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MicroRNA expression and GPX-1 genetic polymorphisms in patients undergoing hemodialysis: Implications for oxidative stress in a cross-sectional study

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Abstract. *MicroRNAs (miRNAs) are crucial regulators of gene expression and have been implicated in renal pathology. The glutathione peroxidase-1 (GPX-1) gene, particularly the rs1050450 single nucleotide polymorphism (SNP), may modulate oxidative stress responses in hemodialysis patients. This study examines the interplay between miRNA expression, oxidative stress, and GPX-1 genetic polymorphisms in hemodialysis patients.*

Methods. A total of 60 hemodialysis patients and 40 healthy controls were recruited. Blood samples were collected and analyzed for miRNA expression (miRNA-143, miRNA-145, miRNA-155, and miRNA-192) using RT-qPCR. GPX-1 rs1050450 polymorphism was detected via conventional PCR and sequencing. Oxidative stress biomarkers, malondialdehyde (MDA), and 8-hydroxy-deoxyguanosine (8-OHDG) were measured using ELISA. Statistical analyses included Pearson correlation and chi-square tests, with significance set at $p < 0.05$.

Results. Hemodialysis patients exhibited significantly upregulated miRNA-143 (4.31-fold) and miRNA-155 (1.79-fold) compared to controls ($p = 0.04$). miRNA-192 expression was downregulated (0.27-fold), though not statistically significant ($p = 0.12$). Pearson correlation analysis showed a significant positive correlation between oxidative stress markers (8-OHDG, MDA) and miRNA-145, miRNA-155, and miRNA-192 ($p \leq 0.001$). Genetic analysis of GPX-1 rs1050450 revealed CC, CT, and TT genotypes in hemodialysis patients, with Hardy-Weinberg equilibrium maintained ($p = 0.46$ for patients, $p = 0.8$ for controls).

Conclusions. The differential expression of miRNAs in hemodialysis patients suggests a role in oxidative stress regulation and renal disease progression. Upregulation of miRNA-143, miRNA-145, and miRNA-155 may contribute to inflammatory and fibrotic pathways, while miRNA-192 downregulation may reflect altered renal function. The GPX-1 rs1050450 polymorphism may modulate oxidative stress responses in these patients. Further studies are needed to explore the therapeutic potential of miRNA-based interventions in CKD management.

Keywords: *gene expression, microRNAs, hemodialysis, GPX-1 gene, hemodialysis.*

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Експресія мікроРНК та генетичний поліморфізм GPX-1 у пацієнтів, які лікуються методом гемодіалізу: зв'язок з оксидативним стресом в рамках поперечного дослідження

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Резюме. *МікроРНК (miRNA) є ключовими регуляторами експресії генів і відіграють важливу роль у розвитку патологій нирок. Ген глутатіонпероксидази-1 (GPX-1), зокрема однонуклеотидний поліморфізм rs1050450 (SNP), може модулювати реакції на окислювальний стрес у пацієнтів, які проходять гемодіаліз. Це дослідження спрямоване на вивчення взаємозв'язку між експресією miRNA, рівнем оксидативного стресу та поліморфізмами гена GPX-1 у пацієнтів, які лікуються методом гемодіалізу (ГД).*

Методи. У дослідженні взяли участь 60 ГД пацієнтів та 40 здорових осіб (контрольна група). Було зібрано зразки крові для аналізу експресії miRNA (miRNA-143, miRNA-145, miRNA-155 та miRNA-192) методом RT-qPCR. Поліморфізм rs1050450 у гені GPX-1 визначали за допомогою традиційної ПЛР та секвенування. Рівень маркерів оксидативного стресу, малонового діальдегіду (МДА) та 8-гідрокси-дезоксигуанозину (8-OHDG), оцінювали методом ІФА. Статистичний аналіз включав кореляцію Пірсона та χ^2-тест, рівень значущості встановлено на рівні $p < 0.05$.

