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α-Klotho, hypoxia-inducible factor-2α, and oxidative stress in hemodialysis – interactions with anemia, secondary hyperparathyroidism, and intradialytic hypotension

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ABSTRACT

Introduction and aim. End-stage renal disease (ESRD) is associated with oxidative stress (OS) and hormonal disturbances linked to anemia, secondary hyperparathyroidism (SHPT), and intradialytic hypotension (IDH). This study evaluated the expression of α-Klotho (α-KL) and hypoxia-inducible factor-2α (HIF-2α) together with OS markers in hemodialysis (HD) patients and explored their interrelationships and associations with anemia, SHPT, and IDH.

Material and methods. Fifty HD patients and 50 healthy controls were evaluated for clinical and biochemical parameters, malondialdehyde (MDA), catalase (CAT) activity, and α-KL and HIF-2α expression. Correlation and network analyses were used to evaluate molecular interactions.

Results. Anemia, SHPT, and IDH were identified in 72%, 44%, and 18% of HD patients, respectively. Compared with controls, HD patients demonstrated significantly higher MDA levels and lower α-KL and HIF-2α expression, and CAT activity (all $p<0.001$). MDA positively correlated with parathyroid hormone (PTH) ($r=0.26$, $p<0.01$) and IDH ($r=0.37$, $p<0.001$), but inversely with hemoglobin (Hb) ($r=-0.24$, $p<0.02$) and CAT activity ($r=-0.36$, $p<0.001$). Lower α-KL and HIF-2α expression levels, together with reduced CAT activity, correlated positively with Hb ($r=0.27–0.71$) and inversely with PTH ($r=-0.27$ to -0.62) and IDH ($r=-0.40$ to -0.92) (all $p<0.01$). Positive intercorrelations were additionally found among α-KL, HIF-2α, and CAT ($r=0.21–0.38$, all $p<0.05$).

Conclusion. Reduced α-KL and HIF-2α expression together with altered OS markers were associated with anemia, SHPT, and IDH in HD patients. The close interrelationships observed among α-KL, HIF-2α, and OS markers may reflect an integrated molecular pattern linked to dialysis-related complications. Additional prospective studies are warranted to further elucidate these relationships.

Keywords. anemia, hemodialysis, hypoxia-inducible factor-2α, α-Klotho, oxidative stress

Introduction

End-stage renal disease (ESRD) represents the terminal stage of chronic kidney disease (CKD) and is associated with a substantial clinical and healthcare burden worldwide. Hemodialysis (HD) remains the most widely used form of renal replacement therapy (RRT).[1] In Egypt, the prevalence of ESRD patients receiving HD was reported as 442 per million population in Sharkia Governorate in 2017.[2]

Patients with ESRD undergoing dialysis frequently develop a broad spectrum of complications, such as anemia, altered mineral balance, cardiovascular instability, and intradialytic hypotension (IDH), which collectively may worsen clinical status and impair quality of life.[3,4] Anemia in ESRD is multifactorial, involving impaired erythropoietin (EPO) production, disrupted oxygen-sensing mechanisms, inflammation, altered iron handling, and the effects of uremia.[5] IDH is another important complication during HD and has been associated with impaired organ perfusion and increased cardiovascular risk.[6]

Growing evidence suggests that oxidative stress (OS) is closely linked to the development and progression of many ESRD-related complications. Excessive oxidative activity may contribute to cellular dysfunction, vascular injury, and chronic inflammation, particularly in patients undergoing HD.[7,8] Catalase (CAT) is a key component of the antioxidant defense system,[9] whereas malondialdehyde (MDA) is a widely recognized indicator of lipid peroxidation and oxidative damage.[10]

The α-Klotho (α-KL) protein is involved in mineral metabolism regulation and exerts cytoprotective effects within the kidney and other tissues.[11] Hypoxia-inducible factor-2α (HIF-2α) plays an essential role in

oxygen sensing and erythropoietic regulation.[12] Increasing evidence suggests that alterations in α-KL, HIF-2α, and oxidative stress pathways may contribute to several complications encountered in HD patients. However, these factors are often investigated separately, and their interrelationships in the context of anemia, secondary hyperparathyroidism (SHPT), and IDH remain insufficiently characterized. Clarifying these associations may provide a broader understanding of the biological processes accompanying ESRD and HD-related complications.

Aim

This study was undertaken to evaluate α-KL and HIF-2α gene expression and oxidative stress status in ESRD patients undergoing HD. It further investigated the associations between these molecular markers and major HD-related complications, including anemia, SHPT, and IDH. In addition, a correlation network was constructed to explore the interrelationships among α-KL, HIF-2α, and oxidative stress markers.

Material and methods

Study design and participants

A case–control design was applied involving 50 Egyptian patients with ESRD undergoing maintenance HD and 50 apparently healthy individuals who served as controls for biomarker and gene expression analyses. Patients were enrolled from the Nephrology Department, Faculty of Medicine, Suez Canal University Hospital, Ismailia. Biochemical and molecular investigations were carried out at the Medical Biochemistry and Molecular Biology Department, Oncology Diagnostic Unit, and Clinical Pathology Department, Faculty of Medicine, Suez Canal University, Ismailia, Egypt.

