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CLINICAL STUDY

Placental growth factor and placental protein 13 in patients with Balkan endemic nephropathy, a worldwide disease

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Abstract

Background: Balkan endemic nephropathy (BEN) is a chronic tubulointerstitial kidney disease occurring in people living in along the tributaries of the Danube River. The aim of the study was to determine serum level and urinary excretion of placental growth factor (PlGF) and placental protein 13 (PP13) in patients with BEN. *Methods*: Thirty patients with BEN from the South Morava River region of Serbia and 18 controls were studied. Age of patients was 74 yr (53–87) and 73 yr (66–83) in controls. *Results*: In patients with BEN, serum creatinine was significantly higher than in controls (129.7 vs. 83.2 μmol/L, respectively), but GFR was lower in patients than in controls (40.7 vs. 54.6 mL/min). Serum PlGF was significantly higher in BEN patients than in controls (9.90 vs. 6.80 pg/mL), urinary excretion being significantly lower in patients (0.20 vs. 0.90 pg/mmol creat.). Serum PP13 was significantly lower in BEN patients (208.2 vs. 291.0 pg/mL). Urinary excretion of PP13 was also significantly lower in BEN patients than in controls (32.5 vs. 182.5 pg/mmol creat). In multivariate regression analysis BEN, sex and age were significant determinants of the observed changes in PlGF and PP13. *Conclusion*: Important changes of PlGF and PP13 in patients with BEN were demonstrated, where kidney disease, female sex, and the age have been significant determinants.

Keywords

Balkan endemic nephropathy, epigenetics, placental growth factor, placental protein 13, tubulointerstitial kidney disease

History

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Introduction

Balkan endemic nephropathy (BEN) is a chronic tubulointerstitial kidney disease with insidious onset and slow progression to terminal renal failure, associated frequently with upper urothelial cancer (UUC).[1,2] It affects people living in the alluvial plains along the tributaries of the Danube River in Bosnia, Bulgaria, Croatia, Romania, and Serbia. Evidence suggest that environmental rather than genetic factors play a decisive role in the etiopathogenesis of BEN.[3] Aristolochic acid and mycotoxins seem to play an important role in BEN etiopathogenesis.[4–6] Since BEN was first described around half a century ago, socioeconomic changes (in housing, farming, living standards, etc.) have been profound and obscured factors responsible for the observed reduction in incidence of BEN and associated UUC.[7] Whatever the causes of BEN, the disease is not be restricted only to southeastern Europe. Rather, the intensity of exposure to risk factors for BEN and, consequently, clustering of cases has more likely determined our knowledge of topographical distribution of an etiological entity that is much more widespread, or that might even be ubiquitous in its sporadic form.[8,9]

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Genetic epidemiology could establish the relative size of the genetic effect in relation to other sources of variation in disease risk (environmental effects such as intrauterine, childhood, or early adulthood environment; and chemical effects as well as behavioral and social aspects) and develop etiologic prevention and treatment.[3,8] NGS nominated CELA1, HSPG2, and KCNK5 as candidate genes for predisposition to BEN were demonstrated.[10] DNA methylation array analysis on DNA samples from Bulgarian and Serbian endemic regions, and histone acetylation levels in BEN and control patients have revealed marked epigenetic changes, important in the pathogenesis of BEN and a opportunity for selective therapeutic interventions in these patients.[11,12]

Placental growth factor (PlGF) is a member of the vascular endothelial growth factor (VEGF) family that also comprises VEGF-A (VEGF), VEGF-B, VEGF-C, and VEGF-D. Unlike VEGF, PlGF has diverse roles in tissue ischemia, malignancy, inflammation, and several other diseases.[13] Placental protein 13 (PP 13) is one of the several placental proteins of importance in many biochemical and physiological effects in the trophoblast membrane related to implantation, blood pressure regulation, and tissue oxygenation. Little is known of its effects not directly related to pregnancy.

The aim of this study was to determine serum level and urinary excretion of PlGF) and PP13 in patients with BEN.