Результати. У ГД пацієнтів спостерігалося значне підвищення експресії miRNA-143 (у 4,31 раза) та miRNA-155 (у 1,79 раза) порівняно з контрольною групою ($p = 0,04$). Експресія miRNA-192 була зниженою (0,27-кратне зменшення), проте ця різниця не була статистично значущою ($p = 0,12$). Кореляційний аналіз Пірсона визначив значущу позитивну асоціацію між рівнями маркерів оксидативного стресу та експресією miRNA-145, miRNA-155 і miRNA-192 ($p \leq 0,001$). Генетичний аналіз rs1050450 у GPX-1 виявив наявність генотипів CC, CT та TT серед ГД пацієнтів, причому рівновага Гарді-Вайнберга зберігалася ($p = 0,46$ для пацієнтів, $p = 0,8$ для контролю).

Висновки. Диференційна експресія miRNA у ГД пацієнтів свідчить про їхню роль у регуляції оксидативного стресу. Підвищена експресія miRNA-143, miRNA-145 і miRNA-155 може сприяти активації запальних і фібротичних шляхів, тоді як зниження рівня miRNA-192 може відображати порушення функції нирок. Поліморфізм rs1050450 у GPX-1 може модулювати реакції на оксидативний стрес у цих пацієнтів. Подальші дослідження необхідні для оцінки терапевтичного потенціалу miRNA-орієнтованих підходів у лікуванні хронічної хвороби нирок.

Ключові слова: *експресія генів, мікроРНК, гемодіаліз, ген GPX-1, гемодіаліз.*

Introduction. Understanding kidney disease's molecular causes improves diagnosis and treatment. Stability and specificity make miRNA biomarkers. In hemodialysis patients, the GPX-1 SNP rs1050450 influences miRNA regulation and disease susceptibility. The CC genotype at rs1050450, the Pro198Pro form of GPX-1, is common in hemodialysis patients and healthy controls, with low or no T allele (198Leu). Over 800 million individuals worldwide suffer from CKD, a degenerative illness that causes death and suffering in the 21st century. CKD affects kidney shape and function [1]. Membrane incompatibility and endotoxin exposure increase oxidative damage during hemodialysis. Pro-oxidant and antioxidant imbalances begin early in renal injury and are especially prominent in dialysis patients [2]. Laboratory tests employ serum creatinine and albuminuria to estimate glomerular filtration rate (GFR) to diagnose CKD. The KDIGO report defines CKD as kidney injury (albuminuria) or impaired kidney function (GFR < 60 mL/min/1.73 m^2) lasting 3 months or more. CKD is divided into five stages based on GFR levels. End-stage kidney disease (ESKD) is stage 5, indicated by a GFR < 15 mL/min/1.73 m^2 and necessitating dialysis due to nephron destruction and functional loss. CKD lowers GFR, reducing the kidneys' ability to eliminate metabolic waste and pollutants [3]. ESKD may expose renal tubular epithelial cells to high quantities of xenobiotics, metabolic wastes, and nephrotoxins, which may damage DNA due to inflammation and chronic illness. ESKD can result from acute or chronic kidney failure and renal replacement treatment [4]. By degrading or blocking mRNA translation into proteins, miRNAs influence gene expression. They are essential for kidney growth and function and cellular activities include dif-

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ferentiation, proliferation, development, and apoptosis [5]. Alterations in miRNA expression are associated with the initiation and progression of kidney illnesses like diabetic nephropathy, renal malignancy, and renal damage, suggesting they play a role in CKD [6]. Glutathione peroxidase-1 (GPX-1), discovered in 1957 as an enzyme in red blood cells that protects hemoglobin from oxidative stress, needs selenium [7]. The GPX-1 gene on chromosome 3p21.31 encodes GPX-1, an enzyme with 1178 base pairs and two exons. GPX-1 is a homotetramer with four identical subunits that are 208 amino acids and 22-23 kDa. The glutathione peroxidase family's GPX-1 converts hydrogen peroxide into water, making it one of the most essential antioxidant enzymes in humans [8]. Many tissues express GPX-1, which protects cells from oxidative damage. Both the cytoplasm and mitochondria contain it [9].