Patients were considered eligible if they had undergone regular HD sessions three times weekly for at least three months. Control participants were matched for age and sex, exhibited normal renal function based on serum creatinine and urea measurements, and had no evidence of chronic systemic illness.

Ethical approval was granted by the Institutional Ethics Committee of Suez Canal University (Approval No. 4724). Written informed consent was secured from all participants prior to study inclusion.

Hemodialysis treatment characteristics

All patients underwent maintenance hemodialysis three times weekly for at least three months, with a four-hour duration per session. Ultrafiltration volume and interdialytic weight gain were documented. Standard management protocols for anemia and CKD–MBD were applied according to institutional protocols and Kidney Disease Improving Global Outcomes (KDIGO) recommendations.[13,14] SHPT was assessed primarily according to parathyroid hormone (PTH) concentrations. Additional biochemical markers associated with bone and mineral metabolism, including calcium (Ca^{2+}), phosphorus (P), and alkaline phosphatase (ALP) were also evaluated to provide indirect evidence of bone-related abnormalities. Direct

evaluation of bone pathology, such as imaging studies or bone mineral density assessment, was not included.

For the purpose of this study, anemia was defined as hemoglobin (Hb) <110 g/L according to KDIGO guidelines.[15] IDH was identified by a reduction in systolic blood pressure of ≥20 mmHg or a decline in mean arterial pressure of ≥10 mmHg during dialysis together with clinical symptoms or the need for therapeutic intervention, according to National Kidney Foundation Kidney Disease Outcomes Quality Initiative (KDOQI) criteria.[16] SHPT was defined as intact PTH levels exceeding 61.7 pmol/L, corresponding to >9-fold above the upper reference limit. KDIGO guidelines recommend maintaining PTH concentrations within approximately 2–9 times the upper normal range in stage 5 CKD patients; therefore, values beyond this range were considered indicative of SHPT in the present analysis.[14] Interdialytic weight gain (IDWG) was determined from the difference between post-dialysis body weight and pre-dialysis body weight at the subsequent dialysis session.

Blood samples collection and laboratory investigation

Peripheral venous blood samples (9 mL) were obtained from all participants. In HD patients, sample collection was performed immediately before dialysis initiation. Samples were processed as follows:

- Serum samples: 5 mL Blood was collected into plain vacutainer tubes, maintained at room temperature until clot formation, then centrifuged at 3,000 rpm for 10 minutes. Serum was subsequently separated into two aliquots:
    - One aliquot was utilized for biochemical investigations, including measurement of serum creatinine, urea, sodium (Na^+), potassium (K^+), Ca^{2+}, P, ALP, iron, and ferritin using the Cobas c 6000 autoanalyzer (Roche Diagnostics, Mannheim, Germany). Serum PTH concentrations were determined by chemiluminescent immunoassay using the Access 2 Immunoassay System (Beckman Coulter, Brea, CA, USA).
    - The second aliquot was stored at −20°C for subsequent assessment of MDA and CAT.
- EDTA samples (4 mL): Divided into two portions:
    - 2 mL was used for complete blood count (CBC) using a 5-part differential hematology analyzer (Siemens AG, Erlangen, Germany).
    - The remaining 2 mL was used for molecular analysis to assess mRNA expression of α-KL and HIF-2α.

Oxidative stress assessment

MDA represents an indicator of lipid peroxidation and membrane oxidative injury, reflecting enhanced oxidative stress and reactive oxygen species generation in CKD.[17] CAT functions as an antioxidant enzyme

responsible for converting hydrogen peroxide into water and oxygen. Simultaneous evaluation of MDA and CAT activity provides an integrated overview of oxidative injury and antioxidant defense status.

Serum levels of both markers were analyzed using a UV–VIS spectrophotometer (Unico S2100 Series, Wixom, MI, USA). Quantification of MDA levels and CAT activity was performed using commercially available Bio-diagnostic assay kits (Giza, Egypt) according to the manufacturer's recommendations and previously published methodologies.[18,19]

Gene expression analysis

RNA extraction was performed from EDTA-treated blood samples using the QIAamp RNA Blood Mini Kit (Qiagen, Germany) in accordance with the manufacturer's protocol. RNA quantity and purity were evaluated using a Nano Drop ND-1000 spectrophotometer V3.1.0 (Nano Drop Technologies Inc., Wilmington, DE, USA) through assessment of A260/A280 and A260/A230 absorbance ratios. Samples demonstrating A260/A280 ratios between 1.8 and 2.1 were considered acceptable for downstream analysis. Equivalent quantities of RNA were reverse transcribed into cDNA using the High-Capacity cDNA Reverse Transcription Kit (Invitrogen, USA) on a Biometra T Advanced thermocycler (Analytik Jena AG, Jena, Germany) according to the supplied protocol.

