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Association Between Serum Pregnancy-Associated Plasma Protein-A and Bicarbonate in Hemodialysis Patients

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Background: Acidosis is associated with protein-energy malnutrition, inflammation, and bone disease, and low bicarbonate levels have been implicated in higher mortality rates in chronic kidney disease. Recently, the concentration of serum pregnancy-associated plasma protein-A (PAPP-A) has become accepted as a prognostic marker in hemodialysis patients. This study determined the relationship between PAPP-A and bicarbonate levels in these patients. *Methods:* The study enrolled 65 hemodialysis patients (41 males, 24 females) and 26 control subjects (11 males, 15 females). Serum PAPP-A, intact parathormone (iPTH), calcium, phosphorus (P), and bicarbonate levels were measured. Correlations between PAPP-A and bicarbonate, iPTH, calcium, and phosphorus were evaluated. *Results:* Median PAPP-A levels were significantly higher in hemodialysis patients [15.1 (<0.03–158.8) ng/ml] than in control subjects [6.6 (<0.03–16.4) ng/ml] ($P < 0.05$). There were statistically significant correlations between serum PAPP-A and bicarbonate, iPTH, and P in hemodialysis patients but not in control subjects. *Conclusion:* Elevation of serum PAPP-A has been found in hemodialysis patients and its significant correlation with bicarbonate suggests that it may be a prognostic factor. J. Clin. Lab. Anal. 28:114–117, 2014.

Key words: acidosis; acute coronary syndrome; bicarbonate; hemodialysis; PAPP-A

INTRODUCTION

Metabolic acidosis is a common condition in renal insufficiency that may cause bone disease and inflammation, and may worsen protein-energy malnutrition (1). Inflammation and protein-energy malnutrition are closely associated in chronic kidney disease (CKD) and together are referred to as malnutrition inflammation complex syndrome (MICS) (1). MICS has been implicated as a major cause of a poor clinical outcome in CKD. Metabolic acidosis is a precipitating factor in MICS and thus may play a major role in the increased mortality in CKD patients. Recently, serum levels of pregnancy-associated plasma protein A (PAPP-A) have been accepted as a prognostic marker in hemodialysis patients. Despite its name, it is found in both males and nonpregnant females (2). During pregnancy, PAPP-A is produced at a high concentration by the trophoblast, but expression has also been reported in endometrium, testis, kidney, colon, bone, and vascular smooth muscle cells (3, 4). PAPP-A is potentially proatherosclerotic, and high levels have been reported in acute coronary syndromes, in asthma, and in renal impairment (5–8). In the present study, we evaluated the association between acidosis and serum PAPP-A levels in hemodialysis patients.

SUBJECTS AND METHODS

Study Population

The study was conducted at the Acibadem Labmed Clinical Laboratories, Istanbul, Turkey. The study protocol was approved by the Ethics Committee of Acibadem University, and all subjects provided written informed

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TABLE 1. Subjects' Characteristics

Characteristic	Patients	Control	*P*
Age, years			
Male	46.6±16.7	36.2 ± 9.2	>0.05
Female	58.9 ± 19	44.2 ± 14.4	>0.05
Male/Female (*n*)	41/24	15/11	
Smoking	4	4	
Diabetes mellitus	8	2	
Hypertension	14	3	
Etiology of renal disease			
Hypertension	12		
Diabetes mellitus	8		
Chronic pyelonephritis	6		
Polycystic disease	8		
Chronic glomerulonephritis	11		
Unknown	20		

consent before participating. The study population consisted of 65 chronic hemodialysis patients, and a control group of 26 healthy subjects of similar age with normal renal function. The demographic characteristics of the subject populations are given in Table 1.

Methods

Blood samples were drawn from the arteriovenous fistulae of hemodialyzed patients and from the antecubital veins of control subjects. Blood gas analyses were performed immediately. After clotting, samples were centrifuged at 1,500 *g*, and sera were divided into two aliquots. Sera to be used for PAPP-A measurements were stored at –80°C until analyzed; other biochemical parameters were analyzed on the same day that each sample was drawn.

Biochemical Assays

At the end of the study, all samples were analyzed for PAPP-A on the same day in one run, using an ELISA kit (ultrasensitive ELISA kit, DRG Instruments, Marburg, Germany). According to the manufacturer, the within-run coefficient of variation was 6.86% and the detection limit of the method was 0.023 ng/ml. Serum intact parathormone (iPTH) levels were determined using a chemiluminescent microparticle immunoassay (CMIA) method (Abbott, ARCHITECT; Wiesbaden, Germany). Serum calcium and phosphorus were determined by standard clinical chemistry methods, using a Beckman Coulter AU 2770 autoanalyzer (Beckman Coulter, CA). Serum bicarbonate levels were measured using a blood gas analyzer (Siemens Rapid Lab, Munchen, Germany).

