

Serum Apelin Peptide Level in Hemodialysis Patients With Pulmonary Arterial Hypertension

Mitra Samareh Fekri,¹ Abbas Etminan,² Alireza Rashidinedjad,³ Aboozar Mojibian,⁴ Yaser Masoomi⁵

¹Department of Pulmonology, Cardiovascular Research Center, Institute of Basic and Clinical Physiology Sciences, Kerman University of Medical Sciences, Kerman, Iran

²Department of Nephrology, Clinical Research Unit, Shafa hospital, Kerman University of Medical Sciences, Kerman, Iran

³Department of Cardiology, Shafa Hospital, Kerman University of Medical Sciences, Kerman, Iran

⁴Department of Internal Medicine, Institute of Basic and Clinical Physiology Sciences, Kerman University of Medical Sciences, Kerman, Iran

⁵Physiology Research Center, Institute of Basic and Clinical Physiology Sciences, Kerman University of Medical Sciences, Kerman, Iran

Keywords. apelin peptide, hemodialysis, pulmonary arterial pressure

Introduction. Pulmonary arterial hypertension (PAH) is a destructive disease that is characterized by vasoconstriction, alterations and abnormal angiogenesis in pulmonary vessels, and right ventricular dysfunction. There is no certain treatment known for this condition. Patients with PAH have a lower level of apelin in their blood and less apelin is secreted in their endothelial cells, but this condition is not investigated in hemodialysis patients. This study aimed to compare apelin level in hemodialysis patients with and without PAH.

Materials and Methods. Forty hemodialysis patients with PAH were compared with 40 patients without the condition. Apelin serum level was measured using an enzyme-linked immunosorbent assay technique. Dialysis adequacy was measured and its relationship with apelin level and the pulmonary arterial pressure was investigated.

Results. The mean level of apelin in the group suffering from PAH was 54.87 ± 23.50 ng/L, while it was 76.85 ± 34.66 ng/L in those without PAH ($P = .001$). It was also found that hemodialysis adequacy had no effect on apelin level or pulmonary arterial pressure.

Conclusion. The findings of our study suggest that in hemodialysis patients with PAH, apelin peptide serum levels are significantly lower than patients with normal arterial pressure and this condition is not affected by hemodialysis.

IJKD 2018;12:209-14
www.ijkd.org

INTRODUCTION

Chronic kidney disease is one of the many chronic diseases that have seen increased prevalence in recent years and is associated with significantly increased risk of cardiovascular disease and stroke.¹ The United States National Kidney Foundation defines chronic kidney disease as kidney damage with a glomerular filtration rate less than 60 mL/min/1.73 m² for a duration of at least 3 months. This is a progressive and irreversible malfunction that will cause uremia.² Serum creatinine levels higher than 2.5 mg/dL can indicate kidney disease. Also, a serum albuminuria level of 30 mg or higher in 24

hours for more than 3 months has been considered a marker for chronic kidney disease.^{3,4}

In patients with chronic kidney failure, hemodialysis is accompanied with an increased rate of cardiovascular diseases. Left ventricular hypertrophy, arteriosclerosis, ischemic heart disease, pericardial diseases, valve disruptions, and congestive heart failure are the most prevalent cardiovascular diseases among these patients.⁵ In recent studies, pulmonary arterial hypertension (PAH) has been reported as an independent and important mortality predictor of hemodialysis patients.^{6,7} Prevalence of this risk factor varied

from 20% to 58% in different studies.⁸ Pulmonary arterial hypertension is diagnosed by a pulmonary arterial pressure (PAP) of higher than 25 mm Hg at rest. If diagnosed in early stages, this disease can be treated before it leads to right ventricle dysfunction by modifying the underlying factors; the mortality rate of hemodialysis cardiac patients can thereby be reduced.⁹

Apelin is a 36 amino acid peptide generated from the cleavage of a pre-proprotein of 77 amino acids (pre-proapelin). It was first isolated in 1998 from bovine stomach extracts, as an endogenous ligand for the G-protein-coupled APJ receptor.¹⁰ It has shown that in humans apelin can affect the cardiovascular performance and glucose homeostasis and energy metabolism, though it seems that its effect on the cardiovascular system is greater, as it increases myocardial contractile force and lowers the blood pressure.¹¹