Quantitative real-time PCR (qRT-PCR) amplification was conducted using SYBR Green chemistry and Maxima SYBR Green qPCR Master Mix (Thermofisher Scientific, USA, cat. no. K0251) following the manufacturer's recommendations. Thermal cycling conditions are summarized in Supplementary Table S1. Amplification reactions were performed using the StepOne™ Real-Time PCR System (Applied Biosystems, USA) for quantification of α-KL and HIF-2α expression levels. GAPDH served as the endogenous reference gene, and all samples were analyzed in duplicate. Melting curve analysis was used to confirm amplification specificity and exclude nonspecific amplification or primer-dimer artifacts. Cycle threshold (Ct) obtained using a uniform fluorescence threshold applied across all experimental runs.

Gene-specific primers (Willowfort, UK) were designed and validated using multiple bioinformatics tools to ensure specificity and efficiency. The nucleotide sequences of all primers used in the present analysis are summarized in Supplementary Table S2. The following platforms were used: Integrated DNA Technologies (IDT) Custom Primer Design,[20] NCBI Primer-BLAST,[21] Sequence Manipulation Suite: PCR Primer Stats,[22] In silico PCR amplification[23] and UCSC In silico PCR.[24]

Relative quantification of gene expression was determined using the $2^{-\Delta\Delta CT}$ method.[25-27] Experimental procedures complied with the Minimum Information for Publication of Quantitative Real-Time PCR Experiments (MIQE) guidelines.[28]

Statistical analysis

All statistical evaluations were carried out using SPSS software version 25 (IBM Corp., Armonk, NY, USA). Data normality was examined with the Shapiro-Wilk test, and continuous data were summarized as mean±SD (SD). Group comparisons were conducted using the independent samples t-test or Mann–Whitney U test according to variable distribution characteristics.

Relationships between variables were examined using Spearman's rank correlation analysis, whereas Pearson correlation was applied exclusively for graphical network visualization. Multivariate logistic regression models were constructed to determine independent predictors of anemia and hyperparathyroidism. Predictor selection was based on biological relevance together with significant associations observed in univariate analyses. To minimize the possibility of overfitting considering the relatively limited sample size, the number of predictors entered into each model was restricted. Findings are presented as ORs with 95% CIs. Statistical significance was considered at a two-sided p-value <0.05.

Results

Demographic and clinical characteristics of the study population

The study cohort comprised 50 HD patients and 50 healthy controls with generally comparable baseline characteristics. No statistically significant differences were identified between the groups regarding age, sex distribution, or body weight (Table 1).

Among HD patients, the mean HD duration was 8.5±5.97 years, whereas the mean IDWG was 2.05±0.73 kg. HCV seropositivity was detected in 28% of patients. Hypertension represented the leading identified cause of ESRD (30%), followed by diabetes mellitus and systemic lupus erythematosus (6% each), while the underlying etiology remained unidentified in 58% of cases. Anemia was the most frequent clinical complication (72%), followed by SHPT (44%) and IDH (18%) (Table 2).

Table 1. Baseline demographic profile of the HD and control groups*

Parameter	HD patients (n=50)	Controls (n=50)	p
Age (years), mean±SD	35.1±17.8	34.7±16.9	0.71
Male, n (%)	25 (50%)	24 (48%)	0.84
Female, n (%)	25 (50%)	26 (52%)	0.84
Weight (kg), mean±SD	55.5±17.1	56.9±15.8	0.63

* Values are expressed as mean±SD or number (%). Group comparisons were conducted using the independent samples t-test for continuous data and the chi-square test for categorical variables, SD – standard deviation, HD – hemodialysis

Table 2. Clinical characteristics, causes of ESRD and HD-related complications among HD patients*

Parameter	HD patients (n=50)
Duration of HD (years), mean±SD	8.5±5.97
IDWG (kg), mean±SD	2.05±0.73
HCV +ve (%)	28%
Patients' co-morbidities causing ESRD	
HTN (%)	30%
DM (%)	6%
SLE (%)	6%
Unknown cause (%)	58%
ESRD and HD-related complications	
Anemia (%)	72%
Hyperparathyroidism (%)	44%
IDH (%)	18%

* Data are expressed as mean±SD or percentage (%), HD – hemodialysis, ESRD – end-stage renal disease, IDWG – interdialytic weight gain, HCV – hepatitis C virus, HTN – hypertension, DM – diabetes mellitus, SLE – systemic lupus erythematosus, IDH – intradialytic hypotension, SD – standard deviation, +ve – positive

Hematological and biochemical profiles of the study groups

HD patients demonstrated significantly lower Hb, serum Ca^{2+}, and Na^{+} levels than controls. Conversely, serum iron, ferritin, P, PTH, ALP, creatinine, urea, and K^{+} concentrations were significantly elevated in the HD group (Table 3).

