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RESEARCH ARTICLE

Distinct Roles of Urinary Liver-Type Fatty Acid-Binding Protein in Non-Diabetic Patients with Anemia

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Abstract

Background

Various stresses including ischemia are known to up-regulate renal L-FABP gene expression and increase the urinary excretion of L-FABP. In diabetic patients with anemia, the urinary excretion of L-FABP is significantly increased. We studied the clinical significance of urinary L-FABP and its relationship with anemia in non-diabetic patients.

Subjects and Methods

A total of 156 patients were studied in this retrospective cross-sectional analysis. The associations between anemia and urinary L-FABP levels, and the predictors of urinary L-FABP levels in non-diabetic patients were evaluated.

Results

Urinary L-FABP levels were significantly higher in patients with anemia compared to those in patients without anemia. Similarly, the urinary L-FABP levels were significantly higher in patients with albuminuria compared to those in patients without albuminuria. Urinary L-FABP levels correlated with urinary albumin-to-creatinine ratios, estimated glomerular filtration rates, body mass index, and hemoglobin levels. Multivariate linear regression analysis determined that hemoglobin levels ($\beta = -0.249$, $P = 0.001$) and urinary albumin-to-creatinine ratios ($\beta = 0.349$, $P < 0.001$) were significant predictors of urinary L-FABP levels.

Conclusions

Urinary L-FABP is strongly associated with anemia in non-diabetic patients.

Introduction

Liver-type fatty acid-binding protein (L-FABP) is a 14 kDa small molecule that is expressed in the proximal tubular cells of the kidney [1]. L-FABP incorporates albumin-bound free fatty acids (FFAs) that are filtered through the glomeruli into proximal tubular cells, and transports these FFAs from the cytosol to the nucleus [2,3]. Various stresses, such as proteinuria, hyperglycemia, hypertension, ischemia, and toxins are known to up-regulate renal L-FABP gene expression and increase the urinary excretion of L-FABP [4–7].

Anemia has a profound effect on patient's mortality, morbidity, and quality of life. It also induces tubular hypoxia [8–10]. Administration of erythropoietin and the subsequent increase in hemoglobin levels decreases urinary L-FABP levels [11]. To date, the association between urinary L-FABP and anemia has only been reported among patients with type 2 diabetes [12]. The objective of this study was to study the association between urinary L-FABP and anemia among non-diabetic patients. We hypothesized that there also would be an association between urinary L-FABP and anemia among non-diabetic patients. Thus we conducted a cross-sectional study to investigate urinary L-FABP levels in non-diabetic patients.

Subjects and Methods

Patients

Between 2007 and 2011, non-diabetic adult patients were consecutively recruited from the outpatient clinic. Patients with history of liver disease, cancer, collagen disease, and hemodialysis were excluded. Patients were also excluded from this study if their medical records contained inadequate amounts of clinical or biochemical information. Ethical approval was obtained from the Institutional Review Board of St. Marianna University Hospital. The study was conducted in accordance with the principles of the Declaration of Helsinki. All patients were provided written informed consent following confirmation of eligibility.

Measurements

This was a retrospective cross-sectional study. Patients' demographic characteristics and laboratory test results were extracted from the electronic patient records and medical notes, including age, sex, body mass index (BMI), hemoglobin (Hb) levels, serum albumin levels, high-sensitive CRP, urinary albumin levels, urinary L-FABP levels, and estimated glomerular filtration rates (eGFR). When immediate analysis is not possible, serum and urine samples were stored at −80°C. Urinary albumin levels were measured using the latex agglutination method. Urinary L-FABP levels were measured using the Human L-FABP ELISA Kit developed by CMIC Co., Ltd. (Tokyo, Japan) [13,14]. Their concentrations were normalized for urine creatinine concentrations. The new equation proposed by the Japanese Society of Nephrology was used to calculate the eGFRs, as follows: $eGFR = 194 \times (\text{creatinine})^{-1.094} \times \text{age}^{-0.287}$ (or × 0.739 if female) [15]. Anemia was defined using the World Health Organization's criteria: Hb <13 mg/dL for men and Hb <12 mg/dL for women [16].

