Protein Z and vitamin K in kidney disease

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Abstract

Purpose: Disturbances in hemostasis are common complications of kidney diseases. Both bleeding diathesis and thromboembolism may complicate the course of chronic uremia. As far as we know, there is a limited data about protein Z in kidney disease.

Material and methods: The aim of our work was to examine plasma protein Z and vitamin K concentrations in nephrotic syndrome (n=34), glomerulonephritis (n=48), kidney transplant recipients (n=80), peritoneally dialyzed patients (n=42) and in the healthy volunteers (n=27).

Results: Vitamin K was significantly lower in nephrotic syndrome when compared to non-nephrotic patients, CAPD and healthy volunteers ($p<0.05$). Protein Z was the highest in CAPD and kidney transplant recipients when compared to any other group. In nephrotic syndrome protein Z was significantly lower when compared to the healthy volunteers, but it did not differ significantly between two groups of patients with chronic renal failure (with and without nephrotic syndrome). Protein Z correlated only with fibrinogen in CAPD, glomerulonephritis and nephrotic patients. Vitamin K correlated with age and albumin in patients with glomerulonephritis, nephrotic syndrome as well as with albumin in CAPD.

Conclusions: Alterations in protein Z might contribute to the enhanced risk of thromboembolic complications in nephrotic syndrome, CAPD and Tx via different and unknown mechanisms. This phenomenon seems to be unrelated to vitamin K status in these patients.

Key words: vitamin K, protein Z, kidney disease.

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Introduction

Hemostasis is a process of blood clot formation at the site of vessel injury. Bleeding or a thrombosis may occur due to missing or dysfunctional moieties of the coagulation or fibrinolytic factors. Abnormal bleeding can result from diminished thrombin generation or enhanced plasmin formation. Conversely, excessive production of thrombin can lead to thrombosis. Renal failure may be associated with a variety of signs and symptoms that are collectively referred to as the uremic state. Both bleeding diathesis and thromboembolism may complicate the course of chronic uremia [1,2]. At present, the incidence of bleeding is apparently declining, whereas thrombotic complications have become the predominant causes of morbidity and mortality in chronic renal failure [2]. Results of both in vitro and in vivo studies suggest that protein Z, a vitamin K-dependent plasma glycoprotein, plays an important role in dampening coagulation [3,4]. Vitamin K is necessary for the posttranslational γ-carboxylation of glutamic acid (Gla), present in several coagulation factors. Gla-mediated Ca^{++} ion binding to the coagulation factors is necessary for their association with phospholipids. This is a critical moment for their hemostatic function. An additional vitamin K-dependent protein, named protein Z, was found to be similar to the coagulation factors VII, IX, X and protein C [3,4]. However, in contrast to them, protein Z is not a serine protesase [3,4]. In the presence of phospholipid vesicles and calcium ions, protein Z serves as a cofactor for the inhibition of activated factor X by protein Z-dependent protease inhibitor. The physiological role of protein Z is still unclear. Controversial data have been published concerning protein Z alterations in different diseases. Low protein Z levels have been associated

with both bleeding tendency [5] and enhanced prothrombotic risk [6]. Recently, a possible role of low protein Z was found to be related to occurrence of acute coronary syndromes [7]. As far as we know, there is a limited data about protein Z in kidney disease. Usalan et al. [8] have reported a high plasma levels of protein Z in hemodialyzed patients. In our preliminary study we found that in nephrotic syndrome vitamin K concentration as well as protein Z were low, but these two findings were unrelated [9]. Since patients with nephrotic syndrome, peritoneally dialyzed and kidney transplant recipients exhibit hypercoagulable state and are particularly prone to thromboembolic complications [10-12], the aim of our work was to examine soluble plasma protein Z and vitamin K concentrations in these populations.

Material and methods

The study was performed on 4 groups of clinically stable patients with renal failure: group I – 48 patients with chronic glomerulonephritis without nephrotic syndrome, group II – 34 patients with nephrotic syndrome, group III – 42 peritoneal dialysis patients maintained on CAPD, group IV – 80 kidney transplant recipients.

In CAPD patients renal failure was due to glomerulonephritis (n=18), chronic interstitial nephritis (n=10), diabetic nephropathy (n=6), polycystic kidney disease (n=5) and other or unknown causes (n=3). All the diabetics were treated with subcutaneous insulin. Blood samples were drawn in the morning when subjects, appeared for routine office assessment of dialysis therapy after an overnight fast. All the CAPD patients were performing four 2 l exchanges. They were using Baxter Twin Bag system or Fresenius Andy Plus system. Dwell times were generally 4-6 h during the day and 8 h overnight. The osmotic pressure of CAPD fluid was adjusted in accordance with the extent of ultrafiltration in each patient.