It should be noted that the working mechanisms of this peptide are not entirely known. There are 2 theories regarding the release of apelin into the blood circulation: The first theory maintains that as the apelin level in blood is significantly correlated to the apelin level in the heart, part of the apelin originates in the cardiovascular system.⁵ However, other researchers believe that an increase in fat tissue can also be the origin of plasma apelin and it is related to insulin and body mass index.¹¹ This peptide hormone broadly affects the metabolism of different organs, including the cardiovascular system, kidney, liver, fat tissues, lungs, digestive system, brain, pancreas, and endothelium. In animals, apelin has a diuretic effect and lowers blood pressure. However, many effects of apelin are still unknown.^{12,13}

In a study of 52 patients with polycystic kidney disease, it was established that the level of peptide apelin was lower in these patients than the normal population, and this had a significant correlation with performance marker of the kidney.¹⁴ In another study, the relationship between peptide apelin and coronary artery disease in patients who had undergone kidney transplant was investigated. It was concluded that among patients who had undergone a kidney transplant, apelin was significantly lower in patients that had coronary artery disease than the group that did not have coronary artery disease.¹⁵

Plasma level of peptide apelin can vary in kidney

diseases and pulmonary arterial hypertension. Based on our literature research, there have not been any studies investigating concentration level of this biomarker in hemodialysis patients and its relationship with PAH to date. This peptide can be used as a biomarker for early diagnosis and prognosis of patients undergoing hemodialysis who have PAH. This study was aimed to measure the plasma apelin level in chronic kidney disease patients under hemodialysis who have PAH.

MATERIALS AND METHODS

For this analytical-descriptive study, 40 hemodialysis patients suffering from PAH and 40 age- and sex-matched hemodialysis patients with normal PAP were randomly selected from patients on hemodialysis. The criteria for exclusion from the study group were known cardiovascular diseases such as valvular cardiac disorders, left ventricle disorders, liver diseases, lung diseases, and collagen vascular diseases. Patients' written consent was obtained, and the study protocol was approved by the Ethics Committee of Kerman University of Medical Sciences (Permission No IR.KMU.AH.REC.95.76).

Patients' data, including demographic data, such as age and sex, medications, comorbidities, and cause of kidney disorder were collected. A venous blood sample was collected from each patient on the same day after dialysis, which were kept at -70°C after separating the serum by centrifuge.

Apelin 13 serum levels were measured using an enzyme-linked immunosorbent assay (Human Apelin 13 Elisa Kit Catalog number E1273Hu, Bioassay Technology Laboratory, China). A specific antibody against each antigen (apelin 13) was coated in the wells and our antigen was sandwiched between primary and secondary HRP-coated antibody, then color progress was assessed within 10 minutes by an enzyme-linked immunosorbent assay reader (450 nm; reference wavelength, 630 nm).

Both groups of cases and control were examined in stable hemodynamic conditions. Diagnosis of PAH was made by a cardiologist using M-mode, 2-dimensional, and Doppler echocardiography, and the tricuspid regurgitation systolic jet was measured using continuous wave Doppler probe from parasternal or apical regions, then the PAP was calculated using the modified Bernoulli equation:

$PAP = 4 \times (\text{tricuspid systolic jet})^2 + 10$ mmHg (estimated right atrial pressure)

The mean PAP was calculated as follows:

Mean PAP = $0.61 \times \text{systolic PAP} + 2$ mm Hg

Pulmonary arterial hypertension was defined as the mean PAP greater than or equal to 25 mm Hg at rest.¹⁶

Based on the literature,¹⁴ which reports apelin level in cases and control groups of hemodialysis patients being 3.6 ng/L and 4.49 ng/L, respectively, the sample size for each group was calculated to be 11. To increase the statistical power of the study, 40 patients were studied in each group.

RESULTS

A total of 80 hemodialysis patients in the two groups of 40 were studied, one group consisted of patients with PAH, the other, of patients without PAH. Of these patients, 32 were women (40%) and 48 were men (60%). The mean age of the patients was 62.63 ± 11.7 years, with the youngest being 30 years old, and the oldest being 85 years old. The mean dialysis duration was 42.34 ± 24.7 months, the mean time from detection of kidney dysfunction was 80.44 ± 12.9 months, the mean value for dialysis adequacy (Kt/V) was 1.5 ± 1.01 , the mean arterial oxygen saturation was $95.11 \pm 1.38\%$, and the mean hemoglobin level was 11.02 ± 1.5 g/dL. Twenty-two patients had a Kt/V of less than 1.2. The mean systolic PAP was 41.85 ± 17.17 mm Hg, and mean PAP was 24.94 ± 8.8 mm Hg. The mean serum apelin level was 65.8 ± 31.4 ng/L (Table 1).