Table 3. Hematological and biochemical findings in HD patients and healthy controls *

Parameter (mean±SD)	Control (n=50)	Patients (n=50)	p
Hematological parameters			
Hb (g/L)	130.2±13.4	97±16.3	<0.001***
MCV (fL)	88.91±5.02	84.97±7.3	0.002**
MCH (pg)	29.99±1.84	27.86±3.16	<0.001***
Iron status			
Iron (µmol/L)	7.20±0.37	10.41±3.02	<0.001***
Ferritin (µg/L)	58.92±22.27	445.8±481.84	<0.001***
Mineral metabolism			

Ca^{2+} (mmol/L)	2.29±0.14	2.01±0.24	*<0.001****
P (mmol/L)	1.11±0.19	1.51±0.41	*<0.001****
PTH (pmol/L)	3.58±0.96	63.8±50.3	*<0.001****
ALP (U/L)	80.2±23.36	309.75±316.79	*<0.001****
Renal function			
Creatinine (µmol/L)	89.3±14.1	925.4±235.1	*<0.001****
Urea (mmol/L)	2.22±0.69	19.25±6.69	*<0.001****
Electrolytes			
Na^{+} (mmol/L)	137.86±3.04	134.67±3.02	*<0.001****
K^{+} (mmol/L)	4.03±0.48	5.18±0.87	*<0.001****

* Results are presented as mean±SD. Statistical comparisons were performed using the independent samples t-test or Mann–Whitney U test according to data distribution., SD – standard deviation, Hb – hemoglobin, MCV – mean corpuscular volume, MCH – mean corpuscular hemoglobin, PTH – parathyroid hormone, ALP – alkaline phosphatase, Na^{+} – sodium, K^{+} – potassium, Ca^{2+} – calcium, P – phosphorus

Gene expression and oxidative status

HD patients exhibited significantly lower expression levels of α-KL, HIF-2α, and CAT activity compared with controls (all $p < 0.001$). In contrast, MDA concentrations were significantly increased in the patient group ($p < 0.001$) (Fig. 1).

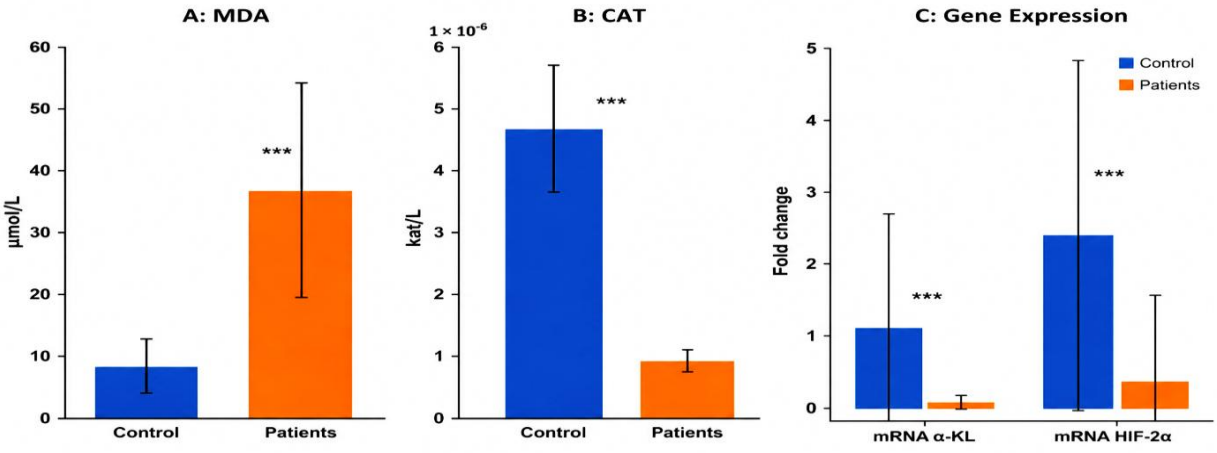

Fig. 1. Gene expression of α-KL and HIF-2α, and oxidative status in patients versus controls, A: Plasma MDA (µmol/L), B: CAT activity (kat/L), C: Relative mRNA expression of α-KL and HIF-2α in peripheral blood samples, bars represent mean±SD for control and patient groups, Group differences were evaluated using the independent samples t-test or Mann-Whitney U test, as appropriate, *** – $p<0.001$ indicates statistical significance, MDA – malondialdehyde, CAT – catalase, α-KL – α-Klotho, HIF-2α – hypoxia-inducible factor-2 alpha

Associations between biomarkers and clinical parameters (Hb, PTH, and IDH)

Hb levels showed inverse associations with P, ALP, PTH, creatinine, urea, MDA, HD duration, IDWG, and IDH, whereas positive associations were observed with iron, ferritin, CAT, α-KL, and HIF-2α.

An opposite trend was observed for PTH, which demonstrated negative correlations with Hb, Ca^{2+}, CAT, α-KL, and HIF-2α, while positive correlations were noted with P, ALP, creatinine, urea, MDA, HD duration, IDWG, IDH, iron, and ferritin.

Likewise, IDH was associated with lower Hb, Ca^{2+}, Na^{+}, CAT, α-KL, and HIF-2α levels, whereas higher P, ALP, PTH, K^{+}, creatinine, urea, MDA, dialysis duration, and IDWG values were observed in affected patients (Figure 2, Table 4, Supplementary Table S3).

