

Supplementary Material

Title: “ α -Klotho, Hypoxia-Inducible Factor-2 α , and Oxidative Stress in Hemodialysis: Interactions with Anemia, Secondary Hyperparathyroidism, and Intradialytic Hypotension”

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This supplementary document provides additional methodological and analytical data supporting the findings of the main manuscript. It includes details of primer sequences used for real-time PCR, the thermal cycling conditions applied during amplification, and a correlation matrix illustrating the relationships between the studied independent variables and Multivariate logistic regression analysis identifying independent predictors of anemia and hyperparathyroidism in hemodialysis patients.

Supplementary tables:

Table S1: Thermal cycling protocol for StepOne™ Real-Time PCR System.

Step	Temperature	Time	Number of cycles
<i>Initial denaturation</i>	95°C	10 min	1
<i>Denaturation</i>	95°C	15 S	40
<i>Annealing</i>	56°C	30 S	
<i>Extension</i>	72°C	30 S	

Thermal cycling protocol was performed according to the manufacturer's instructions of Maxima SYBR Green qPCR master mix (ThermoFisher Scientific, USA, cat. No. K0251). A melting curve analysis was conducted to confirm primer specificity and exclude primer-dimer formation.

Table S2: The nucleotide sequence of primers used in Real-time PCR.

Genes' symbol	Forward primer (5'→3')	Reverse primer (5'→3')
<i>Klotho</i>	AGGGTCCTAGGCTGGAATGT	CCTCAGGGACACAGGGTTTA
<i>Hlf-2α</i>	CAGAGCCCTTCTCGTTTCTTT	GGAGTTGACCCTTCACATTTCT
<i>GAPDH</i>	CTCCTCACAGTTGCCATGTA	GTTGAGCACAGGGTACTTTATTG

Gene-specific primers (Willowfort, UK) were designed using the bioinformatics platforms listed in the Methods section (References from 20 to 24).