However, despite advancements in understanding CKD and its complications, gaps remain in elucidating the molecular mechanisms underlying oxidative stress in hemodialysis patients. While previous studies have highlighted the role of miRNAs in renal pathology and the impact of oxidative stress, limited research has specifically explored the expression patterns of miRNAs (e.g., Mir-143, Mir-145, Mir-155, and Mir-192) in hemodialysis patients. Furthermore, the clinical significance of the GPX-1 SNP rs1050450 (Pro198Leu) in modulating oxidative stress and influencing disease susceptibility in this population remains underexplored. This study addresses the gap by investigating the interplay between miRNA expression, oxidative stress, and genetic polymorphisms in hemodialysis patients.

Materials and Methods. The study was approved by the Ethical Committee of the University of Anbar (Ref: 129, December 11, 2023).

Study population. The study included 60 patients undergoing hemodialysis at the AL-Ramadi Hospital Hemodialysis Center, under the supervision of nephrology specialists. Additionally, 40 healthy individuals without kidney disease were recruited as controls. Blood samples were collected from all participants between October 2023 and January 2024. Each participant completed a brief questionnaire covering demographic and clinical data, including age, height, weight, BMI, treatment history, symptom duration, and other relevant factors.

Inclusion criteria.

Patient Group:

Diagnosed with kidney failure and undergoing hemodialysis at AL-Ramadi Hospital.

Under the care of nephrology specialists.

Age range: 40.17–67.81 years.

Control Group:

Individuals with no history of kidney disease.

Age range: 39.7–57.96 years.

Exclusion criteria.

Presence of other chronic diseases that could significantly influence miRNA levels (e.g., cancer, autoimmune diseases).

History of kidney transplantation.

Acute infections or inflammatory conditions at the time of blood sample collection.

Blood sample collection and processing.

Each participant provided 5 mL of venous blood, which was processed as follows:

miRNA analysis:

300 µL of whole blood was stored in Eppendorf tubes containing 500 µL of TRIzol reagent and preserved at -20 °C for miRNA extraction.

Expression levels of miRNA-143, miRNA-145, miRNA-155, and miRNA-192 were analyzed.

Genetic analysis:

2 mL of blood was collected in sterile EDTA tubes and stored at -20 °C for genetic polymorphism analysis of the GPX-1 gene (SNP rs1050450).

miRNA Expression Analysis via RT-qPCR

Total RNA, including miRNAs, was extracted using the TRIzol Reagent Protocol (TransGen, China). Reverse transcription was performed using the Protoscript® II Reverse Transcription System (NEB, England) following the manufacturer's guidelines.

Quantitative PCR (qPCR) was conducted using a SYBR Green-based assay to measure DNA amplification. Specific lyophilized primers for miRNA targets were obtained from Macrogen (Korea) and prepared as follows:

Stock solution (100 pmol/µL): Primers were reconstituted in nuclease-free water.

Working solution (10 pmol/µL): Prepared by diluting 10 µL of stock solution in 90 µL of nuclease-free water. Table 1 presents the primer sequences used for RT-qPCR.

Table 1

Primer Sequences Used for RT-qPCR Analysis of miRNA Expression

Primer Name	Primer Sequences	Ref.
mir143-RT	5`- GTCGTATCCAGTGCGTGTCGTGGAGTCGGCAATTGCACTGGATACGACGAGCTA-3`	[10]
mir143-F	5`- CACGCATGAGATGAAGCACTG-3`	
mir143-R	5`-CCAGTGCAGGGTCCGAGGTA-3`	
mir145-RT	5`- GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACAGGGAT-3`	[11]
mir145-F	5`-GTCCAGTTTTCCCAGGAATCC-3`	
mir145-R	5`-CAGTGCAGGGTCCGAGGTAT-3`	

