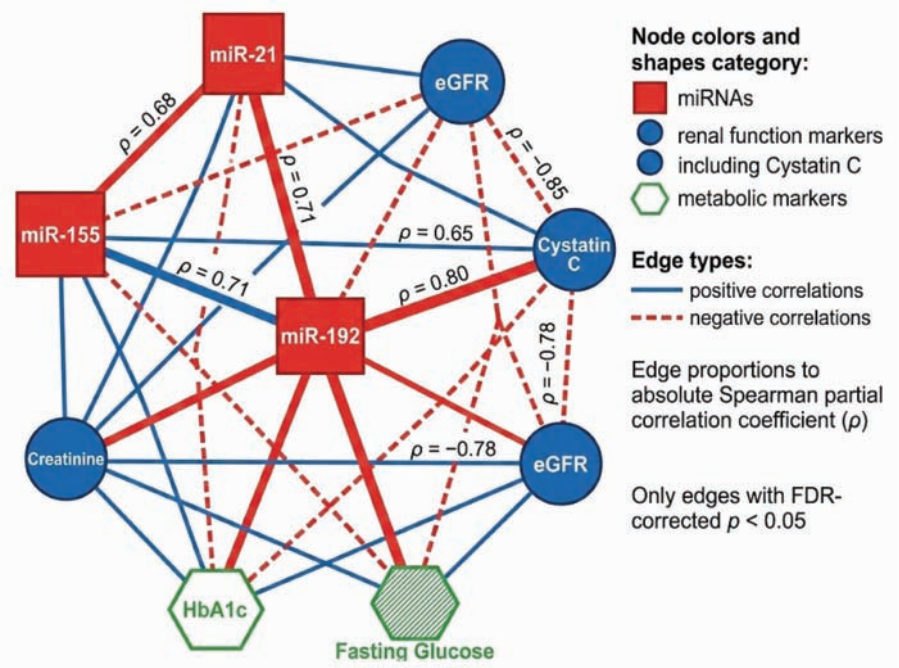

Fig. 3. Network graph of Spearman partial correlations among circulating miRNAs and biochemical biomarkers, adjusted for age, sex, and BMI (n = 100). Node colour denotes biomarker category (red – miRNA; blue – renal function; green – metabolic). Edge thickness is proportional to absolute ρ. Solid blue edges – positive correlation; dashed red edges – negative correlation. Only edges with FDR-corrected P < 0.05 are displayed. Correlation coefficients ≥ 0.70 are annotated on edges

This CKD cohort study, also demonstrates an upregulation of miR-21 that is consistent with the data from Larrue et al. [20], who demonstrated that miR-21 acts as a pro-fibrotic switch silencing PTEN and amplifying the PI3K/Akt pathway, thus driving myofibroblast activation. The finding of current study a moderate yet robust correlation of miR-21 with HbA1c (ρ = 0.55) in our network also reinforces the idea that hyperglycaemia itself, irrespective of overt nephropathy "balloon", mediates renal miR-21 upregulation as an early injury through transcriptional activation mediated by one or more reactive oxygen species. The upregulation of miR-155 in CKD has also been implicated by Pasca et al. [21] in the activation of NF-κB and subsequent macrophage M1 polarisation in the tubulointerstitium. in a mouse model of diabetic nephropathy. In our data, the relatively strong miR-155 signal in CKD+T2DM versus CKD-only (5.20 versus 3.70 fold-change) supports a diabetes-specific inflammatory amplification mechanism.

Cystatin C is by far the most clinically validated alternative to creatinine for GFR estimation, and its value has been well demonstrated in early CKD when muscle mass complicates creatinine-based estimates [22]. Even the new CKD-EPI 2021 equation that includes both creatinine and cystatin C reduces bias in eGFR because of racial ethnic group, allowing more precise prediction of progressive KCD [23]. In the present study, cystatin C (AUC = 0.889) did better than creatinine-based eGFR alone and was the strongest conventional biomarker from the composite panel. The partial-correlation network revealed a particularly strong miR-192–cystatin C axis (ρ = 0.80), possibly underscoring the common TGF-β regulation of both, since TGF-β is hypothesized to increase podocyte damage responsible for reduced glomerular filtration rate (thereby increasing cystatin C) as well as driving miR-192 transcriptional output in surviving tubular epithelial cells.