In terms of the cause of chronic kidney disease, the most frequent cause was hypertension with a frequency of 31 (38.8%; Table 2). Fifty-two patients (65%) had an arteriovenous fistula and 24 patients (30%) were using a catheter (Table 3).

The mean level of apelin in the group suffering

Table 1. Investigated Parameters

Parameter	Mean Value
Age, y	62.63 ± 11.70
Dialysis duration, mo	42.34 ± 24.70
Time from Kidney disease diagnosis, mo	80.44 ± 12.90
Kt/V	1.50 ± 1.01
Arterial oxygen saturation, %	95.11 ± 1.38
Hemoglobin, g/dL	11.02 ± 1.50
Systolic pulmonary arterial pressure, mm Hg	41.85 ± 17.17
Mean pulmonary arterial pressure, mm Hg	24.94 ± 8.80
Peptide apelin level, ng/L	65.80 ± 31.40

Table 2. Causes of Chronic Kidney Disease (CKD)

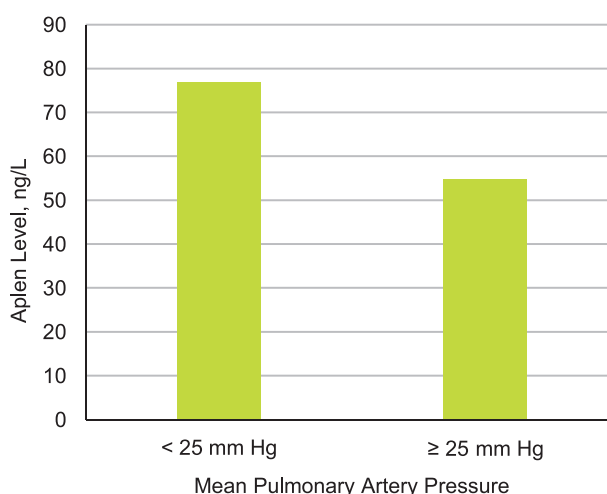
Cause of CKD	Frequency (%)
Autosomal dominant polycystic diseases	3 (3.8)
Diabetes mellitus	11 (13.8)
Diabetes mellitus and hypertension	19 (23.8)
Hypertension	31 (38.8)
Ischemic heart disease	1 (1.3)
Progressive sclerosing glomeronephritis	1 (1.3)
Urinary calculus	1 (1.3)
Uncertain	13 (16.3)
Total	80 (100)

Table 3. Vascular Access Methods for Hemodialysis

Cause of CKD	Frequency (%)
Arteriovenous fistula	52 (65.0)
Arteriovenous fistula and permicath	2 (2.5)
Graft	2 (2.5)
Permicath	24 (30.0)
Total	80 (100)

from PAH (as per mean PAP criterion) was 54.87 ± 23.50 ng/L, and while in the group with normal PAP, it was 76.85 ± 34.66 ng/L (Figure; $P = .001$). Statistical tests based on systolic PAP criterion yield similar results.

The mean value of apelin level in the patients with dialysis adequacy (Kt/V) of less than 1.2 was 67.65 ± 32.90 ng/L and in the patients with the dialysis adequacy of higher than 1.2, it was 62.63 ± 11.70 ng/L, which was not significantly different ($P = .14$). The mean PAP in the patients with dialysis adequacy (Kt/V) of less than 1.2 was 23.43 ± 8.07 mm Hg, and in the patients with the dialysis adequacy of higher than 1.2, it was



Apelin levels comparison between patients with and without pulmonary artery hypertension (PAH)

28.91 ± 9.8 mm Hg, which was not significantly different ($P = .64$).

The correlation between the level of peptide apelin and other parameters was examined and only 1 weak correlation was found between peptide apelin level and oxygen saturation ($r = 0.239$).

DISCUSSION

The finding of our study suggests that apelin serum level in patients with PAH is significantly lower than patients with normal pulmonary arterial pressure who undergo dialysis. According to these results, apelin level measurement can be suggested as a marker for diagnosing PAH. In addition, the results assert that apelin role in reducing pulmonary arterial pressure. The confounding factors in the research, effects of Kt/V on apelin and PAP was assessed and no significant relationship was found. Based on our findings and searches performed in scientific databases, this study is the first that investigates apelin level in hemodialysis patients and its relationship with PAP.

