URINARY ENDOTROPHIN, A PRODUCT OF COLLAGEN TYPE VI FORMATION, PREDICTS DISEASE PROGRESSION AND MORTALITY IN PATIENTS WITH CHRONIC KIDNEY DISEASE

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INTRODUCTION AND OBJECTIVES

Renal fibrosis is the central pathogenic process in progression of chronic kidney disease (CKD).[1] Due to a lack of sufficient tools and biomarkers for a better stratification of CKD patients, precision medicine is currently not available. Collagen type VI (COL VI) is a major component of extra-cellular matrix deposition in systemic fibrosis, including the kidney. Endotrophin, the C-terminal of the alpha-3 chain of COL VI, is cleaved off during formation and is a dynamic product reflecting fibrosis and exerts pleiotropic fibrotic effects.[2,3] However, it is not known if endotrophin is produced in fibrotic kidney disease and whether urinary levels are associated with adverse outcomes in CKD patients.

To investigate the involvement of COL VI formation in kidney pathology we used serial section immunohistochemistry on human kidneys to ascertain endotrophin expression in relationship to COL VI deposition. We furthermore investigated the prognostic potential of urinary endotrophin by measurements in samples from a large prospective study assessing adverse outcomes in CKD patients.

METHODS

Figure 1. Histological and immunohistological assessment of tissue sections from control and fibrotic kidneys

Human renal tissue was obtained from nephrectomized specimens with renal fibrosis from five patients with advanced hydronephrosis and/or chronic pyelonephritis. Control kidney tissue comprised histologically (uninvolved) normal cortex from nephrectomy specimens of five patients with renal cell carcinoma or trauma. The subfigures labelled with a mark (e.g. A') are magnifications of the areas outlined with a square. The scale bars are 250 µm.

Assessing effect of urinary endotrophin on adverse outcomes

Urinary endotrophin was measured with a novel ELISA (Pro-C6, Nordic Bioscience) in urine of 499 patients from a prospective cohort study of patients with high risk CKD, as defined by the UK NICE criteria (2008). Participants were followed for five years to monitor development of ESRD and mortality. Urinary endotrophin was normalized for urinary creatinine (endotrophin:creatinine ratio (ECR)).

Figure 2. Correlation of baseline endotrophin:creatinine ratio with kidney function at baseline and afterone year

Spearman rank correlation analysis of endotrophin:creatinine ratio with kidney function at baseline and one-year. The spearman's rank correlation coefficient has been inserted in each subfigure.

Figure 3. Odds ratios for multivariable analysis of prediction of disease progression within one year

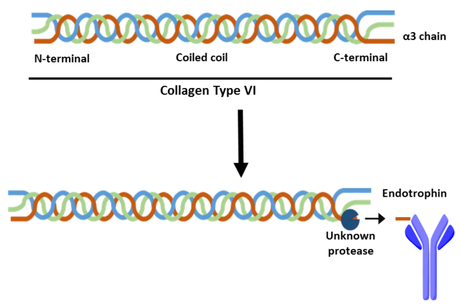
Logistic regression analysis was performed for quartiles of endotrophin:creatinine ratio against one-year disease progression. One-year progression of CKD was defined as a decline in eGFR of more than 30% or development of ESRD. Known important confounding factors (e.g. eGFR and ACR) and any other variables with a p<0.1 on univariable analysis were included in multivariable logistic regression analysis with a forward inclusion strategy. The parameters included in the model analysis, besides quartiles of endotrophin:creatinine ratio, were ethnicity, body mass index, index of multiple deprivation score, renal diagnosis, mean arterial pressure (MAP), estimated glomerular filtration rate (eGFR), and urinary albumin:creatinine ratio (ACR). Presented are Odds ratios with 95% CI.

Figure 4. Kaplan-Meier plot showing (A) development of ESRD and (B) mortality by ECR quartile

Kaplan-Meier curves were made to visualize development of ESRD (**Figure 4A**) and mortality (**Figure 4B**) of patients stratified by quartiles of endotrophin:creatinine ratio. Hazard ratios for quartiles of endotrophin:creatinine ratio and per increase in one standard deviation are presented in the figures.

Endotrophin

Endotrophin is released from the alpha-3 chain of COL VI during COL VI formation. The released fragment is recognized by the Pro-C6 assay.