Table S3: Correlation matrix between all independent variables

Variable		age	weight	Hb	Iron	Ferritin	Ca	P	PTH	ALP	Na ⁺	K ⁺	creatinine	Urea	MDA	CAT	FC- α -KL	FC-HIF- 2α	Duration _of HD	IDH	IDWG
age	r	1	0.55	0.23	-0.18	-0.051	0.31	-0.22	-0.31	-0.3	-0.05	-0.15	-0.25	-0.24	0.01	0.21	0.09	0.03	-0.05	-0.23	-0.12
	p		<.001	0.02	0.077	0.614	0.002	0.025	0.001	0.003	0.644	0.126	0.014	0.018	0.945	0.033	0.392	0.758	0.637	0.02	0.227
weight	r	0.55	1	0.23	-0.18	-0.122	0.29	-0.09	-0.35	-0.28	0.06	-0.16	-0.27	-0.28	-0.04	0.28	0.2	0.14	-0.25	-0.339	-0.1
	p	<.001		0.021	0.074	0.22	0.003	0.395	<.001	0.005	0.54	0.102	0.006	0.005	0.727	0.005	0.052	0.18	0.011	0.0005	0.299
Hb	r	0.23	0.23	1	0.41	0.509	0.36	-0.4	-0.5	-0.45	0.43	-0.5	-0.67	-0.67	-0.24	0.71	0.27	0.35	-0.44	-0.727	-0.68
	p	0.02	0.021		0.000	0.000	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	0.016	<.001	0.007	<.001	<.001	0.000	<.001
Iron	r	-0.18	-0.18	0.41	1	0.324	-0.31	0.27	0.38	0.33	-0.2	0.38	0.6	0.53	0.24	-0.57	-0.25	-0.33	0.36	0.559	0.52
	p	0.077	0.074	0.000		0.001	0.002	0.007	<.001	0.001	0.043	<.001	<.001	<.001	0.017	<.001	0.012	0.001	<.001	0.000	<.001
Ferritin	r	-0.051	-0.122	0.509	0.324	1	-0.039	0.367	0.39	0.292	-0.414	0.436	0.42	0.527	0.078	-0.472	-0.209	-0.133	0.466	0.458	0.43
	P	0.614	0.22	0.000	0.001		0.7	0.0002	0.00006	0.003	0.00002	0.000	0.000	0.000	0.442	0.000	0.037	0.187	0.000	0.000	0.000
Ca	r	0.31	0.29	0.36	-0.31	-0.039	1	-0.24	-0.34	-0.31	0.21	-0.29	-0.61	-0.46	-0.11	0.56	0.29	0.22	-0.44	-0.577	-0.52
	p	0.002	0.003	<.001	0.002	0.7		0.017	0.001	0.002	0.04	0.004	<.001	<.001	0.298	<.001	0.004	0.031	<.001	0.000	<.001
P	r	-0.22	-0.09	-0.4	0.27	0.367	-0.24	1	0.52	0.32	-0.2	0.57	0.64	0.7	0.26	-0.52	-0.25	-0.11	0.24	0.47	0.47
	p	0.025	0.395	<.001	0.007	0.0002	0.017		<.001	0.001	0.05	<.001	<.001	<.001	0.01	<.001	0.014	0.296	0.016	0.000	<.001
PTH	r	-0.31	-0.35	-0.5	0.38	0.39	-0.34	0.52	1	0.76	-0.21	0.4	0.65	0.7	0.26	-0.62	-0.27	-0.3	0.44	0.597	0.49
	p	0.001	<.001	<.001	<.001	0.00006	0.001	<.001		<.001	0.04	<.001	<.001	<.001	0.01	<.001	0.006	0.002	<.001	0.000	<.001
ALP	r	-0.3	-0.28	-0.45	0.33	0.292	-0.31	0.32	0.76	1	-0.15	0.2	0.44	0.43	0.14	-0.43	-0.19	-0.23	0.28	0.464	0.37
	p	0.003	0.005	<.001	0.001	0.003	0.002	0.001	<.001		0.142	0.044	<.001	<.001	0.162	<.001	0.061	0.021	0.005	0.000	<.001
Na ⁺	r	-0.05	0.06	0.43	-0.2	-0.414	0.21	-0.2	-0.21	-0.15	1	-0.41	-0.45	-0.45	-0.25	0.43	0.24	0.23	-0.37	-0.446	-0.52
	p	0.644	0.54	<.001	0.043	0.00002	0.04	0.05	0.04	0.142		<.001	<.001	<.001	0.014	<.001	0.017	0.019	<.001	0.000	<.001
K ⁺	r	-0.15	-0.16	-0.5	0.38	0.436	-0.29	0.57	0.4	0.2	-0.41	1	0.62	0.69	0.18	-0.6	-0.26	-0.24	0.34	0.543	0.6
	p	0.126	0.102	<.001	<.001	0.000	0.004	<.001	<.001	0.044	<.001		<.001	<.001	0.068	<.001	0.009	0.018	0.001	0.000	<.001
creatinine	r	-0.25	-0.27	-0.67	0.6	0.42	-0.61	0.64	0.65	0.44	-0.45	0.62	1	0.9	0.4	-0.89	-0.4	-0.41	0.68	0.876	0.84
	p	0.014	0.006	<.001	<.001	0.000	<.001	<.001	<.001	<.001	<.001	<.001		<.001	<.001	<.001	<.001	<.001	<.001	0.000	<.001
Urea	r	-0.24	-0.28	-0.67	0.53	0.527	-0.46	0.7	0.7	0.43	-0.45	0.69	0.9	1	0.34	-0.84	-0.38	-0.35	0.65	0.787	0.78
	p	0.018	0.005	<.001	<.001	0.000	<.001	<.001	<.001	<.001	<.001	<.001	<.001		0.001	<.001	<.001	<.001	<.001	0.000	<.001
MDA	r	0.01	-0.04	-0.24	0.24	0.078	-0.11	0.26	0.26	0.14	-0.25	0.18	0.4	0.34	1	-0.36	-0.15	-0.19	0.27	0.373	0.42
	p	0.945	0.727	0.016	0.017	0.442	0.298	0.01	0.01	0.162	0.014	0.068	<.001	0.001		<.001	0.148	0.063	0.006	0.000	<.001
CAT	r	0.21	0.28	0.71	-0.57	-0.472	0.56	-0.52	-0.62	-0.43	0.43	-0.6	-0.89	-0.84	-0.36	1	0.38	0.38	-0.69	-0.916	-0.86
	p	0.033	0.005	<.001	<.001	0.000	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001		<.001	<.001	<.001	0.000	<.001
FC- α -KL	r	0.09	0.2	0.27	-0.25	-0.209	0.29	-0.25	-0.27	-0.19	0.24	-0.26	-0.4	-0.38	-0.15	0.38	1	0.21	-0.3	-0.402	-0.37
	p	0.392	0.052	0.007	0.012	0.037	0.004	0.014	0.006	0.061	0.017	0.009	<.001	<.001	0.148	<.001		0.032	0.002	0.000	<.001
FC- HIF- 2α	r	0.03	0.14	0.35	-0.33	-0.133	0.22	-0.11	-0.3	-0.23	0.23	-0.24	-0.41	-0.35	-0.19	0.38	0.21	1	-0.25	-0.436	-0.34
	p	0.758	0.18	<.001	0.001	0.187	0.031	0.296	0.002	0.021	0.019	0.018	<.001	<.001	0.063	<.001	0.032		0.012	0.000	<.001
Duration of HD	r	-0.05	-0.25	-0.44	0.36	0.466	-0.44	0.24	0.44	0.28	-0.37	0.34	0.68	0.65	0.27	-0.69	-0.3	-0.25	1	0.677	0.66
	p	0.637	0.011	<.001	<.001	0.000	<.001	0.016	<.001	0.005	<.001	0.001	<.001	<.001	0.006	<.001	0.002	0.012		0.000	<.001
IDH	r	-0.23	-0.339	-0.727	0.559	0.458	-0.577	0.47	0.597	0.464	-0.446	0.543	0.876	0.787	0.373	-0.916	-0.402	-0.436	0.677	1	0.837
	P	0.02	0.0005	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000			0.000
IDWG	r	-0.12	-0.1	-0.68	0.52	0.43	-0.52	0.47	0.49	0.37	-0.52	0.6	0.84	0.78	0.42	-0.86	-0.37	-0.34	0.66	0.837	1
	p	0.227	0.299	<.001	<.001	0.000	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	0.000	