Apelin peptide was first discovered in 1998 from bovine stomach extracts as an endogenous receptor ligand (APJ). Apelin exerts its physiological effects by linking to this receptor. It had been proven that apelin has effects on cardiovascular function in humans, and glucose hemostasis and energy are affected too. This peptide is present in various organs, although it seems that the cardiovascular system is its main target. This peptide and its receptors are also expressed in endothelial and vascular smooth muscle cells. Histologic studies have clearly identified this peptide and its receptors in the tunica media of the aorta and pulmonary arteries.^{11,17} Cardiovascular stimulation leads to apelin production and eventual activation of its receptors. Recent findings show that when apelin activates its receptors in endothelial cell level through endothelial nitric oxide synthase, and as a result, vascular smooth muscles are relaxed and blood pressure is reduced.¹⁸

Studies have shown that in patients with PAH, endothelial cells are discordant, so that mitosis and migration are increased and angiogenesis is ineffective. Apelin's effect on pulmonary artery is only studied in a few studies.^{19,20} In one study, it was shown that high apelin levels alone could reduce vascular tone by 17% in a reversible way.²¹ In another study, it was shown that apelin

reduced vascular tone up to 11% and this reduction disappeared after apelin removal and blocking endothelial nitric oxide synthase system. Also, it revealed that apelin restrains pulmonary artery contraction in rats with isolated pulmonary arterial hypertension, but does not have this effect in rats that hypoxia is the cause of PAH.²²

An experimental study on pulmonary embolism showed that a single dose of apelin in the amount of 20 mg/kg lowers the mean PAP within 2 minutes. However, the ratio of pulmonary vascular resistance to systemic vascular resistance did not show any changes.²³ Also, studies on animal models of PAH over long periods of time reveals the lowering effect of apelin on pulmonary arterial pressure; however, the mechanism of this effect and whether it is direct or indirect is not known yet.²⁴⁻²⁶

The acute vasodilatory effect of apelin on pulmonary artery pressure is moderate (10% to 17%), and it is even weakened in hypoxia cases. Nevertheless, the long-term effect of apelin on vasodilation cannot be refuted. Apelin effectively decreases secretion of endothelial vasoconstrictive factors.²⁷ In any case, the medications that are presently in use for lowering PAH do not control it effectively. Hence, lately the research on control of PAH has been focused on basic mechanisms that create this condition and factors affecting proliferation and remodeling of pulmonary arteries.²⁸ In this regard, some studies report the chronic effect of apelin on lowering the PAP in animal models of this disease, with proposed mechanisms of stabilization of endothelial cells and preventing the inappropriate division of cells.²⁴⁻²⁶ Apelin can also affect the inflammation process, though the research on has had conflicting results.²⁹

In view of the apelin effects on PAP, which was independent of dialysis adequacy (Kt/V), it is hereby proposed that a study be undertaken to examine the factors influencing increase generation and extend the half-life of apelin in the body.

CONCLUSIONS

The finding of our study suggests that apelin peptide serum levels in patients with PAH who are under hemodialysis are significantly lower than patients who have normal PAP. According to these results, apelin level measurement can be used as a diagnostic marker for PAH. This study also shows that apelin level in patients with chronic

kidney disease under hemodialysis is not related to dialysis adequacy and is significantly lower in patients with PAH. Further research should be undertaken to understand the mechanism that causes PAH and developing medicines that would aid endogenous synthesis of this molecule or control its breakdown so that this peptide could be used in treating PAH and reducing mortality and morbidity from chronic kidney disease.

ACKNOWLEDGMENTS

The authors thank Kerman University of Medical Sciences for financial support of this research.

CONFLICT OF INTEREST

None declared.