The composite panel (miR-192 + cystatin C + eGFR; AUC = 0.971) significantly exceeded the performance of any individual biomarker. This fits with the multi-biomarker paradigm of Kar et al. [24] and adds further support for the emerging consensus that renal injury is best summarized by triangulating molecular, filtration, and damage biomarkers. Practically, this composite could be used as a serum-based

test necessitating a singleton fasting blood draw and thus applicable in the primary care and resource-limited nephrology space.

The current study should be acknowledged for several methodological strengths. First, strict group matching for age, sex and BMI reduced confounding. Second, a cel-miR-39 spike-in control allowed adjustment for variability in extraction efficiency – a common source of pre-analytical bias in serum miRNA studies. Third, the application of Spearman partial correlations – controversially adjusting for age, sex and BMI – and the subsequent FDR correction yields a conservative and biologically interpretable biomarker network. However, the study population consisted mostly of Arabs and Middle Eastern populations which limits the direct generalisability to other ethnic groups where CKD aetiology profiles are different. Lastly, in this study we did not analyze the proteinuric classification in further detail than UACR and there is also a need to establish miRNA expression at specific KDIGO A-categories.

Limitations of the study. This study has several limitations. First, it was a single center study with moderate sample size which may delimited the generalization of the results to population level in regards to larger and ethnically diverse populations. Moreover, the cross-sectional case-control design of study does not permit evaluation of temporal changes in circulating microRNA expression and will also not allow causal inference regarding role of these microRNAs in CKD progression. Third, only three candidate circulating miRNAs (miR-21, miR-155 and miR-192) were examined, and no experiment to validate their biological relevance in renal injury was made on a functional or mechanistic basis. Multicenter, longitudinal studies with larger cohorts and experimental validation are needed to confirm the clinical validity of these biomarkers.

Conclusion. In conclusion, the results of this study show that serum miR-21, miR-155 and miR-192 are markedly increased in early chronic kidney disease with the highest levels occurring in patients with concurrent type 2 diabetes mellitus. All three miRNAs performed better than serum creatinine in detecting AKI and showed similar or improved performance compared to cystatin C. A composite panel of miR-192, cystatin C, and eGFR also produced near-ideal diagnostic accuracy (AUC = 0.971). A partial-correlation network revealed a biologically coherent architecture centered around miR-192 and cystatin C as hubs of high connectivity, with the miR-192–cystatin C axis representing a putative molecular-biochemical sentinel of early glomerular damage. Our data suggest that circulating miRNAs, and specifically miR-192, hold promise of being clinically actionable biomarkers for screening CKD in high-risk populations with and without diabetes. Clinical translation will require prospective longitudinal and multi-centre validation studies.

Author contributions. HHA and RSA helped in the study design, supervised all the biochemical analyses, interpreted the data and revised critically the important intellectual content of this manuscript. SHA played a role in patient recruitment, performed sample collection, some molecular analyses, statistical interpretation and final edits on the manuscript. All authors read and approved the final manuscript and are in agreement with the work.

Conflict of interest. The authors have completed the Unified Conflicts of Interest form at http://ukrbiochemjournal.org/wp-content/uploads/2018/12/coi_disclosure.pdf and declare no conflict of interest.

Funding. No funding related to the research.

Acknowledgment. The authors thank all subjects participated in this study.

ОЦІНКА ДЕЯКИХ мікроРНК ЯК БІОМАРКЕРІВ НА РАННІХ СТАДІЯХ ХРОНІЧНОГО ЗАХВОРЮВАННЯ НИРОК

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Вступ. Хронічна хвороба нирок (CKD) – це прогресуюче захворювання, яке вражає 10–13% населення світу. Традиційні біомаркери, такі як креатинін сироватки крові та розрахункова швидкість клубочкової фільтрації (eGFR), мають низьку чутливість у виявленні CKD на ранній стадії, особливо у разі цукрового діабету 2 типу (T2DM). Недавні дослідження показують, що циркулюючі мікроРНК (miRNAs) є чутливими молекулярними маркерами патології нирок. **Мета.** Визначити рівні miR-21, miR-155 та miR-192 у сироватці крові пацієнтів із CKD