Statistical analysis

Descriptive statistics were used to summarize the demographic characteristics of the patients. For parameters between the two groups, parametric data were compared using unpaired t-tests and non-parametric data were compared using the Mann–Whitney U test. The associations between urinary L-FABP and other variables were evaluated using Spearman's correlation coefficient. Multivariate linear regression analysis was performed to determine the variables independently predict urinary L-FABP levels. Statistical analyses were performed using IBM

SPSS software version 21.0 (IBM Corporation, Armonk, NY, USA). P values <0.05 were considered statistically significant for all analyses.

Results

A total of 156 non-diabetic patients were studied and their demographic characteristics are summarized (Table 1). The mean age (standard deviation) of the patients was 62.2 years (14.8). The mean eGFR of the patients was 56.6 mL/min/1.73m^2 (25.0). The median urinary ACR (interquartile range) was 26.4 mg/gCr (7.2–212.3), and the median urinary L-FABP level was 4.5 μg/gCr (0.7–10.2).

Patients with anemia had significantly higher urinary L-FABP concentrations compared with patients without anemia (5.6 μg/gCr [2.3–20.2] vs. 3.3 μg/gCr [0.2–7.4], P = 0.002) (Fig 1). Also, patients with albuminuria had significantly higher urinary L-FABP levels than patients without albuminuria (7.9 μg/gCr [2.0–21.2]) vs. 2.8 μg/gCr [0.3–6.1], P < 0.001) (Fig 2).

Urinary L-FABP levels correlated with ACR (r = 0.410, P < 0.001), eGFR (r = -0.364, P < 0.001), BMI (r = -0.277, P = 0.001), Hb levels (r = -0.293, P < 0.001), and the presence of anemia (r = 0.250, P = 0.002) (Table 2). Linear regression analysis, using the urinary L-FABP levels as dependent variables, revealed that Hb (β = -0.249, P = 0.001) and ACR (β = 0.349, P < 0.001) were significant and independent predictors of urinary L-FABP levels (Table 3).

Discussion

In this cross-sectional study, we showed for the first time that urinary L-FABP levels are significantly increased in non-diabetic patients with anemia. Similar findings have been reported in experimental models of acute ischemic injury and in diabetic patients [5,12,17]. In the present study, the urinary L-FABP levels were significantly higher (approximately 2-fold) in patients with anemia compared to those in patients without anemia. Urinary L-FABP levels were similar to previously reported levels in diabetic patients with anemia [12]. Also, patients with albuminuria had urinary L-FABP levels that were significantly higher (approximately 3-fold)

Table 1. Baseline patient characteristics.

	All patients (n = 156)
Age (years)	62.2 ± 14.8
Female, n (%)	67 (42.9)
Body mass index (kg/m^2)	24.0 ± 3.6
Systolic blood pressure (mmHg)	130.6 ± 14.5
Diastolic blood pressure (mmHg)	79.1 ± 10.0
eGFR (mL/min/1.73m^2)	56.6 ± 25.0
Total cholesterol (mg/dL)	183.7 ± 34.7
HDL-cholesterol (mg/dL)	49.5 ± 15.5
High-sensitive CRP (mg/dL)	0.09 (0.05–0.16)
ACE/ARB, n (%)	98 (62.8)
Statin, n (%)	26 (16.7)
Urinary ACR (mg/gCr)	26.4 (7.2–212.3)
Urinary L-FABP (μg/gCr)	4.5 (0.7–10.2)

Data are mean (SD), median (IQR), or number of patients (%). ACR, albumin-to-creatinine ratio; ACE/ARB, angiotensin-converting enzyme/angiotensin-receptor blocker; HDL, high-density lipoprotein; eGFR, estimated glomerular filtration rate.