80 kidney transplant recipients, with stable graft function (serum creatinine 1.74 ± 0.80 mg/dl) without any infection (C-reactive protein within the normal ranges), and liver dysfunction (normal prothrombin time and normal activities of alanine and asparaginine aminotransferases) were enrolled in the study. The immunosuppressive agents used in the transplants recipients consisted of cyclosporine (Neoral), azathioprine/mycophenolate mofetil and prednisone. The average dose of cyclosporine was 290 ± 85 mg/day, concentration measured using monoclonal antibodies in the whole blood was 158 ± 62 ng/ml). The average dose of prednisone was 5 mg/day and the highest dose was 10 mg/day. The azathioprine (Imuran) dose was on average 100 mg/day, with a range of doses between 50 and 150 mg/day, the mycophenolate mofetil (CellCept) doses ranged from 1 g/d to 2 g/d. Patients were engrafted for a period of 7 months to 12 years. The underlying renal pathology in patients was chronic glomerulonephritis (n=38), pyelonephritis (n=9), adult polycystic kidney disease (n=8), unknown (n=19), diabetic nephropathy (n=6).

Nephrotic syndrome was due to IgA nephropathy (n=15), focal and segmental glomerulosclerosis (n=11), membrano-proliferative glomerulonephritis in 3 cases, membranous nephropathy in 2 cases, submicroscopic glomerulonephritis in 1 case. Biopsy was not diagnostic in 2 cases. During the study none of the patients have received prednisone, anticoagulants or cytotoxic drugs. Twenty seven sex-and age-matched healthy volunteers served as a control group.

All the patients were informed about the aim of the study and gave their consent. The study was approved by Local Ethical Committee.

The following parameters were assessed: total protein, albumin, cholesterol, triglycerides, urea, total calcium, prothrombin time and activated partial thromboplastin time-APTT by means of standard laboratory methods. Vitamin K concentration was analyzed by HPLC as described previously [13]. Protein Z was assyaed using commercially available kits from Diagnostica Stago, France.

Data expressed as means ± SD and analyzed using Statistica 5.0 computer software. ANOVA or Kruskal-Wallis ANOVA, Pearson or Spearman correlations were used in statistical analysis, when appropriate.

Results

Concentrations of vitamin K were not significantly related to either age or gender in patients on renal replacement therapy (CAPD, Tx), but in GN+NS vitamin K correlated positively with age ($r=0.64$, $p<0.01$). We found that vitamin K concentrations did not differ significantly between all of the groups studied, when all the patients with chronic renal failure were taken together (NS+GN). When they were divided into patients with and without nephrotic syndrome, vitamin K was significantly lower in nephrotic syndrome when compared to non-nephrotic patients ($p<0.05$). In nephrotic patients vitamin K concentration was lower when compared to the patients maintained on CAPD and the healthy volunteers ($p<0.05$). Protein Z was the highest in CAPD patients when compared to any other group. In nephrotic syndrome protein Z was significantly lower when compared to the healthy volunteers, but it did not differ significantly between two groups of patients with chronic renal failure (with and without nephrotic syndrome). Protein Z correlated only with fibrinogen in CAPD patients ($r=-0.78$, $p<0.01$) and NS+GN ($r=-0.73$, $p<0.01$). Vitamin K correlated with albumin in CAPD patients ($r=0.52$, $p<0.05$) and in NS+GN ($r=0.58$, $p<0.05$).

Discussion

Protein Z (PZ) is a 6.2 kDa vitamin K-dependent protein, synthesized in the liver, first described in bovine plasma by Prowse and Esnouf [14]. Later human protein Z, was isolated in 1984 but its physiological function has remained uncertain [15]. It is a 62 kD glycoprotein, highly homologous to factors VII, IX, X, protein C, S and prothrombin. However, protein Z is not a serine protease due to lack of the active centre in its amino acid sequence [3,4]. It has been suggested that protein Z could promote coagulation [16], but since protein Z circulates in complex with protein Z-dependent protease inhibitor (ZPI),

Table 1. **Vitamin K, protein Z and some biochemical markers in patients with nephrotic syndrome (NS), peritoneally dialyzed patients (CAPD), kidney transplant recipients (Tx), patients with chronic glomerulonephritis (GN) and healthy volunteers (CG)**