Continuation of Table 1

Primer Name	Primer Sequences	Ref.
mir155-RT	5`-GCGAGGCGGTGGCAGTGGAAGCGTGATTTATTCACCGCCTCGCACCCCTAT-3`	[12]
mir155-F	5`-CTCAGACTCGGTTAATGCTAATCGTGATAGG-3`	
mir155-R	5`-GCTGTGGCAGTGGAAGCGTGATTTATT-3`	
mir192-RT	5`- GTTGGCTCTGGTGCAGGGTCCGAGGTATTCGCACCAGAGCCAACGGCTGT -3`	[13]
mir192-F	5`-CGGTCCTGACCTATGAATTG-3`	
mir192-R	5`-GTGCAGGGTCCGAGGTATTC-3`	

Realtime quantitative polymerase chain reaction protocol. This project consists of two phases. In the first phase, a Protoscript cDNA synthesis kit was used to synthesise miRNA cDNA with primers for miRNA-143, -145, -155, and -192 (Table 1). The PCR mixture was prepared by combining the components in a sterile 1.5 mL microcentrifuge tube on ice, excluding the cDNA sample. The cDNA mixture was then added to the PCR mixture, resulting in a final reaction volume of 29 µL. The mixture was incubated in a thermocycler at 42 °C for 1 hour, followed by enzyme inactivation at 80 °C. The cDNA product was stored for relative quantitative PCR analysis after measurement using a Qubit 4.0 fluorometer.

In the second phase, patient and control cDNA samples were processed simultaneously. Each sample was tested in two PCR tubes: one for the target miRNAs (143, 145, 155, and 192) and one for U6 snRNA, the housekeeping gene. The Luna® Universal QPCR Master Mix (M3003S) (NEB, England) was used to quantify sample expression levels based on fluorescence intensity. Gene expression levels of miRNA-143, miRNA-145, miRNA-155, and miRNA-192 were quantified using the comparative Ct method. U6 was used as the housekeeping gene (HKG) for normalization. The relative expression levels of the miRNAs were calculated using the formula 2^-ΔCt, where ΔCt represents the difference between the mean Ct of the gene of interest (GOI) and the mean Ct of the housekeeping gene (HKG).

Molecular detection of the GPX-1 gene rs1050450 by conventional PCR and sequencing. Genomic DNA was extracted using the EasyPure Genomic DNA Kit (with RNase A) from TransGen (China). Following extraction, DNA fragments were confirmed by electrophoresis, verifying their presence and integrity. The GPX-1 gene rs1050450 polymorphism was detected using conventional PCR and sequencing. The primers were designed by the author and obtained as lyophilised primers from Macrogen. To prepare the working primer solution, 10 µL of the stock primer was diluted with 90 µL of nuclease-free water. Each lyophilised primer was dissolved according to the manufacturer's instructions (Macrogen, Korea) in an appropriate volume of nuclease-free water. Stock and diluted primer solutions were stored at -20 °C until use, as shown in Table 2.

Table 2

Primer sequences for detecting the GPX-1 gene rs1050450

SNP	Primer Sequences	Reference
GPX-1 rs1050450 F	5'-AGAGATTCTGAATTCCCTCAA-3'	New Synthesis
GPX-1 rs1050450 R	5'-CGAGGTGGTATTTTCTGTAAG-3'	New Synthesis

Following DNA extraction from the patient and control groups, GPX-1 was amplified with a fresh primer set to produce a 471 bp fragment for sequencing. A 25 µL PCR mixture included 12.5 µL OneTaq® Master Mix (NEB, England), 4 µL DNA sample, 1 µL primers, and 6.5 µL nuclease-free water Response followed optimal PCR conditions (Table 3).

