ERYTHROPOIETIN TREATMENT MODULATES SERUM KLOTHO LEVELS IN KIDNEY TRANSPLANT RECIPIENTS

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BACKGROUND AND AIM

Klotho protein exists in two forms: a type I transmembrane protein exerting its function on mineral bone metabolism as co-receptor of FGF23; and a circulating soluble secreted protein that seems to play a role as humoral anti aging factor with pleiotropic activities. Data on soluble Klotho in Chronic Kidney Disease (CKD) are contradictory and even less is known about its expression after renal transplantation. Few studies evaluated the pharmacological modulation of Klotho. In vivo experimental studies demonstrated that the observed Klotho reduction caused by renal damage can be mitigated by recombinant human erythropoietin (rhEPO) treatment.
The aim of this study was to determine soluble Klotho serum levels in a population of kidney transplant patients and to evaluate whether rhEPO treatment can modulate these levels.

MATERIALS AND METHODS

117 renal transplant recipients (TX) aged 23-79 years who had received their transplants at least 6 months previously were enrolled in the study. Fasting serum samples and 24-h urine samples were collected at enrollment. We discontinued the use of rhEPO for five weeks in all transplant patients with stable Hb level above 11 g/dl. Whole blood was collected before and after the EPO interruption to measure changes in soluble Klotho levels.

	KTR n=117	CKD n=31	HS n=22
Age (years)	51.6 ± 11.49	57.09 ± 12.6	48.64 ± 13.19
Males	73 (62.39 %)	19 (61.29 %)	13 (59.10 %)
eGFR (ml/min/1.73m²)	52.98 (36.29-63.12)	50.28 (32.31-67.68)	97.32 (86.78-113.9) ‡
iPTH (pg/ml)	136 (100.5-218)	96.5 (61-148.2) *	49 (40-56) ‡
Ca (mg/dl)	9.87 ± 0.53	9.25 ± 0.37 ‡	9.26 ± 0.53 ‡
Ph (mg/dl)	3.41 ± 0.86	4.08 ± 0.62 †	3.81 ± 0.44 *
U-Ph (mg/24h)	729.1 ± 259.87	648.8 ± 229.75	429.7 ± 435.64 *
25 OH vit D (ng/ml)	9.67 (5.91-15.4)	13.27 (8.88-24.4) *	20.52 (17.23-23.5) ‡
FGF23 (pg/ml)	4.01 (1.4-22.96)	3.19 (0.25-20.65)	8 (2-24.37)
Klotho (ng/ml)	0.68 (0.37-1.07)	0.6 (0.48-1.12)	0.37 (0.27-0.52) †
Hb (g/dl)	13.39 ± 1.81	12.94 ± 2.03	13.87 ± 1.43
RBC (10^9/ml)	4.95 ± 0.9	4.44 ± 0.64 *	5.03 ± 0.66
Epo (mUI/ml)	13 (9-22)	11.5 (8.5-23.75)	9 (8-13.25) *
TSAT (%)	21.47 ± 9.4	19.25 ± 7.81	20.93 ± 6.54
rhEPO treatment	17 (14.53 %)	5 (16.12 %)	
Active vit. D treatment	26 (22.22 %)	11 (35.48 %) *	
RAAS blocker treatment	47 (40.17 %)	15 (48.396 %)	
CyA treatment	45 (38.46 %)		
Transplant Age (years)	5.22 (2.63-12.16)		

Table 1: Characteristics of the study population. RBC: Red Blood Cells; TSAT: Transferrin Saturation. Differences with Transplanted patients: * p < 0.05;† p < 0.001; ‡ p < 0.0001.

	PRE	POST
Klotho (ng/ml)	1.17 (0.99-2.175)	0.76 (0.625-1.51) *
FGF23 (pg/ml)	1.12 (1.04-2.215)	0.67 (0.37-1.5)
25 OH vit D (ng/ml)	4.44 (3-9.975)	8.3 (5.035-13.74)
eGFR (ml/min)	30.78 (20.34-56.1)	30.78 (18.43-56.86)
Epo (mUI/ml)	40 (14-46)	6.5 (5.25-10.75) *
Hb (g/dl)	12.3 (11.7-12.45)	11.6 (11.1-11.8) *
Ph (mg/dl)	3.6 (3.3-4.1)	3.5 (3.3-4.1)
U-Ph (mg/24h)	512.4 (441.8-604.6)	481.6 (403.8-524.4)
Ca (mg/dl)	9.5 (9.35-9.65)	9.5 (9.15-9.7)
iPTH (pg/ml)	228.0 (115.5 - 343.4)	202.7 (128.3 - 331.1)