Patients and methods

BEN was diagnosed at the Institute of Nephrology, Clinical Centre Nis (Serbia), using criteria proposed by an international panel.[14] Impairment of kidney function was defined as glomerular filtration rate estimated using MDRD equation (GFR)[15] lower than 60 mL/min/1.73 m^2. Thirty BEN patients were recruited from the endemic settlements around the South Morava River. Control patients, 18, came from neighboring non-endemic regions, after exclusion of any with a family history of kidney disease. Controls had no kidney disease, no anamnesis data for other chronic illnesses, hypertensive disease and were clinically healthy at the time of blood sampling. They were matched according to age and sex to BEN patients. First morning urine was collected, centrifuged at 3000 rpm, and frozen at −20 °C. Blood and urine chemistries were done on an Automatic analyzer A24 for In Vitro Diagnostics (manufactured by Biosystems SA), which performed spectrometric measurements. Serum level and urinary excretion of PlGF and PP 13 in patients with BEN were determined with commercial ELISA kits from Cusabio (CUSABIO BIOTECH, Toronto, Canada). The microtiter plate provided in this kit had been pre-coated with an antibody specific to PlGF/PP13. Standards or samples were then added to the appropriate microtiter plate wells with a biotin-conjugated polyclonal antibody preparation specific for PlGF/PP13, and Avidin conjugated to Horseradish Peroxidase and incubated. Then, a TMB (3,3′,5,5′ tetramethyl-benzidine) substrate solution was added to each well. The enzyme-substrate reaction was terminated by the addition of a sulfuric acid solution, and the color change is measured spectrophotometrically at a wavelength of 450 nm. The concentration of PlGF/PP13 in the samples is then determined by comparing the O.D. of the samples to the standard curve.

This study protocol was approved by the Ethical Committee of the Faculty of Medicine, University of Nis, and the research was carried out in compliance with the Declaration of Helsinki.

Statistical analysis

Results are expressed as Means ± SD, Median (Minimum–Maximum) or Number (Percentage), as appropriate. To compare values of continuous variables between two groups Student's t-test was used for normally distributed data and Mann–Whitney's *U*-test for non-normally distributed data. Pearson's Chi-Square test and Fisher's exact test were used to compare categorical variables between groups. Univariate and multivariate regression models using enter method were performed to estimate associations between PP13 and PlGF with factors of interest. To evaluate a correlation between all investigated characteristics Spearman's rank correlation coefficients were calculated. A p-value < 0.05 was considered statistically significant. Data were analyzed using statistical software SPSS for Windows Version 18.0 (Chicago, IL).

Results

Laboratory parameters and serum level and urinary excretion of PlGF and PP 13 in patients with BEN and controls are shown in Table 1. No significant difference between the male and the female parameters of investigated groups was observed. Glomerular filtration rate (GFR) in BEN patients was found decreased by testing serum creatinine and estimated GFR. Hemoglobin level was significantly lower in BEN patients; serum glucose was higher in controls. There were five type II diabetic patients (2/18 controls and 3/30 BEN patients). SPlGF of BEN patients was significantly higher than in controls ($p < 0.05$) and correlated significantly with the age of BEN patients ($p < 0.05$) (Table 2). SPlGF of healthy controls correlated significantly with the serum creatinine level ($p < 0.01$). UPlGF did not correlate with any of the tested parameters of BEN patients and controls. SPP13 was significantly lower in BEN patients than in controls ($p < 0.022$), and significantly correlated with the sex ($p < 0.01$), age ($p < 0.05$), SCr ($p < 0.01$), CCr ($p < 0.01$), UP ($p < 0.01$), UA ($p < 0.01$) and the hemoglobin level ($p < 0.01$). UPP 13 of healthy controls correlated significantly with the serum creatinine level ($p < 0.01$). Univariate regression analysis revealed association between UPlGF as dependent variable with SCr ($p < 0.038$), UP ($p < 0.001$) and UA ($p < 0.001$); SP13 as dependent variable with the BEN group ($p < 0.008$), CCr ($p < 0.001$), and hemoglobin level ($p < 0.001$); UPP13 as dependent variable with women sex ($p < 0.009$), BEN group ($p < 0.001$), SCr ($p < 0.003$), CCr ($p < 0.032$), UP ($p < 0.046$), hemoglobin ($p < 0.023$) and glucose level ($p < 0.037$) (data not presented in table). Multivariate regression analysis for estimating the relationships among serum and urine PlGF and PP13, and clinical-biochemical variables was done (Table 3). In multivariate regression analysis, BEN and age were significant determinants of SPlGF, UP, and UA for UplGF; women sex and BEN group were significant determinants of UPP13 as the dependent variable.

Table 1. Statistical difference between the distribution of parameters in investigated groups.