Table 3

PCR conditions for amplification of GPX-1 gene

Cycle Step	Temperature	Time	Cycles
Initial Denaturation	95 °C	3 Minutes	1
Denaturation **Annealing** **Extension**	95 °C 51 °C 68 °C	30 Seconds 45 Seconds 30 Seconds	30
Final Extension	68 °C	5 Minutes	1

At Macrogen, Korea, Sanger sequencing was used to detect single nucleotide polymorphisms in over 20 µL of PCR output from each sample. Geneious Prime matched the sequences to GPX-1 (NG-051308). This molecular technique is widely applied in the medical field, it is used to diagnose urinary tract disorders [16-18], In addition, polymerase chain reaction (PCR) has been utilized to research the functions that enzymes play in the interactions between cancer cells in vivo [19, 20], to investigate mutations in gastric cancer [21], and to evaluate the levels of gene expression of biomarkers in a variety of disorders [22, 23]. Furthermore, the molecular techniques have also been used to investigate metabolic reprogramming of small nuclear RNA as a breast cancer therapeutic target [24], quantify mitochondrial DNA for blastocyst transfer potential [25], and use miRNA-126 as a cancer stem cell biomarker [26].

Measurement of oxidative stress markers. The measurement of malondialdehyde (MDA) and 8-hydroxy-deoxyguanosine (8-OHDG) was conducted according to standardized procedures, as summarized in Tables 4 and 5.

The standards were diluted in small tubes before pipetting 50 µL from each dilution into the corresponding microplate wells. Each standard dilution was tested in duplicate, requiring a total of ten wells.

Table 4

Standard dilution for malondialdehyde (MDA)

Concentration	Standard No.	Preparation Method
360 ng/mL	1	300 µL Original Standard + 150 µL Standard Diluent
240 ng/mL	2	300 µL Standard No.1 + 150 µL Standard Diluent
120 ng/mL	3	150 µL Standard No.2 + 150 µL Standard Diluent
60 ng/mL	4	150 µL Standard No.3 + 150 µL Standard Diluent
30 ng/mL	5	150 µL Standard No.4 + 150 µL Standard Diluent

Table 5

Standard dilution for 8-hydroxy-deoxyguanosine (8-OHDG)

Concentration	Standard No.	Preparation Method
2400 pg/mL	1	300 µL Original Standard + 150 µL Standard Diluent
1600 pg/mL	2	300 µL Standard No.1 + 150 µL Standard Diluent
800 pg/mL	3	150 µL Standard No.2 + 150 µL Standard Diluent
400 pg/mL	4	150 µL Standard No.3 + 150 µL Standard Diluent
200 pg/mL	5	150 µL Standard No.4 + 150 µL Standard Diluent

ELISA procedure:

1. Sample Preparation: In the microplate strip wells, one well was left empty as a blank control. In the sample wells, 40 µL of sample dilution buffer and 10 µL of the sample were added (dilution factor: 5). The samples were carefully loaded onto the bottom of the wells without touching the walls and mixed gently.
2. Incubation: The plate was sealed with a closure membrane and incubated at 37°C for 30 minutes.
3. Washing Buffer Preparation: The concentrated washing buffer was diluted with distilled water (30-fold for 96T plates and 20-fold for 48T plates).
4. Washing Procedure: The closure membrane was removed, and the wells were aspirated and refilled with the wash solution. After a 30-second resting period, the solution was discarded. This washing step was repeated five times.
5. HRP-Conjugate Addition: 50 µL of HRP-conjugate reagent was added to each well, except the blank control.
6. Second Incubation: The plate was incubated again at 37°C for 30 minutes, as described in Step 2.
7. Second Washing Step: The washing process was repeated as described in Step 4.
8. Color Development: 50 µL of Chromogen Solution A and 50 µL of Chromogen Solution B were added to each well. The plate was gently shaken and incubated at 37°C for 15 minutes in the dark to prevent light interference.
9. Reaction Termination: 50 µL of stop solution was added to each well, causing the color to change from blue to yellow.
10. Optical Density (OD) Measurement: The absorbance was read at 450 nm using a microplate reader. The OD value of the blank control well was set to zero. The assay was completed within 15 minutes after adding the stop solution (Sunlong, China).

Statistical analysis. Statistical analyses were performed using SPSS version 20. In addition to Pearson correlation analysis to examine the association between

biochemical variables and miRNA expression, genotype distribution and allele frequencies were assessed using the chi-square test. Hardy-Weinberg equilibrium was tested, with p-values < 0.05 considered statistically significant.