Table 2: Clinical measurements in renal transplantation patients before (PRE) and after (POST) rhEPO discontinuation.
Differences with pre wash-out assessment:
* p < 0.05

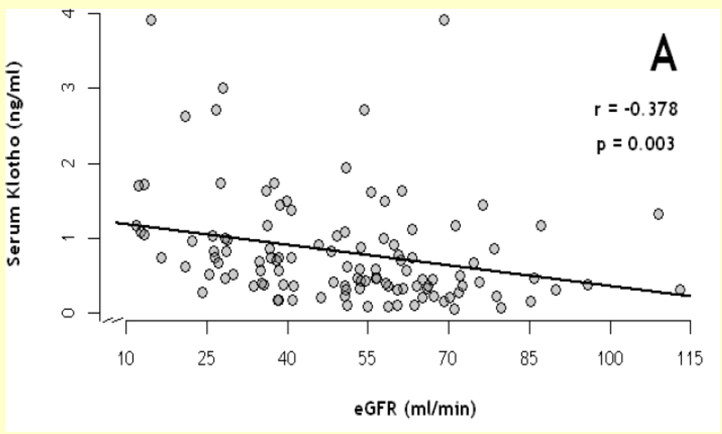

Figure 1 : Relationship among eGFR (A), serum FGF23 (B) with serum Klotho. Data are Pearson product moment correlation coefficient and P value. Because FGF23 had a positively skewed distribution, this variable were log transformed (ln) before the correlation study.

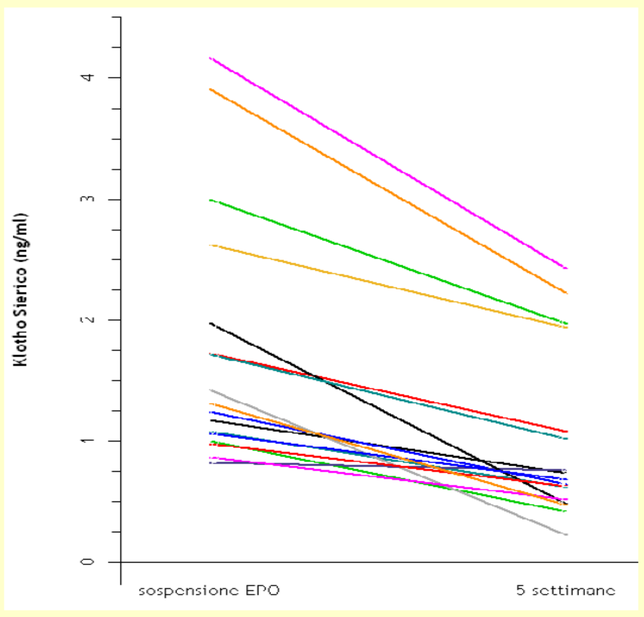

Figure 2: Serum Klotho levels in 17 KTR on rhEPO treatment before and after rhEPO discontinuation

RESULTS

Our analyses revealed that HK-2 cells express ADPN both in terms of mRNA and protein. These results were confirmed by the observed cytoplasmatic HK-2 intense immunoreactivity for ADPN antibody and by immunohistochemical analysis showing a diffuse ADPN distribution in normal kidney tissue. We also confirmed that HK-2 cells express both best-characterized receptors for ADPN, adipoR1 and adipoR2 although, the results revealed that adipoR1 is the predominant isoform. Furthermore we observed that tubular cells secrete ADPN in basal condition and, more interestingly, this secretion significantly increases (p < 0.05) upon LPS treatment in a time dependent manner. Finally, immunohistochemical analysis of kidney biopsies obtained from patients affected by membranous and rapidly progressive glomerulonephritis showed a similar pattern of ADPN staining observed in healthy control.

CONCLUSIONS

Our study demonstrates, for the first time, that tubular renal cells express and secretes ADPN, which concentration increases upon inflammatory stimulus. These results suggest that in renal inflammatory diseases, tubular cells may contribute to the increasing ADPN circulating levels, triggering a feedback response in order to self-mitigate the inflammatory process.

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