	NS n=34	CAPD n=42	Tx n=80	GN n=48	CG n=27
age (years)	52±19	49±17	48±12	55±18	45±16
BMI (kg/m^2)	25.4±4.2	23.9±3.4	25.2±297	23.5±4.1	24.9±4.8
cholesterol (mg/dl)	238±58^{o}	223±47**	219±41**	209±65**	178±38
triglycerides (mg/dl)	254±69*	159±72***	201±44***	127±52**	93±29
total protein (g/l)	6.19±0.52*	6.22±0.69**	6.89±0.67	6.48±0.71**	6.95±0.61
albumin (g/l)	3.49 ±0.50*^{oo}	3.51±0.54**^{oo}	4.26±0.44	3.73±0.42**^{oo}	4.35±0.56
urea (mg/dl)	85.3±28.8***	127.5±41.2***	99.4±37.1***	85.7±42.3***	29.1±5.5
Vitamin K (ng/L)	93.8±17.5*^{o}	108.9±20.9$^{\&}$	109.4±21.5	108.8±17.2#	109.5±13.1
Protein Z (ng/ml)	0.48±0.27**$^{\&\&\&}$	1.58±0.44*^{o}	0.83±0.35*$^{\&\&}$	1.05±0.27$^{\&}$	1.19±0.32$^{\&}$
prothrombin time (secs)	12.5±0.7	12.4±0.9	13.0±1.0	13.2±0.5	12.3±1.0
aptt (secs)	30.8±7.9	34.5±7.8	37.0±8.1	36.2±10.1	36.4±8.2
total Ca (mmol/l)	2.14±0.31^{oo}##	2.09±0.31^{oo}##	2.29±0.33	2.29±0.23	ND

Values given are means ± SD,
* p<0.05, ** p<0.01, *** p<0.001 vs control group
#p<0.05 vs NS
$^{\&}$ p<0.05, $^{\&\&}$ p<0.01, $^{\&\&\&}$ p<0.001 vs CAPD
^{o}p<0.05, ^{oo}p<0.01, vs Tx
ND – not done

this limits the action of protein Z to a cofactor for the inhibition of activated factor X [17]. In the last few years, limited and controversial data were published on the changes of protein Z in various diseases. Very low plasma levels of protein Z were observed under oral anticoagulant treatment [18]. The cause of this phenomenon might be increased protein Z binding on the surface of endothelial cells or its consumption during coagulopathy. In our study we chose population of kidney patients prone to hypercoagulability. Nephrotic syndrome is known to carry an increased risk of thrombosis by a number of mechanisms. Continuous ambulatory peritoneal dialysis (CAPD) with its tendency to hypoalbuminemia and hypertriglyceridemia as well as transperitoneal protein loss appears to mimic the metabolic abnormalities that account for the hypercoagulability in the nephrotic syndrome [19]. Similarly stable kidney transplant recipients manifest a chronic hypercoagulable state with an increased risk of thrombotic complications, which also appears to be multifactorial. In our study we found that in patients with nephrotic syndrome, protein Z concentration was significantly lower than in the healthy volunteers and in CAPD and kidney transplant recipients it was significantly higher than in the control group. Significant protein losses through the peritoneum may be counterbalanced by the possible increase in protein synthesis in CAPD as a result of a nonspecific stimulation of the liver to protein production due to a lowered oncotic pressure. Thus, protein Z synthesis in CAPD patients may be increased via this mechanism. In nephrotic syndrome urinary protein loss is usually lesser than in transperitoneal protein loss in CAPD patients. On the other hand, preliminary reports suggest that protein Z could be a negative acute phase reactant [20]. In fact, inflammation (very often subclinical with CRP within normal ranges) is a frequent finding in dialyzed patients as a one of the components of MIA (malnutrition, inflammation, atherosclerosis) syndrome. In our study we observed a negative correlation between protein Z and fibrinogen in nephrotic syndrome and CAPD. Similarly, Fedi et al. [7] observed a lower protein Z levels in patiens with acute coronary syndromes and fibrinogen levels over 400 mg/dL. In the only one paper regarding protein Z levels in dialyzed patients, Usalan et al. [8] compared plasma protein Z levels in 10 hemodialysis patients (6 M, 4 F, mean age 36±11) and 10 healthy normal controls (5 M, 5 F, mean age 34±8). They found increased mean plasma protein Z levels in haemodialysis patients over healthy controls. They suggested that increased level of protein Z, which influenced the action of thrombin on its protein substrates and inhibitors might contribute to the haemostatis alterations in end-stage renal failure patients, in addition to other well known abnormalities in the coagulation and fibrinolytic system. In contrast to our study, the number of patients was relatively small and they were significantly younger.

It is known that vitamin K is needed for the carboxylation of osteocalcin, matrix Gla protein in bone, prothrombin, protein S and protein Z [21]. However, the specific function of these proteins is still unknown. Therefore, we tried to find correlations between vitamin K status and protein Z. In none of the group of patients studied vitamin K was related to protein Z levels.

Conclusions

Our results add further weight to the possible role of protein Z in the hemostatic disturbances in kidney diseases. The most interesting finding of our study is the observation

that in nephrotic syndrome vitamin K concentration is low as well as protein Z. Protein Z alterations might contribute to the enhanced risk of thromboembolic complications in nephrotic syndrome, CAPD and stable kidney transplant recipients, however, these findings seems to be unrelated to vitamin K status in these patients.

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