Results. Gene expression of miRNAs. The housekeeping gene (U6) was assessed in both patients (30 samples) and controls (15 samples) after the correct primer was bound to the appropriate target microRNAs (miRNA-143, -145, -155, and -192) at the optimal temperature. The results showed that, in this study, patients had 4.31 times more miRNA-143, 1.79 times more miRNA-145, and 2.27 times more miRNA-155 compared to controls. Table 6 indicates that patients downregulated miRNA-192 by 0.27-fold relative to controls.

Table 6

Fold change in gene expression for miRNA-143, miRNA-145, miRNA-155, and miRNA-192

Groups	Gene	Mean Ct of GOI (Gene of Interest)	Mean Ct of HKG (Housekeeping Gene)	ΔCt (GOI - HKG)	Relative Expression (2^- Ct)	2^- Ct Patients / 2^- Ct Controls	Fold Change in Expression (Patients vs. Controls)	P-value
Patients	miRNA-143	29.76	20.54	9.22	0.00168	0.00240 / 0.00097	4.31	0.04
Controls		29.49	18.17	11.32	0.00039	0.00039 / 0.00039	1.00	
Patients	miRNA-145	31.66	20.57	11.09	0.000459	0.000459 / 0.000821	1.79	0.92
Controls		28.36	18.11	10.25	0.000821	0.000821 / 0.000821	1.00	
Patients	miRNA-155	17.25	20.54	-3.29	9.79	9.79 / 4.32	2.27	0.04
Controls		16.06	18.17	-2.11	4.32	4.32 / 4.32	1.00	
Patients	miRNA-192	32.22	21.50	10.73	0.00059	0.00059 / 0.002176	0.27	0.12
Controls		27.43	18.58	8.84	0.002176	0.002176 / 0.002176	1.00	

Relationship among miRNAs with products of oxidative stress. The Pearson correlation test was used to compare the mean ΔCt values of the four miRNAs of interest with two oxidative stress indicators (8-OHDG and MDA). The results showed that:

- 8-OHDG and MDA were positively correlated ($p \leq 0.001$) with miRNA-145 and miRNA-192 ($r = 0.705$, $r = 0.639$, $r = 0.586$, $r = 0.589$).
- miRNA-155 was positively correlated with 8-OHDG and MDA ($p = 0.023$, $r = 0.414$; $p = 0.018$, $r = 0.428$).
- miRNA-143 had a small, non-significant positive correlation with 8-OHDG and MDA ($p = 0.131$, $r = 0.282$; $p = 0.137$, $r = 0.278$).

Genetic polymorphisms of the GPX-1 gene. Figure 1 presents the 471-bp gel electrophoresis band for the GPX-1 rs1050450 SNP identified by conventional PCR.

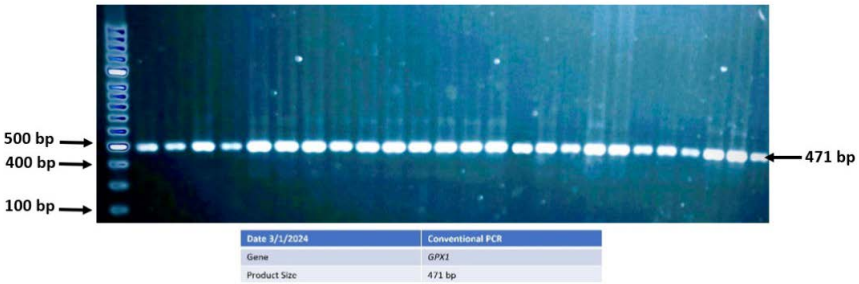

Fig. 1. Gel Electrophoresis of GPX-1 rs1050450 SNP. Agarose gel electrophoresis (1%) of the 471-bp GPX-1 rs1050450 amplicon from patient and control groups. The gel was run in 1X TAE buffer at 100V for 1 hour and stained with red stain. A 100-bp DNA ladder was used as a marker.