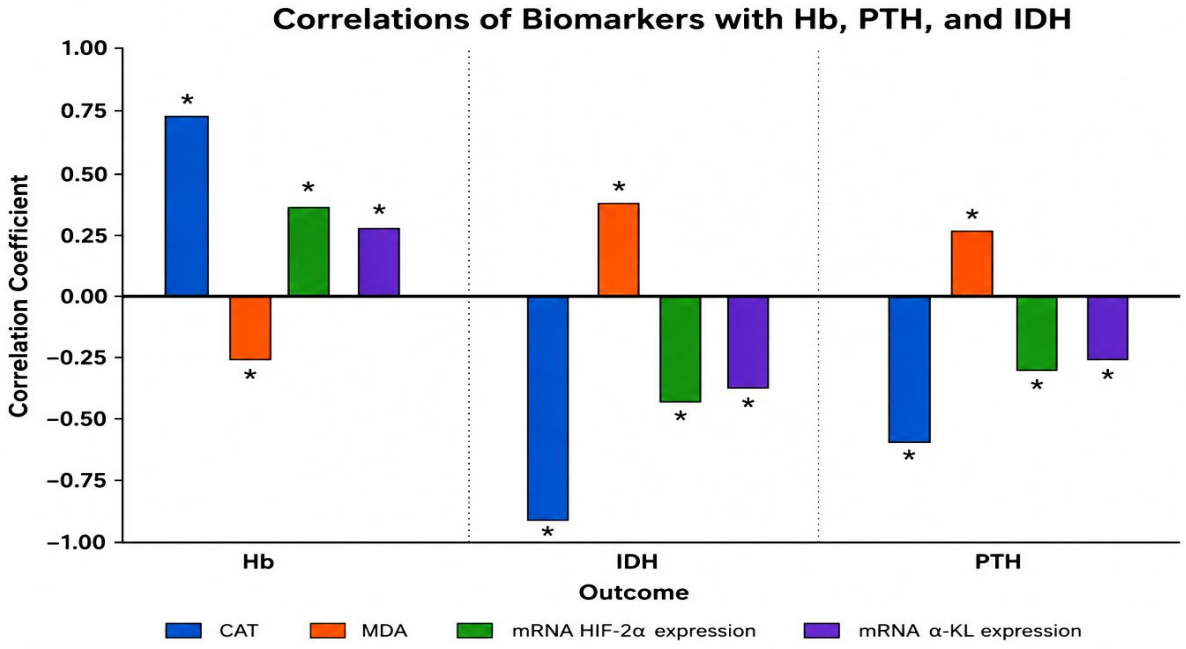

Fig. 2. Correlations between oxidative stress markers, gene expression parameters, and clinical outcomes, grouped bar chart showing correlation coefficients between MDA, CAT, mRNA α-KL expression, and mRNA HIF-2α expression with Hb, PTH, and IDH, positive and negative correlation values are shown on the y-axis, distinct colors represent different biomarkers, Correlations were analyzed using Spearman's rank correlation test, * – statistically significant correlations ($p<0.05$), MDA – malondialdehyde, CAT – catalase, α-KL – α-Klotho, HIF-2α – hypoxia-inducible factor-2 alpha, Hb – hemoglobin, PTH – parathyroid hormone, IDH – intradialytic hypotension

Table 4. Correlations of MDA, CAT, α-KL, and HIF-2α with clinical and biochemical parameters*

Variable		MDA	CAT	mRNA α-KL expression	mRNA HIF-2α expression
Hb	Correlation	-0.24	0.71	0.27	0.35
	p	0.016	<0.001	0.007	<0.001
Ferritin	correlation	0.078	-0.472	-0.209	-0.133
	p	0.442	<0.001	0.037	0.187
Ca^{2+}	Correlation	-0.11	0.56	0.29	0.22
	p	0.298	<0.001	0.004	0.031
P	Correlation	0.26	-0.52	-0.25	-0.11
	p	0.01	<0.001	0.014	0.296
PTH	Correlation	0.26	-0.62	-0.27	-0.3
	p	0.01	<0.001	0.006	0.002
ALP	Correlation	0.14	-0.43	-0.19	-0.23
	p	0.162	<0.001	0.061	0.021
Creatinine	Correlation	0.4	-0.89	-0.4	-0.41
	p	<0.001	<0.001	<0.001	<0.001
MDA	Correlation	1	-0.36	-0.15	-0.19
	p		<0.001	0.148	0.063
CAT	Correlation	-0.36	1	0.38	0.38
	p	<0.001		<0.001	<0.001
mRNA α-KL expression	Correlation	-0.15	0.38	1	0.21
	p	0.148	<0.001		0.032
mRNA HIF-2α expression	Correlation	-0.19	0.38	0.21	1
	p	0.063	<0.001	0.032	
IDH	Correlation	0.373	-0.916	-0.402	-0.436
	p	<0.001	<0.001	<0.001	<0.001