на ранній стадії з супутнім T2DM або без нього та визначити діагностичну ефективність цих miRNAs порівняно з цистатином C та eGFR. **Методи.** Проведено дослідження випадок-контроль за участю 100 учасників, розділених на три групи: здорові контрольні особи (n = 30), пацієнти з CKD і з T2DM (n = 35) та пацієнти з CKD без T2DM (n = 35). Експресію мікроРНК у сироватці крові вимірювали за допомогою кількісної ПЛР у реальному часі з використанням методу $2^{-\Delta\Delta Ct}$, використовуючи U6 snRNA як внутрішній стандарт. Цистатин C визначали за допомогою імунонефелометрії з посиленням частинок; eGFR розраховували за допомогою рівняння CKD-EPI. Статистичний аналіз проводили з використанням тесту Краскела-Уолліса, часткових кореляцій Спірмена, ROC-аналізу та багатовимірної логістичної регресії. **Результати.** Було виявлено значне підвищення регуляції всіх трьох мікроРНК в обох групах пацієнтів з CKD (P < 0,001) із різною величиною кратності зміни порівняно з контрольною групою, а також виявлено найвищу ампліфікацію для miR-192. ROC-аналіз визначив miR-192 як найкращий окремий біомаркер (AUC = 0,937; 95% CI: 0,880–0,978). Мережі часткової кореляції Спірмена показали сильні позитивні зв'язки мікроРНК з цистатином C (ρ = 0,74–0,80) та сильніші негативні зв'язки з eGFR (ρ = від −0,69 до −0,78). **Висновки.** Циркулюючі miR-21, miR-155 та miR-192 є перспективними малоінвазивними біомаркерами, кращими за звичайні маркери для раннього виявлення CKD. Цистатин C вимірювався разом з ним, що забезпечило високоточну діагностичну панель, особливо у субфенотипі діабетичної нефропатії. Клінічна застосовність вимагає перевірки у великих багатоцентрових когортах.

Ключові слова: мікроРНК, хронічна хвороба нирок, біохімічні маркери, цистатин C, швидкість клубочкової фільтрації, ROC-аналіз.

References

1. Al Rashdi F, Tabche C, Atwan Z, Al-Qanubi H, Al Khaldi S, Al-Zadjali N, Rawaf S. Chronic kidney disease in hypertensive patients: the urgent need for targeted interventions in Arab countries: a systematic review. *Front Nephrol*. 2026; 6: 1735217.
2. Kostev K, Lang M, Tröbs SO, Urbisch S, Gabler M. The Underdiagnosis of Chronic Kidney Disease in Patients with a Documented Estimated Glomerular Filtration Rate and/or Urine Albumin-Creatinine Ratio in Germany. *Medicina (Kaunas)*. 2025; 61(5): 843.
3. Alicic RZ, Rooney MT, Tuttle KR. Diabetic Kidney Disease: Challenges, Progress, and Possibilities. *Clin J Am Soc Nephrol*. 2017; 12(12): 2032-2045.
4. Thomas HY, Ford Versypt AN. Pathophysiology of mesangial expansion in diabetic nephropathy: mesangial structure, glomerular biomechanics, and biochemical signaling and regulation. *J Biol Eng*. 2022; 16(1): 19.
5. Neama ED, Al-Azawi RS, Kzar HH, Naji HH, Al-Alwany, EAH. Assessment of alpha-1-acid glycoprotein (AGP) and alpha-1-antitrypsin (AAT) in patients with type 2 diabetes mellitus. *Ro J Med Pract*. 2025; 20(4): 396.
6. Obert LA, Elmore SA, Ennulat D, Frazier KS. A Review of Specific Biomarkers of Chronic Renal Injury and Their Potential Application in Nonclinical Safety Assessment Studies. *Toxicol Pathol*. 2021; 49(5): 996-1023.
7. O'Brien J, Hayder H, Zayed Y, Peng C. Overview of MicroRNA Biogenesis, Mechanisms of Actions, and Circulation. *Front Endocrinol (Lausanne)*. 2018; 9: 402.
8. Lohajová Behulová R, Bugalová A, Bugala J, Struháŕňanská E, Šafranek M, Juráš I. Circulating exosomal miRNAs as a promising diagnostic biomarker in cancer. *Physiol Res*. 2023; 72(S3): S193-S207.
9. Rodzoń-Norwicz M, Kogut P, Sowa-Kućma M, Gala-Błądzińska A. What a Modern Physician Should Know About microRNAs in the Diagnosis and Treatment of Diabetic Kidney Disease. *Int J Mol Sci*. 2025; 26(14): 6662.
10. Kozomara A, Birgaoanu M, Griffiths-Jones S. miRBase: from microRNA sequences to function. *Nucleic Acids Res*. 2019; 47(D1): D155-D162.
11. Margaritis K, Margioula-Siarkou G, Giza S, Kotanidou EP, Tsinopoulou VR, Christoforidis A, Galli-Tsinopoulou A. Micro-RNA Implications in Type-1 Diabetes Mellitus: A Review of Literature. *Int J Mol Sci*. 2021; 22(22): 12165.
12. Ren H, Wang Q. Non-Coding RNA and Diabetic Kidney Disease. *DNA Cell Biol*. 2021; 40(4): 553-567.