The analysis revealed that hemodialysis patients exhibited CC, CT, and TT genotypes. The distribution of these genotypes in patients and controls was consistent with Hardy-Weinberg equilibrium ($P = 0.46$ for patients, $P = 0.8$ for controls), indicating genetic stability in the population (Table 7).

Table 7

Genotypes of GPX-1 rs1050450 and allele frequencies in patients and controls

GPX-1 Genotyping	Patients		Controls	
	Observed (%)	Expected (%)	Observed (%)	Expected (%)
CC	33 (68.75)	32 (66.7)	23 (76.7)	23.41 (78.0)
CT	12 (25.00)	15 (31.22)	7 (23.3)	6.2 (20.7)
TT	3 (6.25)	1 (2.08)	0 (00.0)	0.3 (1.3)
Total	78 (100.0)	78 (100.0)	30 (100.0)	30 (100.0)
p-HWE	P = 0.46		P = 0.8	

Discussion. MiRNAs contribute to fibrosis in various organs [27], including renal fibrosis, which has been extensively studied. Certain miRNAs induce mRNA degradation and/or inhibit protein translation, playing a role in kidney pathophysiology, including inflammation, nephritic syndrome, renal fibrosis, lupus nephritis (LN), and cancer [28, 29].

The miR-143/145 cluster is now recognized as multifunctional. MiRNAs 143 and 145 inhibit cancer progression by promoting cell death, cell cycle regulation, and apoptosis [30]. In this study, renal failure patients exhibited a 4.31-fold higher expression of miRNA-143 than controls. MiRNA-143 is essential for cell growth, differentiation, and apoptosis, and its upregulation is associated with the progression of chronic kidney disease (CKD) and renal failure, which are driven by fibrosis and inflammation [31]. Similarly, miRNA-145 expression was 1.79 times higher in patients than in controls, consistent with findings by Brignat et al. (2017), who reported elevated miRNA-145 levels in CKD patients compared to healthy individuals [32]. Hemodialysis patients have also been shown to overexpress miRNA-145 compared to healthy controls [33].

MiRNA-155, encoded by the MIR155 host gene, is involved in various physiological and pathological processes, including cancer, viral infections, and cardiovascular diseases. It plays a crucial role in hematopoiesis, immunity, and inflammation [34]. Hypoxia-induced overexpression of miRNA-155 in proximal tubule cells contributes to renal fibrosis while increasing evidence links miRNA-155 to hematopoiesis, inflammation, and immunological responses. Renal failure patients demonstrate increased miRNA-155 expression, which correlates with inflammatory responses and immune regulation. IL-6 and hs-CRP levels are significantly elevated in the serum of uremic dialysis patients expressing miRNA-155 [35].

MiRNA-192 regulates differentiation, proliferation, apoptosis, epithelial-mesenchymal transition (EMT), angiogenesis, metabolism, inflammation, oxidative stress, and drug resistance by affecting mRNA degradation and protein translation [36, 37]. In this study, patients exhibited a 0.27-fold lower expression of miRNA-192 than controls. The kidneys express miRNA-192, which regulates renal function and structural genes, such as E-cadherin. In CKD mouse models, miRNA-192 downregulation promotes fibrosis and reduces renal function, as profibrotic and proinflammatory genes become upregulated [38].

The differential expression of miRNA-143, miRNA-145, miRNA-155, and miRNA-192 in renal failure patients compared to controls suggests their role in disease pathogenesis. The overexpression of miRNA-143, miRNA-145, and miRNA-155 in patients may indicate their involvement in renal failure progression through inflammation, fibrosis, or apoptosis [39]. Conversely, the downregulation of miRNA-192 in patients may serve a protective or regulatory function in renal pathology [38]. These findings underscore the complexity of miRNA dysregulation in renal failure and highlight the need for further research to elucidate the underlying mechanisms.