Variables	BEN	Control group	*p*
Male	17 (56.7%)	11 (61.1%)	0.866
Female	13 (43.3%)	7 (38.9%)	
Age, yrs	74 (53–87)	73 (66–83)	0.411
SCr, μmol/L	129.7 (67.6–187.1)	83.2 (67.8–107.4)	0.001
GFR, ml/min	40.7 (9.4–96.0)	64.6 (45.9–99.9)	0.004
UP, mg/mmol Creat.	15.49 (3.25–462.4)	11.62 (1.93–26.39)	0.001
UA, mg/mmol Creat.	1,73 (0.18–257.0)	0.98 (0.08–51.2)	0.045
HGB, g/L	11.8 ± 1.8	13.5 ± 1.4	0.003
SGlucose, mmol/L	4.80 (3.54–6.60)	5.10 (4.20–8.12)	0.033
SPlGF, pg/ml	9.90 (0.63–37.37)	6.80 (0.75–34.94)	0.050
SPP13, pg/ml	208.2 ± 114.0	291.0 ± 57.2	0.022
UPlGF, pg/ml	2.30 (0.075–98.70)	9.55 (0.075–32.74)	0.005
UPP13, pg/ml	575.7 (0.75–3024.0)	1959.0 (0.75–3439.5)	0.001
UPlGF, pg/mmol Creat.	0.20 (0.075–15.10)	0.90 (0.75–2.09)	0.003
UPP 13, pg/mmol Creat.	32.50 (0.75–324.4)	182.5 (075–381.2)	0.001

Notes: SCr – Serum creatinine; GFR – Glomerular filtration rate; UP – Urine protein; UA – Urine albumin; HGB – Hemoglobin; SPlGF – Serum Placenta Growth Factor; SPP13 – Serum Placenta Protein 13; UPlGF – Urine Placenta Growth Factor; UPP 13 – Urine Placenta Protein 13.

Discussion

The present study showed important changes of PlGF and PP13 in serum and urine of patients with BEN. Higher serum

Table 2. Spearman's rank correlation coefficient for statistical dependence between serum/urine PlGF and PP 13, and clinical-biochemical parameters tested in patients with BEN and control healthy subjects.

Groups	Variable	SPlGF, pg/ml	SPP13, pg/ml	UPlGF, pg/ml	UPP13, pg/ml
BEN	Sex	−0.212	−0.127	−0.071	0.487**
	Age	0.408*	−0.190	0.154	−0.414*
	SCr	0.139	−0.258	0.066	−0.687**
	CCr	−0.070	−0.258	0.016	0.686**
	UP	−0.10	−0.345	0.219	−0.734**
	UA	−0.019	−0.335	−0.018	−0.479**
	HGB	0.098	0.472*	−0.156	0.494**
	SPlGF, pg/ml		−0.003	−0.009	0.027
	SPP13, pg/ml			−0.071	0.244
	UPlGF, pg/ml				−0.310
Control	Sex	−0.269	−0.220	−0.297	0.319
	Age	−0.255	0.041	−0.149	0.117
	SCr	0.519**	−0.574**	−0.282	−0.679**
	CCr	0.187	0.284	0.436	−0.293
	UP	−0.126	0.346	0.178	0.185
	UA	−0.115	−0.121	0.0217	−0.007
	HGB	0.075	0.402	0.402	−0.062
	SPlGF, pg/ml		0.228	0.094	−0.183
	SPP13, pg/ml			0.323	0.314
	UPlGF, pg/ml				−0.183

Notes: SCr – Serum creatinine; GFR – Glomerular filtration rate; UP – Urine protein; UA – Urine albumin; HGB – Hemoglobin; SPlGF – Serum Placenta Growth Factor; SPP13 – Serum Placenta Protein 13; UPlGF – Urine Placenta Growth Factor.
*$p < 0.05$; **$p < 0.01$.

Table 3. Multivariate regression analysis for estimating the relationships among serum and urine PlGF and PP 13, and clinical-biochemical variables.