13. Motshwari DD, Matshazi DM, Erasmus R, Kengne AP, Matsha TE, George C. MicroRNAs associated with chronic kidney disease in the general population and high-risk subgroups: protocol for a systematic review and meta-analysis. *BMJ Open*. 2022; 12(2): e057500.
14. AbdAli MR, Hamzah EF, Neama ED, Hadi SJ, Kzar HH, Al-Azawi RS, Kadhim ZH, Aboktifa MA, Ibrahim NJ, Jassim AM, Al-Gazally ME. (2023). Investigation of the PON1 (C> T rs705379) SNP and its correlation with physiological adiponectin levels in insulin resistance T2D patients induced by obesity. *Int J Chem Biochem Sci*. 2023; 23(1): 314-319.
15. Zou Z, Zhou N, Zhang C. miRNA in the Progression of Diabetic Kidney Disease: New Insight. *Int J Mol Sci*. 2025; 27(1): 420.
16. Rao X, Huang X, Zhou Z, Lin X. An improvement of the 2ˆ(-delta delta CT) method for quantitative real-time polymerase chain reaction data analysis. *Biostat Bioinforma Biomath*. 2013; 3(3): 71-85.
17. Motawea M, Khalel MS, Kandil I, Faheem AM, Zaki MES, Abdelsalam M, Kyrillos F. Study of MicroRNA-192 as an Early Biomarker for Diagnosis of Diabetic Nephropathy. *Diagnostics (Basel)*. 2025; 15(12): 1504.
18. Wan X, Liao J, Lai H, Zhang S, Cui J, Chen C. Roles of microRNA-192 in diabetic nephropathy: the clinical applications and mechanisms of action. *Front Endocrinol (Lausanne)*. 2023; 14: 1179161.
19. Wang L, Wang HL, Liu TT, Lan HY. TGF-Beta as a Master Regulator of Diabetic Nephropathy. *Int J Mol Sci*. 2021; 22(15): 7881.
20. Larrue R, Fellah S, Van der Hauwaert C, Hennino MF, Perrais M, Lionet A, Glowacki F, Pottier N, Cauffiez C. The Versatile Role of miR-21 in Renal Homeostasis and Diseases. *Cells*. 2022; 11(21): 3525.
21. Pasca S, Jurj A, Petrushev B, Tomuleasa C, Matei D. MicroRNA-155 Implication in M1 Polarization and the Impact in Inflammatory Diseases. *Front Immunol*. 2020; 11: 625.
22. Tsai CW, Grams ME, Inker LA, Coresh J, Selvin E. Cystatin C- and creatinine-based estimated glomerular filtration rate, vascular disease, and mortality in persons with diabetes in the U.S. *Diabetes Care*. 2014; 37(4): 1002-1008.
23. Naji HH, Al-Azawi RSA, Ibrahim NJ, Kzar HH. Investigation of the Role of Zn/Cu Index and its Correlation with Physiological Activity of SOD 1 and GRx in Males with Acne Vulgaris. *Arch Razi Inst*. 2022; 77(2): 623-628.
24. Kar S, Paglialunga S, Islam R. Cystatin C Is a More Reliable Biomarker for Determining eGFR to Support Drug Development Studies. *J Clin Pharmacol*. 2018; 58(10): 1239-1247.