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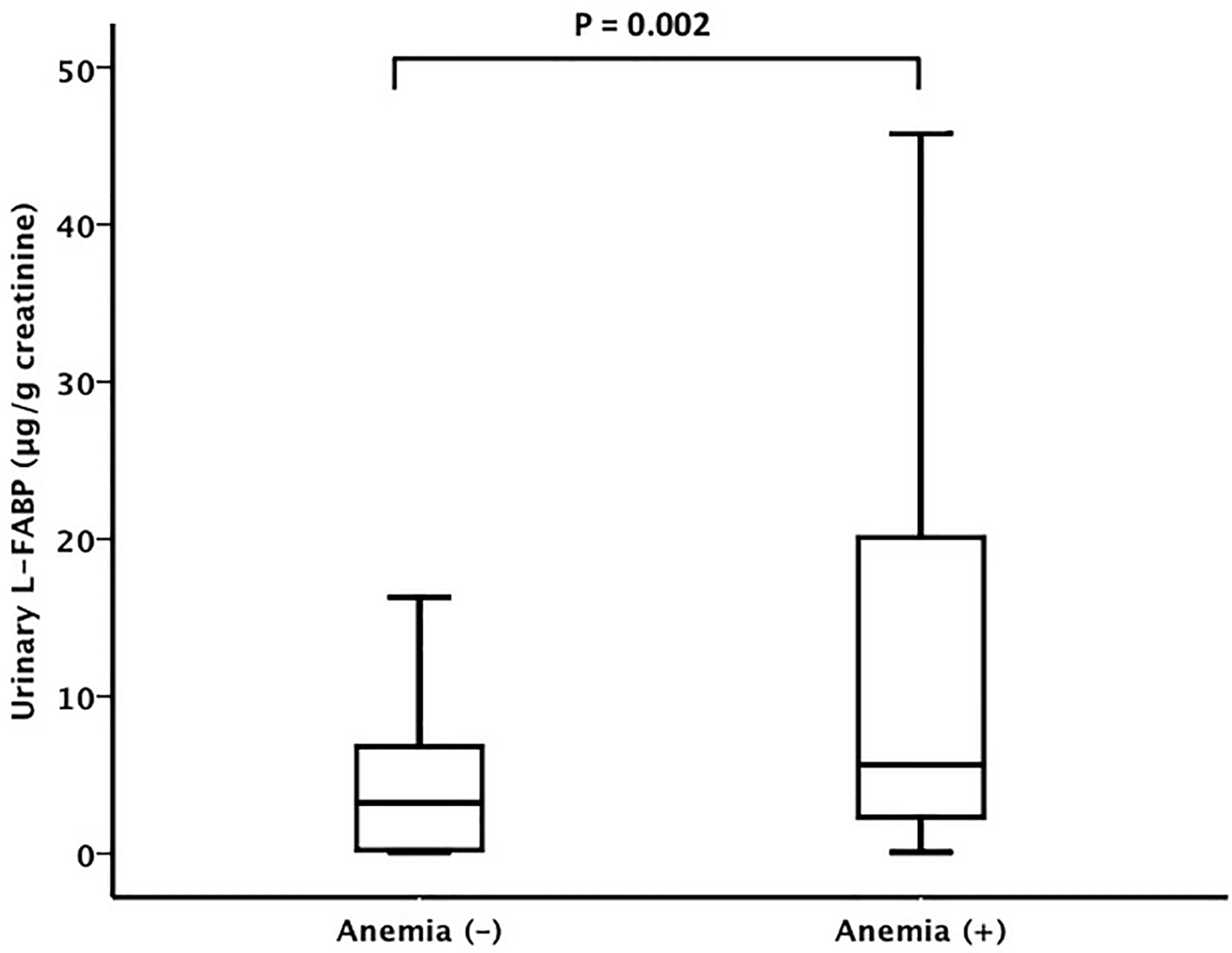

Fig 1. Urinary L-FABP levels and anemia. Patients with anemia had significantly higher urinary L-FABP levels than patients without anemia (5.6 μg/gCr [2.3–20.2] vs. 3.3 μg/gCr [0.2–7.4], P = 0.002).

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compared to those in patients without albuminuria. Multivariate linear regression analysis identified Hb levels and the ACR as significant predictors of urinary L-FABP levels. These results are similar to those reported in the study of diabetic patients, which reported that Hb levels and the ACR were significant predictors of urinary L-FABP levels [12].