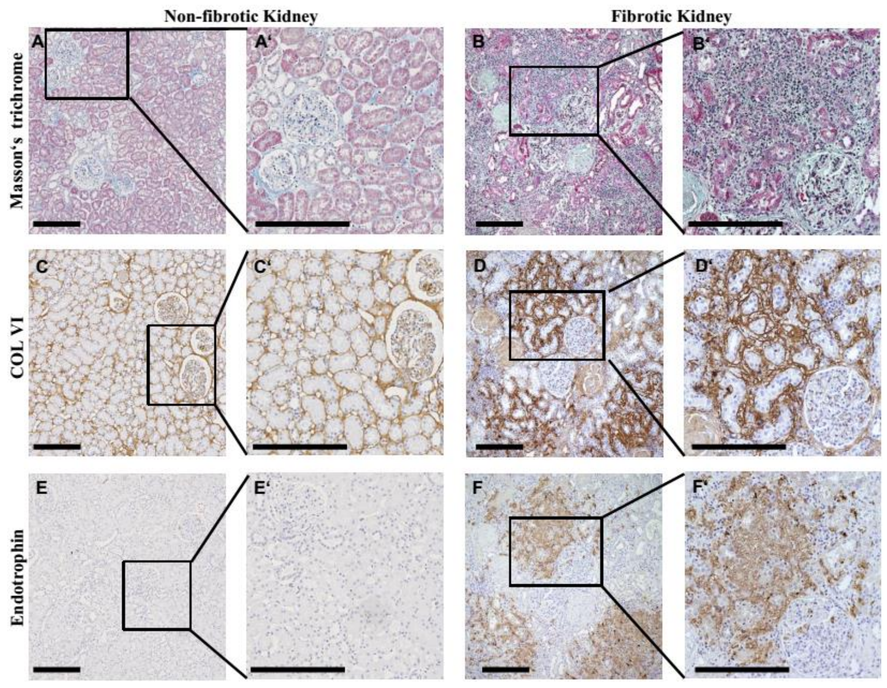


CONCLUSIONS

Our study suggests that endotrophin reflects renal fibrosis and that elevated levels of urinary endotrophin:creatinine ratio are associated with disease progression and mortality.

RESULTS

- In fibrotic kidneys, a prominent staining for endotrophin was observed, in areas that co-localized with high COL VI staining (**Figure 1**)
- Endotrophin:creatinine ratio correlated with eGFR at baseline and one year (**Figure 2**)
- Patients with high levels of endotrophin:creatinine ratio were more likely to progress within one year. Adjustment for albumin:creatinine ratio, mean arterial pressure, and eGFR showed an independent association of both Q3 (OR 3.62, p=0.04) and Q4 of endotrophin:creatinine ratio with progression of CKD (OR 8.96, p=0.0007) (**Figure 3**)
- Endotrophin:creatinine ratio was associated with development of ESRD (per increase in one standard deviation; HR 1.58, p<0.0001) (**Figure 4A**)
- Endotrophin:creatinine ratio was associated with mortality (per increase in one standard deviation; HR 1.37, p=0.0013) (**Figure 4B**)



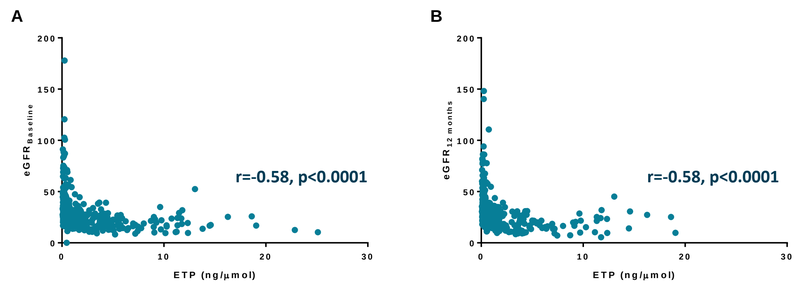


Figure 2. Correlation of baseline urinary endotrophin:creatinine ratio with kidney function at baseline and after one year