significant at p<0.05. r: Correlation, Hb: hemoglobin, MCH: mean corpuscular hemoglobin, HCT: Hematocrit, PLT: platelet count, WBC: white blood count, Ca²⁺: calcium, P: phosphorus, PTH: parathyroid hormone, ALP: alkaline phosphatase, ALT: alanine transaminase, AST: aspartate aminotransferase, Na⁺: sodium, K⁺: potassium, MDA: malondialdehyde, CAT: catalase, KL: Klotho, HIFs: Hypoxia-inducible factors, HD: hemodialysis , IDH: intradialytic hypotension, , IDWG: interdialytic weight gain.

Table S4: Multivariate logistic regression analysis identifying independent predictors of anemia and hyperparathyroidism in hemodialysis patients.

predictability of Anaemia			
Predictor variables	Odds Ratio	95% conf. interval	p
FC- α -KL	0.44	0.21 - 0.93	0.032*
FC- HIF-2 α	1.67	1.16 - 2.4	0.006*
CAT	0.98	0.98 - 0.99	< 0.001*
MDA	1.01	0.99 - 1.02	0.31
predictability of Hyperparathyroidism			
Predictor variables	Odds Ratio	95% conf. interval	p
FC- α -KL	0.01	0 - 0.93	0.046*
FC- HIF-2 α	0.42	0.24 - 0.76	0.004*
CAT	0.97	0.94 - 0.99	0.008*
MDA	1.1	1.06 - 1.14	< 0.001*

Results are presented as odds ratios (ORs) with 95% confidence intervals (CIs). A p-value < 0.05 was considered statistically significant.

CI: confidence interval; FC: fold change, α -KL, α -Klotho; HIF-2 α , hypoxia-inducible factor-2 α ; CAT, catalase; MDA, malondialdehyde.