Serum LFABP levels do not affect urinary L-FABP levels. A previous study has reported that the estimated contribution of serum L-FABP to urinary L-FABP is only 3% [18]. This suggests that there is no transglomerular passage of L-FABP, and that it is the tubular cells that primarily produce urinary L-FABP. It is shown that administration of erythropoietin and the subsequent increase in hemoglobin levels decreases urinary L-FABP levels [11]. Tubular hypoxia induced by anemia likely up-regulate expression of the LFABP gene and promote the urinary excretion of LFABP [5]. On the other hand, albumin is transported with FFAs to the proximal tubules, where the tubular cells absorb the FFAs. Subsequently, L-FABP transports the FFAs to

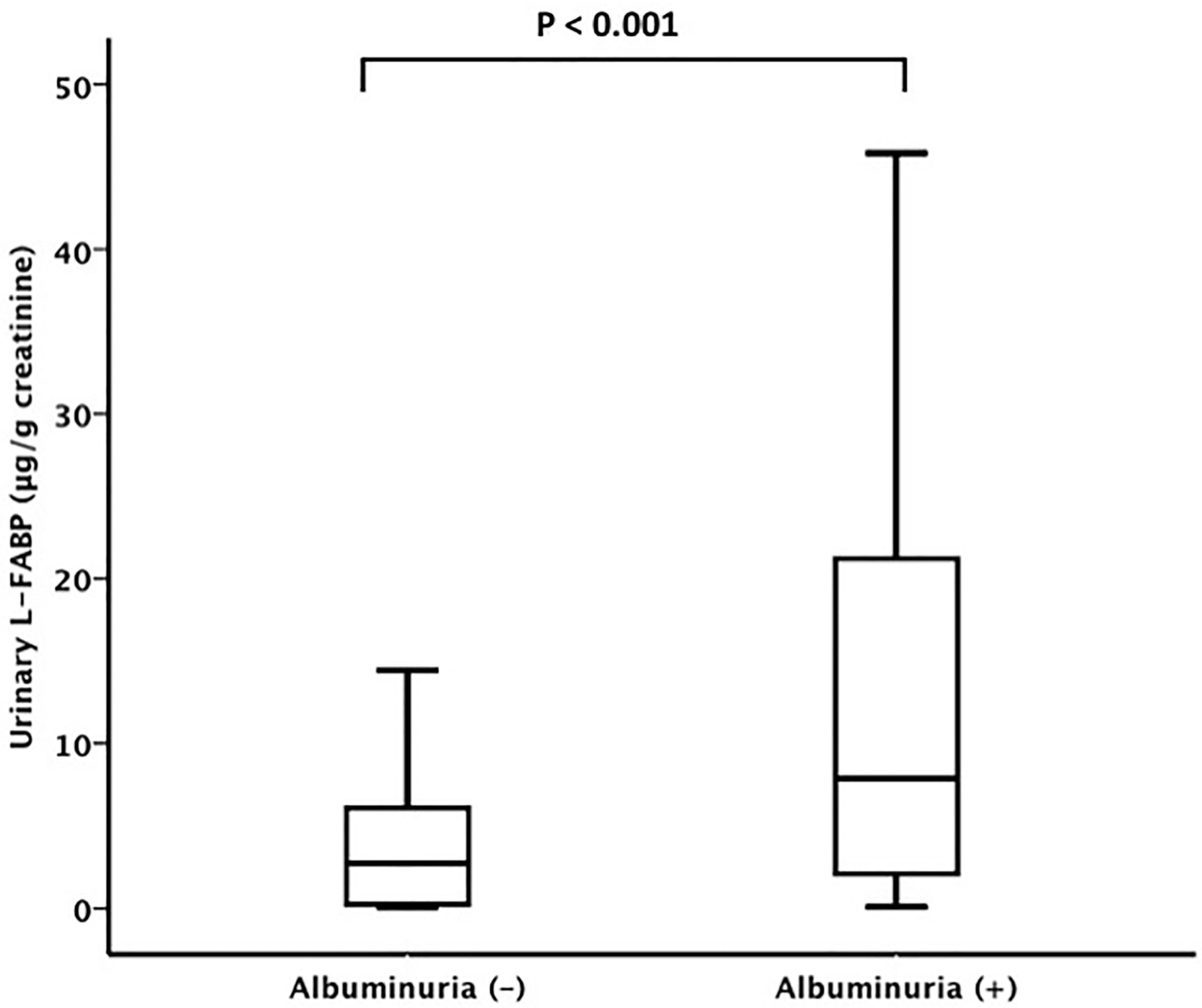

Fig 2. Urinary L-FABP levels and albuminuria. Patients with albuminuria had significantly higher urinary L-FABP levels than patients without albuminuria (7.9 μg/gCr [2.0–21.2] vs. 2.8 μg/gCr [0.3–6.1], P < 0.001).

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the mitochondria. Hence, when the severity of albuminuria increases, the L-FABP gene is up-regulated, and more LFABP is excreted in the urine [19,20].

In the present study, statins and angiotensin receptor blockers (ARBs) were administered to 17% (n = 26) and 63% (n = 98) of patients, respectively. Statin use has been shown to decrease proliferation, increase apoptosis, and enhance the fibrinolytic activity of renal tubular cells, while ARB use has been shown to prevent vascular damage, ameliorate tubular hypoxia, and reduce oxidative stress [21,22]. Previous studies have reported a significant decrease in urinary L-FABP when the patients were treated with statins or ARBs [19,23,24]. Therefore, statins and/or ARBs might have influenced the changes in the urinary L-FABP levels observed among our patients. Other reports have also suggested that angiotensin-converting enzyme inhibitors (ACEi) and

Table 2. Spearman's correlation coefficients between urinary L-FABP levels with clinical characteristic of patients.

	r	P
Age	0.134	0.096
Female sex	-0.062	0.439
Body mass index	-0.277	0.001
Hemoglobin	-0.293	<0.001
Prevalent anemia	0.250	0.002
Total cholesterol	-0.145	0.071
High-sensitive CRP	-0.097	0.228