* Correlations involving MDA (μmol/L), CAT (kat/L), mRNA α-KL expression, and mRNA HIF-2α expression with clinical and biochemical parameters, Spearman's rank correlation analysis was applied to examine relationships between variables, and the results are expressed as correlation coefficients (r) and corresponding p-values, Hb – hemoglobin, Ca^{2+} – calcium, P – phosphorus, PTH – parathyroid hormone, ALP – alkaline phosphatase, MDA – malondialdehyde, CAT – catalase, α-KL – Klotho, HIF-2α – hypoxia-inducible factor, IDH – intradialytic hypotension

Correlation analysis

Oxidative stress markers demonstrated significant relationships with several clinical and biochemical parameters. MDA showed positive correlations with P, PTH ($r=0.26$, $p<0.01$), creatinine, HD duration, and IDH ($r=0.37$, $p<0.001$), while inverse correlations were observed with Hb ($r=-0.24$, $p<0.02$) and CAT ($r=-0.36$, $p<0.001$). CAT was positively associated with Hb ($r=0.71$, $p<0.001$), α-KL, and HIF-2α expression ($r=0.38$, $p<0.001$ for both), whereas negative associations were identified with PTH ($r=-0.62$, $p<0.001$), MDA, HD duration, and IDH ($r=-0.32$, $p<0.001$).

HIF-2α expression demonstrated positive correlations with Hb ($r=0.35$, $p<0.001$), Ca^{2+}, CAT, and α-KL expression, while inverse correlations were observed with PTH ($r=-0.3$, $p<0.002$), creatinine, HD duration, IDH ($r=-0.44p<0.001$), and IDWG. Similarly, α-KL expression was positively associated with Hb ($r=0.27$, $p<0.007$), CAT ($r=0.38$, $p<0.001$), and HIF-2α, whereas negative correlations were identified with P, PTH ($r=-0.27$, $p<0.006$), creatinine, HD duration, IDH ($r=-0.4$, $p<0.001$), and IDWG. Detailed correlation coefficients and significance values are presented in Figure 2, Table 4 and Supplementary Table S3.

Correlation network

To further explore the relationships among OS and gene expression markers, a graphical correlation network was constructed (Figure 3). α-KL, HIF-2α, and CAT formed a closely related cluster, whereas MDA demonstrated inverse relationships with CAT and HIF-2α. In addition, α-KL and HIF-2α exhibited a positive correlation pattern, suggesting potential interrelated regulatory mechanisms within oxidative stress pathways. These findings were generally consistent with the correlation matrix presented in Table 4 and Supplementary Table S3.