A significant moderate positive correlation ($p \leq 0.001$) was observed between miRNA-145, miRNA-192, 8-OHDG, and MDA, indicating their essential role in oxidative stress responses. The overexpression of protective miRNAs may have therapeutic potential in chronic kidney disease, where oxidative damage is a key contributor [40]. Although miRNA-155 showed a moderate correlation with oxidative stress markers, miRNA-143 did not exhibit a significant association with oxidative stress indicators ($r=0.282$ for 8-OHDG, $r=0.278$ for MDA) [41, 42]. However, miRNA-143 may influence renal vascular disease by affecting vascular smooth muscle cell proliferation and apoptosis. The lack of correlation with oxidative stress remains unexplored. Further research is necessary to determine whether this is due to miRNA-143's role in fibrosis or apoptosis, which may be unrelated to oxidative stress, or if other factors influence its expression [43, 44].

The moderate positive correlation between miRNA-155 and both oxidative stress markers ($r=0.414$ for 8-OHDG; $r=0.428$ for MDA) suggests that miRNA-155 may contribute to renal injury via inflammation. MiRNA-155 is associated with kidney disease, macrophage activation, and cytokine production [45].

Hardy-Weinberg equilibrium (HWE) analysis confirmed that genotype frequencies in both patient and control groups align with HWE expectations, suggesting genetic stability. The CC genotype was more prevalent in the control group (76.7%) than in the patient group (68.75%), while the CT genotype was

slightly more frequent in patients (25.00%) compared to controls (23.3%). The TT genotype was absent in controls but detected in 6.25% of patients. Both groups had more C alleles than T alleles, with the control group exhibiting a higher frequency of the C allele [46].

These findings suggest that GPX-1 Pro198Pro (CC genotype at rs1050450) is the predominant variant, which is associated with normal GPX-1 activity. Since all participants—both patients and controls—carry this genotype [46], differences in oxidative stress are unlikely due to SNP variations. Instead, genetic, environmental, or disease-specific factors may contribute to the increased oxidative stress observed in hemodialysis patients [47].

The absence of CT and TT genotypes in some hemodialysis patients and controls suggests that the T allele (198Leu) is rare in this population. Genotype distribution is significantly influenced by population genetics. According to HWE principles, genotype frequencies remain constant unless affected by natural selection, genetic drift, gene flow, or mutations. The low frequency of the CT and TT genotypes in this study sample may be due to population-specific allele distributions [48].

Previous research indicates that the T allele is associated with reduced GPX-1 activity and increased oxidative stress. However, because the CC genotype (Pro198Pro) is predominant in this study, GPX-1 enzyme activity remains stable, offering protection against oxidative stress [49].

Despite this, hemodialysis patients still exhibit elevated oxidative stress levels, likely due to uremic conditions and treatment-related factors. Additional contributors may include reduced antioxidant levels, increased ROS production, or impaired antioxidant defense mechanisms [50]. Given the high oxidative stress levels observed in hemodialysis patients without SNP polymorphisms, other factors must be responsible. This underscores the complex nature of oxidative stress regulation and the need for comprehensive genetic, environmental, and biochemical investigations in hemodialysis patients [50].

This study has several limitations. First, the small sample size may have influenced the results. Second, the study focused solely on patients from Ramadi, limiting the generalizability of the findings; future research should include diverse populations. Third, while real-time PCR was used for gene expression analysis, incorporating conventional PCR, electrophoresis, and sequencing could provide additional insights into the effects of GPX-1 SNP rs1050450 in hemodialysis patients. Finally, this study did not investigate immunological changes in hemodialysis patients, which could be explored in future research.

Conclusion. Hemodialysis patients exhibited significantly higher overexpression of miRNA-143, miRNA-145, and miRNA-155 compared to healthy controls. The downregulation of miRNA-192 in patients suggests its potential role in renal function modification. Additionally, hemodialysis patients without the rs1050450 mutation experienced elevated oxidative stress. The overexpression of miRNA-143, miRNA-145, and miRNA-155 in renal failure patients indicates their potential as diagnostic biomarkers. Their high expression levels may facilitate non-invasive monitoring of disease progression and therapeutic responses. A deeper understanding of the roles of miRNA-143, miRNA-145, miRNA-155, and miRNA-192 in renal failure could pave the way for personalized treatment approaches.

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