eGFR	-0.364	<0.001
Urinary ACR	0.410	<0.001

ACR, albumin-to-creatinine ratio; eGFR, estimated glomerular filtration rate.

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ARBs might have a negative impact on Hb levels [25,26]. Although a high proportion of our patients (66%, n = 103) were receiving these drugs, their use did not appear to be independently associated with lower Hb levels in our patients, as has been previously reported [27,28].

Our study has several limitations. First, this was a single-center study and the sample size was relatively small. Second, as in every cross-sectional study, no clear conclusions can be reached regarding the associations between the parameters studied. As well, bias by indication is also possible. Third, erythropoietin and iron levels were not measured for any of the subjects. Since patients are more elderly and more male subjects are included, an iron deficiency might not be the reason for anemia in some of the patients. Fourthly, we were not able to compare urinary L-FABP with other emerging markers of kidney dysfunction such as kidney injury molecule (KIM) -1, N-acetyl-β-glucosaminidase (NAG), and neutrophil gelatinase-associated lipocalin (NGAL). Further study is needed to evaluate the clinical value of urinary L-FABP. Finally, there are no marker of hypoxia, such as lactate and pH, available in the present study. Anemic patients with high urinary L-FABP levels may benefit from therapeutic interventions that address renal tubular hypoxia. This also would be the area where further research is needed.

Based on the findings of this study, urinary L-FABP levels have a strong association with anemia. Further prospective studies are needed to clarify the utility of measuring urinary L-FABP levels in anemic patients.

Table 3. Independent predictors of urinary L-FABP* in multivariate linear regression models.

	β	t	P
Age	-0.131	-1.477	0.142
Sex	-0.146	-1.929	0.056
Body mass index	-0.141	-1.690	0.093
Hemoglobin	-0.249	-3.377	0.001
Urinary ACR*	0.349	4.728	<0.001
ACE/ARB	0.128	1.748	0.083
Statin	0.039	0.479	0.632
r^2			0.197

*Log-transformed variables.

ACR, albumin-to-creatinine ratio; eGFR, estimated glomerular filtration rate; ACE/ARB, angiotensin-converting enzyme/angiotensin-receptor blocker.