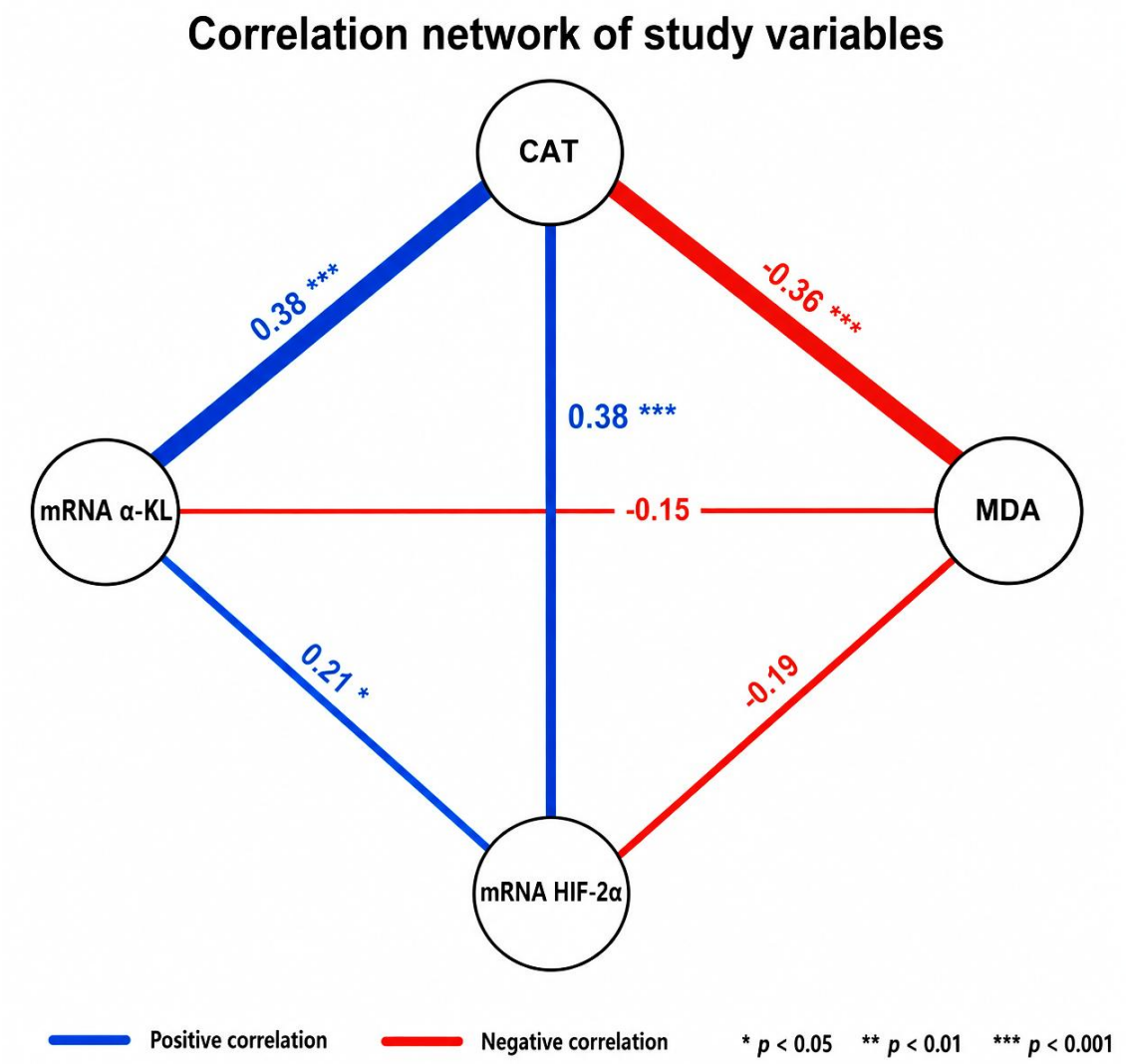

Fig. 3. Correlation network of study variables, graphical representation of the correlations among MDA, CAT, mRNA α-KL, and mRNA HIF-2α, Pearson correlation coefficients were used for network visualization, Line colors indicate correlation direction (blue, positive correlation; red, negative correlation), while edge thickness reflects correlation strength (|r|), numbers indicate correlation coefficients, and statistical significance is indicated by asterisks (* – p<0.05, *** – p<0.001), MDA – malondialdehyde, CAT – catalase, α-KL – α-Klotho, HIF-2α – hypoxia-inducible factor-2 alpha

Multivariate logistic regression analysis identified reduced α-KL, HIF-2α, and CAT levels as independent predictors of anemia. In addition, SHPT was independently associated with lower α-KL, HIF-2α, and CAT levels, together with higher MDA concentrations (Supplementary Table S4).

Discussion

ESRD patients undergoing HD commonly present with anemia, SHPT, and IDH, accompanied by disturbances in oxidative and hormonal homeostasis. In the present study, anemia was identified in 72% of patients, SHPT in 44%, and IDH in 18%, findings that are generally consistent with previous reports among HD populations.[29-31] These observations confirm the persistent burden of these complications in ESRD and provide the clinical context for interpreting the molecular and oxidative alterations observed in this study.

α-Klotho in ESRD: correlations and clinical significance

α-KL mRNA expression was markedly reduced in HD patients, consistent with previous reports demonstrating progressive KL downregulation across CKD stages.[32] α-KL showed positive correlations with Hb, Ca^{2+}, Na^{+}, CAT, and HIF-2α, and negative correlations with iron, ferritin, P, PTH, ALP, K^{+}, creatinine, urea, HD duration, IDH, and IDWG.

These associations are consistent with the established role of α-KL in mineral metabolism and its relationship with erythropoiesis-related pathways. The inverse correlations with phosphorus and PTH agree with its recognized involvement in phosphate homeostasis and regulation of mineral balance.[33,34] The observed associations of α-KL with Na^{+} and K^{+} are also consistent with previous reports suggesting links between α-KL expression and pathways involved in electrolyte regulation.[35,36]

Furthermore, the inverse relationship with IDH is in line with reports associating reduced α-KL levels with vascular dysfunction and impaired cardiovascular health.[37] The positive association with CAT also supports a link between α-KL and antioxidant defense mechanisms.[38] Collectively, these findings suggest that reduced α-KL expression is associated with disturbances in mineral metabolism, oxidative balance, and dialysis-related complications and may represent part of a broader network involving HIF-2α and OS markers.

HIF-2α and its role in erythropoiesis and mineral disorders

HIF-2α expression was significantly reduced in HD patients and showed positive correlations with Hb, Ca^{2+}, Na^{+}, CAT, and α-KL, while negative correlations were observed with PTH, ALP, K^{+}, creatinine, urea, dialysis duration, IDH, and IDWG.

The positive association between HIF-2α and Hb is consistent with its established role in hypoxia-responsive erythropoiesis and iron utilization.[12] Its correlation with α-KL is consistent with experimental findings suggesting a link between α-KL and HIF-2α pathways.[33] Associations with electrolyte parameters and IDH may reflect links with adaptive responses to metabolic and hemodynamic stress, although the underlying mechanisms remain incompletely understood. In addition, previous studies have implicated HIF-2α in bone remodeling processes, which may explain its association with markers of mineral metabolism.[39]

Overall, the observed associations support a potential link between HIF-2α, α-KL, and oxidative stress pathways in ESRD, highlighting their possible involvement in oxygen sensing, redox balance, and mineral metabolism.