TABLE 2. Serum PAPP-A, Bicarbonate, iPTH, Calcium, and Phosphorus Levels in Dialysis Patients and Control Subjects

Parameter	Hemodialysis ($n = 65$)	Control group ($n = 26$)	*P*
PAPP-A, ng/ml	15.2 (<0.03–158.8)	6.2 (<0.03–16.4)	<0.01
Bicarbonate, mmol/l	21.0 ± 2.6	23.5 ± 26.1	<0.01
iPTH, ng/l	400.0 (20.5–2,395)	40.6 (16.1–141.6)	<0.01
Calcium, mmol/l	2.23 ± 0.25	2.33 ± 0.1	<0.05
Phosphorus, mmol/l	1.68 ± 0.52	0.94 ± 0.19	<0.01

Data are medians (minimum–maximum) or means ± SD.

Statistical Analysis

Data are expressed as means ± SD or (for PAPP-A and iPTH) median and range (min–max). The Kolmogorov–Smirnov test was used to evaluate the normality of the data. Correlations between variables were assessed using Spearman's correlation analysis. The Mann–Whitney *U*-test was used to evaluate the significance of differences between the control and hemodialysis patients. Curve estimation logistic regression analysis was used to evaluate the influence of the study variables on PAPP-A. Values of $P < 0.05$ were considered to indicate statistical significance.

RESULTS

Serum PAPP-A, bicarbonate, iPTH, calcium, and phosphorus levels in dialysis patients and control subjects are shown in Table 2. PAPP-A was not measurable (below the detection limit of the method) in two patients and two control subjects. As shown in Figure 1, the median serum PAPP-A level of hemodialysis patients [15.2 (<0.03–158.8) ng/ml] was significantly higher than that of control subjects [6.6 (<0.03–16.4) ng/ml] ($P < 0.01$). On the other hand, serum bicarbonate levels in patients (21.0 ± 2.6) were lower than in control subjects (23.5 ± 26.1) ($P < 0.01$). There was a significant negative correlation

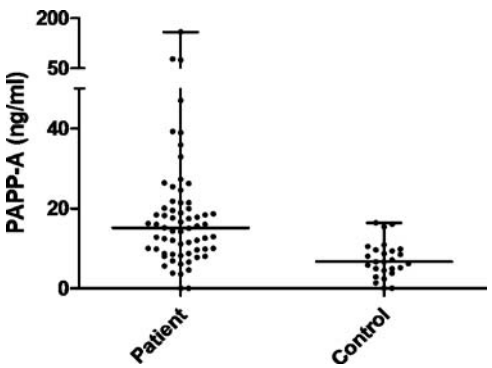

Fig. 1. Serum PAPP-A levels of all hemodialysis patients and control subjects. Horizontal bars are medians with minimum–maximum.

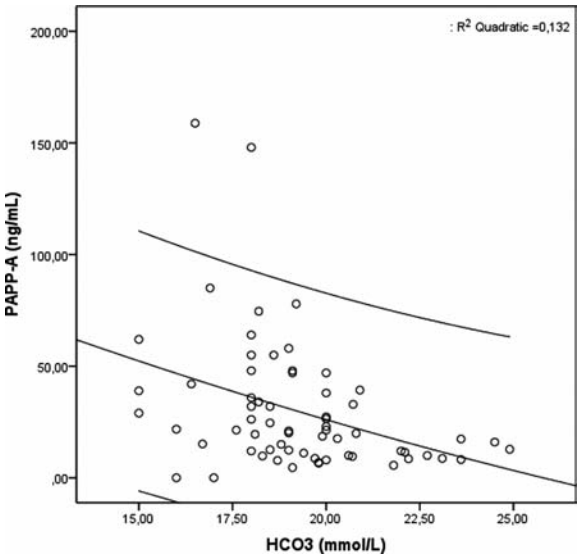

Fig. 2. Correlation between serum PAPP-A and bicarbonate levels in hemodialysis patients.

between serum PAPP-A and bicarbonate levels ($r = -0.291$; $P < 0.01$) (Fig. 2). Additionally, there was a significant positive correlation between levels of serum PAPP-A and iPTH ($r = 0.273$; $P < 0.01$), and phosphorus ($r = 0.230$; $P < 0.05$). However, in control subjects the correlation between serum PAPP-A and bicarbonate levels was not statistically significant ($r = 0.069$; $P = 0.739$). We also performed correlation analysis between serum PAPP-A and other variables in control subjects. The correlations between serum PAPP-A and iPTH ($r = 0.014$; $P = 0.947$), and phosphorus ($r = 0.030$; $P = 0.884$) were not statistically significant.