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Author Contributions

Conceived and designed the experiments: NI TY. Performed the experiments: NI TY. Analyzed the data: NI TY. Contributed reagents/materials/analysis tools: NI TY AK. Wrote the paper: NI TY YS KK.

References

1. Maatman RG, Van Kuppevelt TH, Veerkamp JH (1991) Two types of fatty acid-binding protein in human kidney. Isolation, characterization and localization. Biochem J 273 (Pt 3): 759–766.
2. Huang H, Starodub O, McIntosh A, Kier AB, Schroeder F (2002) Liver fatty acid-binding protein targets fatty acids to the nucleus. Real time confocal and multiphoton fluorescence imaging in living cells. J Biol Chem 277: 29139–29151. doi: 10.1074/jbc.M202923200 PMID: 12023965
3. Lawrence JW, Kroll DJ, Eacho PI (2000) Ligand-dependent interaction of hepatic fatty acid-binding protein with the nucleus. Journal of lipid research.
4. Kamijo A, Sugaya T, Hikawa A, Okada M, Okumura F, Yamanouchi M, et al. (2004) Urinary excretion of fatty acid-binding protein reflects stress overload on the proximal tubules. Am J Pathol 165: 1243–1255. doi: 10.1016/S0002-9440(10)63384-6 PMID: 15466390
5. Yamamoto T, Noiri E, Ono Y, Doi K, Negishi K, Kamijo A, et al. (2007) Renal L-Type Fatty Acid Binding Protein in Acute Ischemic Injury. Journal of the American Society of Nephrology 18: 2894–2902. doi: 10.1681/ASN.2007010097 PMID: 17942962
6. Yokoyama T, Kamijo-Ikemori A, Sugaya T, Hoshino S, Yasuda T, Kimura K (2009) Urinary excretion of liver type fatty acid binding protein accurately reflects the degree of tubulointerstitial damage. Am J Pathol 174: 2096–2106. doi: 10.2353/ajpath.2009.080780 PMID: 19435794
7. Matsui K, Kamijo-Ikemori A, Sugaya T, Yasuda T, Kimura K (2011) Renal Liver-Type Fatty Acid Binding Protein (L-FABP) Attenuates Acute Kidney Injury in Aristolochic Acid Nephrotoxicity. Am J Pathol 178: 1021–1032. doi: 10.1016/j.ajpath.2010.12.002 PMID: 21356355
8. McCullough PA, Lepor NE (2005) The deadly triangle of anemia, renal insufficiency, and cardiovascular disease: implications for prognosis and treatment. Rev Cardiovasc Med 6: 1–10. PMID: 15741920
9. Babazono T, Hanai K, Suzuki K, Kiuchi Y, Inoue A, Tanaka N, et al. (2006) Lower haemoglobin level and subsequent decline in kidney function in type 2 diabetic adults without clinical albuminuria. Diabetologia 49: 1387–1393. doi: 10.1007/s00125-006-0247-y PMID: 16612589
10. Nangaku M (2005) Chronic Hypoxia and Tubulointerstitial Injury: A Final Common Pathway to End-Stage Renal Failure. Journal of the American Society of Nephrology 17: 17–25. doi: 10.1681/ASN.2005070757 PMID: 16291837
11. Nakamura T, Sugaya T, Kawagoe Y, Suzuki T, Ueda Y, Hikaru K, et al. (2006) Effect of Erythropoietin on Urinary Liver-Type Fatty-Acid-Binding Protein in Patients with Chronic Renal Failure and Anemia. Am J Nephrol 26: 276–280. doi: 10.1159/000093934 PMID: 16772708
12. Eynatten von M, Baumann M, Heemann U, Zdunek D, Hess G, Nawroth P, et al. (2010) Urinary L-FABP and anaemia: distinct roles of urinary markers in type 2 diabetes. European Journal of Clinical Investigation 40: 95–102. doi: 10.1111/j.1365-2362.2009.02220.x PMID: 19912308
13. Kamijo A, Kimura K, Sugaya T, Yamanouchi M, Hikawa A, Hirano N, et al. (2004) Urinary fatty acid–binding protein as a new clinical marker of the progression of chronic renal disease. Journal of Laboratory and Clinical Medicine 143: 23–30. doi: 10.1016/j.lab.2003.08.001 PMID: 14749682
14. Kamijo-Ikemori A, Sugaya T, Kimura K (2014) Novel urinary biomarkers in early diabetic kidney disease. Curr Diab Rep 14: 513. doi: 10.1007/s11892-014-0513-1 PMID: 24919751
15. Matsuo S, Imai E, Horio M, Yasuda Y, Tomita K, Nitta K, et al. (2009) Revised equations for estimated GFR from serum creatinine in Japan. Am J Kidney Dis 53: 982–992. doi: 10.1053/j.ajkd.2008.12.034 PMID: 19339088
16. World Health Organization (2011) Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity.
17. Portilla D, Dent C, Sugaya T, Nagothu KK, Kundi I, Moore PP, et al. (2008) Liver fatty acid-binding protein as a biomarker of acute kidney injury after cardiac surgery. Kidney Int 73: 465–472. doi: 10.1038/sj.ki.5002721 PMID: 18094680
18. Kamijo A, Sugaya T, Hikawa A, Yamanouchi M, Hirata Y, Ishimitsu T, et al. (2006) Urinary liver-type fatty acid binding protein as a useful biomarker in chronic kidney disease. Mol Cell Biochem 284: 175–182. doi: 10.1007/s11010-005-9047-9 PMID: 16532260