Therapeutic perspective: hypoxia-inducible factor–prolyl hydroxylase inhibitors (HIF-PHIs)

HIF-PHIs have emerged as a promising therapeutic option for renal anemia through activation of HIF signaling, stimulation of endogenous erythropoietin production, and improvement of iron utilization. Their potential effects on inflammation and oxidative stress remain under investigation.[40]

Oxidative stress: antioxidant defense

A marked oxidative imbalance was evident in HD patients. CAT activity was significantly reduced, whereas MDA levels were significantly elevated. CAT was positively correlated with Hb, Ca^{2+}, Na^{+}, α-KL, and HIF-2α and negatively correlated with several markers of disease burden. In contrast, MDA showed the opposite pattern of associations.

These findings indicate a close association between OS, anemia, mineral metabolism abnormalities, and hemodynamic instability. The inverse relationship between CAT and MDA reflects impaired antioxidant defense and increased oxidative burden in ESRD, consistent with previous reports.[41]

Inflammation and OS interplay

The observed increase in MDA together with reduced CAT activity may also reflect an underlying pro-inflammatory state. Inflammation and OS are closely interconnected and have been implicated in impaired erythropoiesis and CKD progression.[42,43] Although inflammatory markers were not directly measured, the observed biochemical profile is compatible with enhanced inflammatory activity. Reduced α-KL expression has also been associated with increased inflammatory signaling.[44]

Previous studies have demonstrated a bidirectional interaction between OS and inflammation during CKD progression.[42,43,45,46] Experimental evidence showing attenuation of oxidative injury and inflammation following KL supplementation further supports the biological relevance of the associations observed in the present study.[47]

Applicability to peritoneal dialysis (PD)

Although the present study focused on HD patients, some of the observed molecular associations may also be relevant in PD. However, differences between dialysis modalities may influence these relationships, and direct evaluation in PD populations is required.[48,49]

Overall, a key finding of this study was the concurrent reduction of α-KL, HIF-2α, and antioxidant activity together with increased OS, accompanied by significant interrelationships among these markers. The observed associations suggest the involvement of interconnected pathways related to oxidative balance, erythropoiesis, mineral metabolism, and dialysis-related complications in ESRD. However, given the cross-sectional design, these findings should be interpreted as associations rather than evidence of causality, and further longitudinal studies are required to clarify the nature of these interactions.

Study limitations

This study is limited by its cross-sectional design, single-center recruitment, and relatively modest sample size. In addition, α-KL and HIF-2α were evaluated at the mRNA level, while inflammatory and treatment-related variables were not specifically analyzed. Nevertheless, the findings provide novel insights into the associations among α-KL, HIF-2α, OS markers, and HD-related complications. Further studies are warranted to validate these observations

Conclusion

This study identified significant associations among α-KL, HIF-2α, and OS markers in ESRD patients undergoing HD. Reduced α-KL and HIF-2α expression, together with decreased CAT activity and elevated MDA levels, were associated with anemia, SHPT, and IDH. The observed interrelationships among these biomarkers highlight potential links between oxidative balance, erythropoiesis, mineral metabolism, and dialysis-related complications. Further large-scale prospective studies are needed to confirm these associations and clarify their clinical significance.

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Supplementary material

Supplementary Tables associated with this article are provided in a single PDF file.

- **Supplementary Table S1** describes the thermal cycling protocol applied using the StepOne™ Real-Time PCR System.
- **Supplementary Table S2** presents the nucleotide sequences of primers used for real-time PCR analysis.

- **Supplementary Table S3** shows the correlation matrix between all independent variables, with statistically significant correlations defined at $p<0.05$.
- **Supplementary Table S4** presents Multivariate logistic regression analysis identifying independent predictors of anemia and hyperparathyroidism in hemodialysis patients**.**

Declarations

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Author contributions

Conceptualization, H.M.Z.; Methodology, H.M.Z. and M.M.H.; Software, R.E.E.; Validation, H.M.Z. and M.M.H.; Formal Analysis, R.E.E. and M.M.H.; Investigation, H.M.Z., S.A.A., M.M.H. and M.A.; Resources, H.M.Z.; Data Curation, H.M.Z., S.A.A. and M.A.; Writing – Original Draft Preparation, H.M.Z.; Writing – Review & Editing, H.M.Z., R.E.E., S.A.A., M.A. and M.M.H.; Visualization, R.E.E.; Supervision, M.M.H.; Project Administration, H.M.Z. and M.M.H.

Conflicts of interest

The authors declare that they have no conflicts of interest relevant to this article.

Data availability

All data generated or analyzed during this study are included in this published article and its Supplementary Information files.

Ethics approval

This study was approved by the Research Ethics Committee of the Faculty of Medicine, Suez Canal University (Approval No. 4724).

Use of AI and AI-assisted technologies in the writing process

During the preparation of this manuscript, QuillBot was used by H.M.Z. for language refinement and paraphrasing of selected text sections. All content was carefully reviewed and edited by the authors, who take full responsibility for the final version of the manuscript.

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