To assess the impact of the correlated parameters on PAPP-A, we used curve estimation regression analysis and defined PAPP-A as an independent variable. All of the variables influenced PAPP-A levels independently. In contrast, when PAPP-A was included as a constant; age, time on dialysis, or calcium level did not affect the constant, while levels of phosphorus ($r = 0.225$, $P < 0.05$), iPTH ($r = 0.305$, $P < 0.01$), and HCO_3^- ($r = 0.301$, $P = 0.05$) influenced it strongly.

DISCUSSION

The present study has shown, for the first time, that serum PAPP-A is significantly but inversely correlated with the bicarbonate level in hemodialysis patients. This finding supports the suggestion of Kalusova et al. that PAPP-A is an independent prognostic marker in dialysis patients (9).

PAPP-A was isolated by Lin et al. in 1974 as one of four placental proteins circulating in high concentration in pregnant females (10). It is part of the insulin-like growth factor (IGF) axis (11). This axis integrates growth hormone (GH), IGF-1 and IGF-2, six IGF-binding proteins (IGFBP-1–6), and IGFBP proteases. PAPP-A is a zinc-binding protease (pappalysin-1, EC 3.4.24.79) of IGFBPs. Its primary substrate is IGFBP-4, although it also cleaves IGFBP-2 and IGFBP-5 (11–13), and increases the local concentrations of IGFs, which are involved in the regulation of growth, proliferation, and differentiation of various cell types. PAPP-A protein (PAPP-A monomer) contains 1,547 amino acids and has a molecular weight of 200 kDa. Two forms are found, free and complexed. Free PAPP-A exists as a homodimer of 400 kDa that is proteolytically active. However, complexed forms are proteolytically inactive, associating with pro-major basic protein as a heterotetrameric complex of 500 kDa. In pregnancy, the major form is complexed PAPP-A, while in acute coronary syndromes only the free form is relevant (2,14). In hemodialysis, patient's levels of both complexed and noncomplexed forms of PAPP-A are increased (15).

In the present study, PAPP-A levels were higher in patients than in control subjects and were also correlated with the severity of acidosis and iPTH levels. However, in control subjects there was not a statistically significant correlation between PAPP-A and bicarbonate, iPTH and phosphorus. Metabolic acidosis is a well recognized and common complication of CKD (16). Animal and human studies have shown that chronic metabolic acidosis associated with CKD has several deleterious effects, including chronic inflammation, increased protein catabolism, uremic bone disease, muscle wasting, and accumulation of β-2 microglobulin (17,18). These conditions may contribute to the higher cardiovascular burden among CKD patients.

Bone buffering of some of the excess hydrogen ions is associated with the release of calcium and phosphate from bone (19). Consequently, metabolic acidosis stimulates a gradual reduction in bone mineral stores. Preventing acidosis may minimize the degree of negative calcium balance and prevent or delay the progression both of osteopenia and of hyperparathyroid bone disease (20). In humans, there is evidence that maintenance dialysis that achieves a normal plasma bicarbonate concentration with alkali therapy can slow the rate of progression of uremic bone disease (21,22). Correction of acidosis may act in part by diminishing the stimulus to hyperparathyroidism (23).

In a previous study, we found that PAPP-A levels are increased in dialysis patients and may reflect a greater degree of chronic inflammation than osteodystrophy in uremic patients (7). In the current study, we investigated the association between serum PAPP-A, bicarbonate levels, and uremic bone disease parameters in hemodialysis patients. Taken together, we conclude that the current findings suggest a strong correlation between PAPP-A

and osteodystrophy, and that the severity of osteodystrophy depends upon the presence of acidosis, which can be detected by measuring the serum bicarbonate level.

CONFLICT OF INTEREST

The authors report no conflicts of interest.