19. Nielsen SE, Sugaya T, Tarnow L, Lajer M, Schjoedt KJ, Astrup AS, et al. (2009) Tubular and Glomerular Injury in Diabetes and the Impact of ACE Inhibition. Diabetes Care 32: 1684–1688. doi: 10.2337/dc09-0429 PMID: 19502542

20. Mayer GL, Sugaya T (2006) Urinary L-FABP: A novel biomarker for renal disease and its role in the diagnosis and prognosis of chronic and acute kidney disease. Fats of Life 20: 4–12.

21. Vrtovsnik F, Essig M, Iimura O, Friedlander G (1999) Effect of lipid-lowering strategies on tubular cell biology. Kidney Int Suppl 71: S92–S96. PMID: 10412747

22. Izuhara Y, Nangaku M, Inagi R, Tominaga N, Aizawa T, Kurokawa K, et al. (2005) Renoprotective properties of angiotensin receptor blockers beyond blood pressure lowering. J Am Soc Nephrol 16: 3631–3641. doi: 10.1681/ASN.2005050522 PMID: 16236804

23. Nakamura T, Sugaya T, Koide H (2007) Angiotensin II receptor antagonist reduces urinary liver-type fatty acid-binding protein levels in patients with diabetic nephropathy and chronic renal failure. Diabetologia 50: 490–492. doi: 10.1007/s00125-006-0545-4 PMID: 17171364

24. Nakamura T, Sugaya T, Kawagoe Y, Ueda Y, Osada S, Koide H (2005) Effect of pitavastatin on urinary liver-type fatty acid–binding protein levels in patients with early diabetic nephropathy. Diabetes Care 28: 2728–2732. PMID: 16249547

25. Dikow R, Schwenger V, Schömig M, Ritz E (2002) How should we manage anaemia in patients with diabetes? Nephrol Dial Transplant 17 Suppl 1: 67–72. PMID: 11812916

26. Ertürk S, Ateş K, Duman N, Karatan O, Erbay B, Ertug E (1996) Unresponsiveness to recombinant human erythropoietin in haemodialysis patients: possible implications of angiotensin-converting enzyme inhibitors. Nephrol Dial Transplant 11: 396–397. PMID: 8700371

27. Thomas MC, MacIsaac RJ, Tsalamandris C, Power D, Jerums G (2003) Unrecognized Anemia in Patients With Diabetes: A cross-sectional survey. Diabetes Care 26: 1164–1169. doi: 10.2337/diacare.26.4.1164 PMID: 12663591

28. Hayashi K, Hasegawa K, Kobayashi S (2001) Effects of angiotensin-converting enzyme inhibitors on the treatment of anemia with erythropoietin. Kidney Int 60: 1910–1916. doi: 10.1046/j.1523-1755.2001.00028.x PMID: 11703610