		Multivariate regression			
			95% CI for B		
Dependent variable	Factor	*B*	Lower Bound	Upper Bound	*p*
SPlGF	Women	4.00	−3.62	11.62	0.291
	BEN group	8.40	0.14	11.65	**0.046**
	Age	0.58	0.03	1.14	**0.040**
	SCr	0.03	−0.05	0.11	0.420
	GFR	0.10	−0.13	0.33	0.370
	UP	0.00	−0.05	0.05	0.983
	UA	−0.18	−0.52	0.16	0.296
	HGB	0.96	−1.53	3.45	0.436
SPP13	Women	−24.22	−101.92	53.49	0.527
	BEN group	−20.89	−104.82	63.04	0.615
	Age	2.59	−3.11	8.29	0.360
	SCr	−0.75	−1.03	0.46	0.200
	GFR	0.72	−1.54	2.99	0.518
	UP	−0.20	−0.74	0.33	0.448
	UA	0.62	−2.85	4.10	0.717
	HGB	14.20	−11.24	39.64	0.263
UPlGF	Women	2.24	−5.26	9.73	0.548
	BEN group	−5.90	−14.06	2.26	0.151
	Age	0.39	−0.18	0.96	0.172
	SCr	−0.03	−0.10	0.04	0.406
	GFR	0.14	−0.08	0.37	0.204
	UP	0.37	0.32	0.42	**0.001**
	UA	−0.51	−0.85	−0.16	**0.005**
	HGB	0.30	−2.06	2.66	0.798
UPP13	Women	765.46	179.17	1351.75	**0.012**
	BEN group	−753.64	−1393.56	−113.73	**0.022**
	Age	−5.39	−70.45	59.66	0.867
	SCr	−3.10	−6.70	0.50	0.090
	GFR	−11.52	−37.03	13.99	0.365
	UP	2.62	−3.45	8.70	0.386
	UA	−16.59	−55.94	22.75	0.397
	HGB	69.94	−200.43	340.32	0.602

Notes: SCr – Serum creatinine; GFR – Glomerular filtration rate; UP – Urine protein; UA – Urine albumin; HGB – Hemoglobin; SPlGF – Serum Placenta Growth Factor; SPP13 – Serum Placenta Protein 13; UPlGF – Urine Placenta Growth Factor; UPP 13 – Urine Placenta Protein 13.
Statistically significant values are shown in bold.

level of PlGF was associated with the lower urinary excretion of PlGF. Serum level of PP13 was higher in the control and was associated with a significantly increased excretion of this protein.

PlGF is a member of the VEGF sub-family molecule involved in angiogenesis, in particular during embryogenesis. Treatment with PlGF blockade offers the potential to stem ongoing disease.[16] The role of PlGF blockade on tumor angiogenesis in 15 models during primary tumor growth was modest in most models and suggest that clinical evaluation of anti-PlGF antibodies may be challenging.[17]

There appears to be a polygenic susceptibility to the disease in interaction with multiple environmental factors.[3,4] AA is confirmed as the etiologic agent of BEN; however, it may not be the sole risk factor, and also includes mycotoxins.[4] Mutant genes (CELA1, HSPG2, and KCNK5) in BEN patients encode proteins involved in the basement membrane/extracellular matrix and vascular tone, tightly connected to process of angiogenesis.[10] In BEN patients, the CpG islands of SEC61G, IL17RA, HDAC11 genes were hypomethylated compared to controls.[11] Deregulation of these genes, involved in immunological response, was suggested as a common mechanism in BEN pathogenesis. The acetylation of histone lysine residues was found to be increased at specific sites of H3 and total H4 histones isolated from urothelial cells of patients with BEN. Due to a possible mechanism and biological role of epigenetic chromatin modification in urothelial tumor development, the obtained results may open opportunity for selective therapeutic interventions in patients with BEN.[12] Epigenetic mechanisms of gene regulation, such as DNA methylation and chromatin modification, are also influenced by the environment and play an important role in the fetal basis of adult disease susceptibility.

The vastly different environments are all able to alter gene expression and change phenotype, in part by impinging on and modifying the epigenome. In addition, if these environmentally induced epigenetic adaptations occur at crucial stages of life, as it is in BEN etiopathogenesis, they can potentially change the behavior, disease susceptibility, and survival. A sub-optimal in utero environment can impair the development of many organs including the kidney. This vulnerability may present as a reduction in the number of nephrons.[18] As all nephrons are formed before birth in the human, this congenital nephron deficit is permanent and has been strongly correlated with increased risk of hypertension[19] and renal disease in later life.[20]

Here we hypothesize that BEN changes occur early in the life, possibly during the intrauterine period of development.

In addition, some of these environmental effects seem to be passed on through subsequent generations.

Our conclusions are limited by sample size and sampling at one-time point. Longitudinal study of these proteins in patients with BEN, from the early stages, and in families with BEN, could bring more light to their role in the pathogenesis of the disease.

Conclusion

Important changes of PlGF and PP13 in patients with BEN were demonstrated, where kidney disease-BEN, female sex and the age have been significant determinants. Epigenetic mechanisms in developmental programming of adult disease-BEN were discussed and a screened for epigenetic biomarkers during the early life, and the possible reversibility of epigenetic action providing a promising therapy intervention was suggested.

Declaration of interest

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