REFERENCES

1. Wu DY, Shinaberger CS, Regidor DL, McAllister CJ, Kopple JD, Kalantar-Zadeh K. Association between serum bicarbonate and death in hemodialysis patients: Is it better to be acidotic or alkalotic? Clin J Am Soc Nephrol 2006;1:70–78.
2. Kalousová M, Tesar V, Muravská A, Zima A. Pregnancy-associated plasma protein A: Spotlight on kidney diseases. Clin Chem Lab Med 2012;50:1183–1190.
3. Schindler AM, Bischof B. Histochemical localization of pregnancy associated plasma protein-A in fetal, infant, and adult organs and comparison between antisera. Gynecol Obstet Invest 1984;18:88–94.
4. Overgaard MT, Oxvig C, Christiansen M, et al. Messenger ribonucleic acid levels of pregnancy-associated plasma protein-A and the proform of eosinophil major basic protein: Expression in human reproductive and nonreproductive tissues. Biol Reprod 1999;61:1083–1089.
5. Coskun A, Balbay O, Duran S, et al. Pregnancy-associated plasma protein-A and asthma. Adv Ther 2007;24:362–367.
6. Coskun A, Duran S, Apaydin S, Bulut I, Sariyar M. Pregnancy associated plasma protein-A: Evaluation of a new biomarker in renal transplant patients. Transplant Proc 2007;39:3072–3076.
7. Coskun A, Bicik Z, Duran S, et al. Pregnancy-associated plasma protein A in dialysis patients. Clin Chem Lab Med 2007;45: 63–66.
8. Bayes-Genis A, Conover CA, Overgaard MT. Pregnancy-associated plasma protein A as a marker of acute coronary syndromes. N Engl J Med 2001;345:1022–1029.
9. Kalousová M, Benáková H, Kuběna AA, Dusilová-Sulková S, Tesař V, Zima T. Pregnancy-associated plasma protein-A as an independent mortality predictor in long-term hemodialysis patients. Kidney Blood Press Res 2012;35:192–201.
10. Lin TM, Galbert SP, Kiefer D, Spellacy WN, Gall S. Characterization of four human pregnancy-associated plasma proteins. Am J Obstet Gynecol 1974;118:223–226.
11. Lawrence JB, Oxvig C, Overgaard MT, et al. The insulin-like growth factor (IGF)-dependent IGF binding protein-4 protease secreted by human fibroblasts is pregnancy associated plasma protein-A. Proc Natl Acad Sci USA 1999;96:3149–153.
12. Laursen LS, Overgaard MT, Soe R, et al. Pregnancy-associated plasma protein-A (PAPP-A) cleaves insulin-like growth factor binding protein (IGFBP)-5 independent of IGF: Implications for the mechanism of IGFBP-4 proteolysis by PAPP-A. FEBS Lett 2001;504:36–40.
13. Monget P, Mazerbourg S, Delpuech T, et al. Pregnancy-associated plasma protein-A is involved in insulin-like growth factor binding protein-2 (IGFBP-2) proteolytic degradation in bovine and porcine preovulatory follicles: Identification of cleavage site and characterization of IGFBP-2 degradation. Biol Reprod 2003;68:77–86.
14. Qin QP, Kokkala S, Lund J, Tamm N, Voipio-Pulkki LM, Pettersson K. Molecular distinction of circulating pregnancy-associated plasma protein A in myocardial infarction and pregnancy. Clin Chem 2005;51:75–83.
15. Tertti R, Wittfooth S, Porela P, Airaksinen KE, Metsärinne K, Pettersson K. Intravenous administration of low molecular weight and unfractionated heparin elicits a rapid increase in serum pregnancy-associated plasma protein A. Clin Chem 2009;55:1214–1217.
16. Kraut JA, Kurtz I. Metabolic acidosis of CKD: Diagnosis, clinical characteristics, and treatment. Am J Kidney Dis 2005;45:978–993.
17. Raphael KL, Wei G, Baird BC, Greene T, Beddhu S. Higher serum bicarbonate levels within the normal range are associated with beter survival and renal outcomes in African Americans. Kidney Int 2011;79:356–362.
18. Bailey JL, Wang X, England BK, Price SR, Ding X, Mitch WE. The acidosis of chronic renal failure activates muscle proteolysis in rats by augmenting transcription of genes encoding proteins of the ATP-dependent ubiquitin-proteasome pathway. J Clin Invest 1996;97:1447–1453.
19. Bushinsky DA. The contribution of acidosis to renal osteodystrophy. Kidney Int 1995;47:1816–1832.
20. Mathur RP, Dash SC, Gupta N, et al. Effects of correction of metabolic acidosis on blood urea and bone metabolism in patients with mild to moderate chronic kidney disease: A prospective randomized single blind controlled trial. Ren Fail 2006;28:1–5.
21. Silver J, Levi R. Cellular and molecular mechanisms of secondary hyperparathyroidism. Clin Nephrol 2005;63:119–126.
22. Krieger NS, Frick KK, Bushinsky DA. Mechanism of acid-induced bone resorption. Curr Opin Nephrol Hypertens 2004;13:423–436.
23. Graham KA, Hoenich NA, Tarbit M, et al. Correction of acidosis in hemodialysis patients increases the sensitivity of the parathyroid glands to calcium. J Am Soc Nephrol 1997;8:627–631.