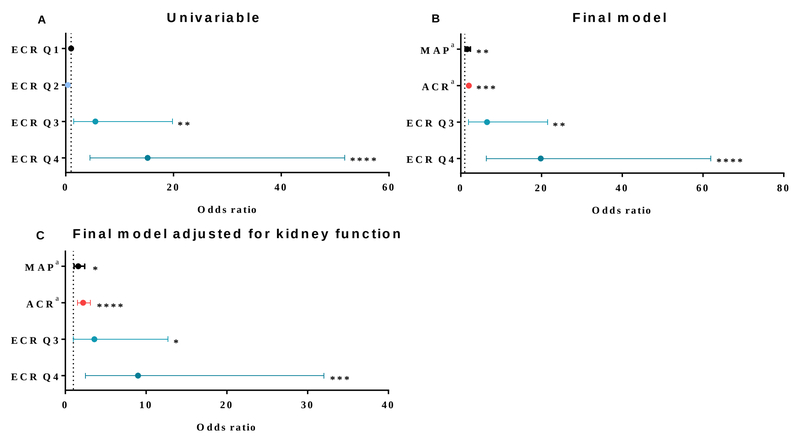


Figure 3. Univariable and multivariable logistic regression analysis for prediction of one-year disease progression

Out of 416 patients alive and with one year follow-up data, 46 (11%) had progressed. ECR; Endotrophin:creatinine ratio.
[a]per increase in one standard deviation (1SD).
Statistical significance: *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Table 1. Clinical characteristics of study subjects stratified by quartiles of urinary endotrophin:creatinine ratio (ECR)

	Endotrophin:creatinine Quartile 1 (n=125)	2 (n=126)	3 (n=123)	4 (n=125)	P value	Completeness of data
Age (years)	60 (49-72)	65 (50-78)	66 (52-78)	66 (54-76)	0.02	100%
Sex (male)	79 (63.2)	71 (56.3)	79 (64.2)	78 (62.4)	0.57	100%
Ethnicity					0.12	
White	97 (77.6)	95 (75.4)	85 (69.1)	83 (66.4)		
Black	13 (10.4)	11 (8.7)	13 (10.6)	7 (5.6)		100%
South Asian	14 (11.2)	19 (15.8)	23 (18.7)	34 (27.2)		
Other	1 (0.8)	1 (0.8)	2 (1.6)	1 (0.8)		
Primary renal diagnosis					0.0002	
Ischaemia/hypertension	30 (24.0)	35 (27.8)	31 (25.2)	34 (27.2)		
Diabetes mellitus	11 (8.8)	4 (3.2)	11 (8.9)	22 (17.6)		100%
Glomerulonephritis	34 (27.2)	23 (18.3)	18 (14.6)	8 (6.4)		
Polycystic kidney disease	6 (4.8)	10 (7.9)	10 (8.1)	3 (2.4)		
Other/Unknown	44 (35.2)	54 (42.8)	53 (43.2)	58 (46.4)		
Co-morbidities						
Malignancy	15 (12.0)	19 (12.7)	17 (13.8)	21 (16.8)	0.74	
Diabetes mellitus	39 (31.2)	37 (29.4)	47 (38.2)	61 (48.8)	0.006	
COPD	16 (12.8)	22 (17.5)	11 (8.9)	11 (8.8)	0.12	100%
Cerebrovascular disease	15 (12.0)	13 (10.3)	11 (8.9)	15 (12.0)	0.84	
Ischaemic heart disease	30 (24.0)	30 (23.8)	22 (17.9)	30 (24.0)	0.58	
Peripheral vascular disease	12 (9.6)	11 (8.7)	16 (13.0)	12 (9.6)	0.69	
Age-adjusted CCI (score ≥5)	52 (41.6)	73 (57.9)	72 (58.5)	81 (64.8)	0.0004	100%
Smoking (Current)	21 (16.8)	20 (15.9)	15 (12.2)	11 (8.8)	0.31	100%
IMD Score	28.7 (16.3-44.1)	25.5 (15.3-45.1)	33 (19.3-44.6)	27 (17-47)	0.66	100%
Body mass index(kg/m²)	30 (26-33)	29 (25-32)	28 (25-33)	28 (24-35)	0.53	100%
Systolic blood pressure (mmHg)	119 (109-129)	123 (113-140)	126 (116-142)	127 (116-148)	<0.0001	100%
Diastolic blood pressure (mmHg)	74 (66-82)	75 (67-81)	74 (67-84)	75 (68-83)	0.92	100%
Mean arterial pressure (mmHg)	90 (83-96)	91 (84-98)	94 (84-101)	94 (86-100)	0.02	100%
Pulse pressure (mmHg)	42 (34-53)	49 (36-66)	51 (39-65)	51 (40-68)	<0.0001	100%
Serum creatinine (µmol/L)	158 (137-197)	189 (149-225)	216 (181-260)	270 (222-345)	<0.0001	99%
Cystatin C (mg/L)	1.9 (1.6-2.3)	2.3 (1.8-2.8)	2.5 (2.0-3.0)	3.1 (2.6-3.7)	<0.0001	94%
eGFR (mL/min/1.73m²)	36 (28 -46)	28 (23-38)	25 (20-29)	18 (14-24)	<0.0001	99%
ACR (mg/mmol)	10 (2-76)	23 (6-129)	47 (9-163)	77 (15-184)	<0.0001	98%
C-reactive protein (mg/L)	2.8 (1.1-6.5)	3.6 (1.5-8.0)	2.7 (1.5-5.0)	3.9 (1.9-8.1)	0.18	93%
ECR (ng/µmol creatinine)	0.2 (0.1-0.2)	0.4 (0.3-0.5)	1.3 (0.9-1.7)	5.0 (3.6-8.8)	<0.0001	100%

Categorical variables are expressed as number (%), and continuous variables as median (IQR). Abbreviations: COPD, chronic obstructive pulmonary disease; CCI, Charlson's comorbidity index; IMD, index of multiple deprivation; eGFR, estimated glomerular filtration rate; ACR, albumin:creatinine ratio; ECR, endotrophin:creatinine ratio.

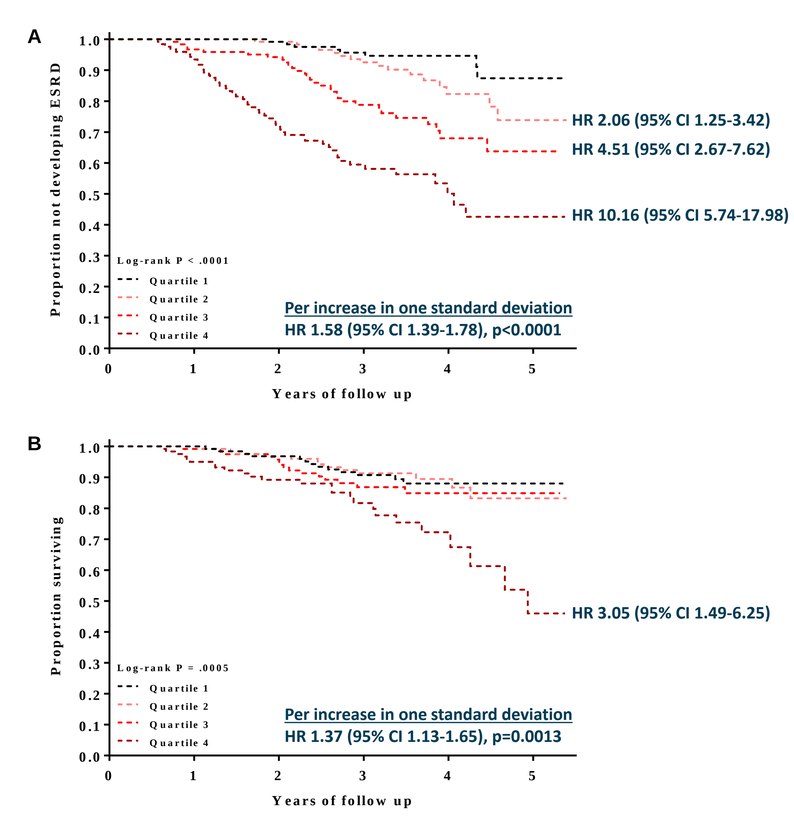


Figure 4. Kaplan-Meier plot showing (A) development of ESRD and (B) mortality stratified by quartiles of urinary endotrophin:creatinine ratio

Out of the 499 patients, 105 (21.0%) developed ESRD and 66 (13.2%) died during the follow-up period.

REFERENCES

1) Liu Y. Nat Rev Nephrol. 2015;7(12):684–96.
2) Aigner T et al. Biochem Biophys Res Commun. 2002;748:743–8.
3) Sun K et al. Nat Commun. 2014;5:3485.

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