REFERENCES

- Baumgarten M, Gehr T. Chronic kidney disease: detection and evaluation. *Am Fam Physician*. 2011;15:1138-48.
- Abassi MA, Chertow G M, Hall Y. End Stage Renal Disease. *Am Fam Physician*. 2010;82:1512-4.
- Weiss RH, Kim K. Metabolomics in the study of kidney diseases. *Nat Rev Nephrol*. 2012 25;8:22-33.
- KDIGO 2012 Clinical Practice Guideline for the Evaluation and management of Chronic Kidney Disease. *Kidney Int Suppl*. 2013;3:1-150.
- Foldes G, Horkay F, Szokodi I, et al. Circulating and Cardiac Levels of Apelin, the Novel Ligand of the Orphan Receptor APJ, in Patients with Heart Failure. *Biochem Biophys Res Commun*. 2003;308:480-5.
- Baldi F, Fuso L, Arrighi E, Valente S. Optimal management of pulmonary arterial hypertension: prognostic indicators to determine treatment course. *Ther Clin Risk Manag*. 2014;7:825-39.
- Yigla M, Fruchter O, Aharonson D, Yanay N, Reisner SA, Lewin M. Pulmonary hypertension is an independent predictor of mortality in hemodialysis patients. *Kidney Int*. 2009;75:969-75.
- Fabbian F, Cantelli S, Molino C, Pala M, Longhini C, Portaluppi F. Pulmonary hypertension in dialysis patients: A cross-sectional Italian study. *Int J Nephrol*. 2011;2011:283475.
- McDonald JE, Deloney LA, Jambhekar K. Pulmonary arterial hypertension: Part 1: A review for an internist. *J Ark Med Soc*. 2014;111:62-4.
- Tatemoto K, Hosoya M, Habata Y, et al. Isolation and characterization of a novel endogenous peptide ligand for the human APJ Receptor. *Biochem Biophys Res Commun*. 1998;251:471-6.
- Castan-Laurell I, Michaela V, Danièle D, et al. Effect of hypocaloric diet-induced weight loss in obese women on plasma Apelin and adipose tissue expression of Apelin and APJ. *Eur J Endocrinol*. 2008;158:905-10.
- Lee LK, Geoge SR, O'Dowd BF. Unravelling the roles of the Apelin system: prospective therapeutic applications in heart failure and obesity. *Trends Pharmacol Sci*. 2006;27:190-4.
- Lv D, Li H, Chen L. Apelin and APJ, a novel critical factor and therapeutic target for atherosclerosis. *Acta Biochim Biophys Sin (Shanghai)*. 2013;45:527-33.
- Lacquaniti A, Chirico V, Lupica R, et al. Apelin and copeptin: two opposite biomarkers associated with kidney function decline and cyst growth in autosomal dominant polycystic kidney disease. *Peptides*. 2013;49:1-8.
- Malyszko J, Malyszko JS, Pawlak K, Wolczynski S, Mysliwiec M. Apelin, a novel adipocytokine, in relation to endothelial function and inflammation in kidney allograft recipients. *Transplant Proc*. 2008;40:3466-9.
- Reisne SA, Azzam Z, Halmann M, et al. Septal/free wall curvature ratio: a noninvasive index of pulmonary arterial pressure. *J Am Soc Echocardiogr*. 1994;7:27-35.
- Leal VO, Lobo JC, Stockler-Pinto MB, et al. Apelin: a peptide involved in cardiovascular risk in hemodialysis patients?. *Ren Fail*. 2012;34:577-81.
- El-Shehaby AM, El-Khatib MM, Battah AA, Roshdy AR. Apelin: a potential link between inflammation and cardiovascular disease in end-stage renal disease patients. *Scand J Clin Lab Invest*. 2010;70:421-7.
- Masri FA, Xu W, Comhair SA, et al. Hyperproliferative apoptosis-resistant endothelial cells in idiopathic pulmonary arterial hypertension. *Am J Physiol Lung Cell Mol Physiol* 2007;293:548-54.
- Tuder RM, Chacon M, Alger L, et al. Expression of angiogenesis-related molecules in plexiform lesions in severe pulmonary hypertension: Evidence for a process of disordered angiogenesis. *J Pathol* 2001;195:367-74.
- Andersen CU, Markvardsen LH, Hilberg O, Simonsen U. Pulmonary apelin levels and effects in rats with hypoxic pulmonary hypertension. *Respir Med* 2009;103:1663-71.
- Huang P, Fan XF, Pan LX, et al. Effect of apelin on vasodilatation of isolated pulmonary arteries in rats is concerned with the nitric oxide pathway. *Zhongguo Ying Yong Sheng Li Xue Za Zhi* 2011;27:1-5.
- Feng JH, Li WM, Wu XP, et al. Hemodynamic effect of apelin in a canine model of acute pulmonary thromboembolism. *Peptides*. 2010;31:1772-8.
- Alastalo TP, Li M, Perez JP, et al. Disruption of PPARgamma/beta-catenin-mediated regulation of apelin impairs BMP-induced mouse and human pulmonary arterial EC survival. *J Clin Invest*. 2011;121:3735-46.
- Fan XF, Wang Q, Mao SZ, et al. Protective and therapeutic effect of apelin on chronic hypoxic pulmonary hypertension in rats. *Zhongguo Ying Yong Sheng Li Xue Za Zhi*. 2010;26:9-12.
- Falcao-Pires I, Goncalves N, Henriques-Coelho T, Moreira-Goncalves D, Roncon-Albuquerque R Jr, Leite-Moreira AF. Apelin decreases myocardial injury and improves right ventricular function in monocrotaline-induced pulmonary hypertension. *Am J Physiol Heart Circ Physiol* 2009;296: 2007-14.
- Schannwell CM, Steiner S, Strauer BE. Diagnostics in pulmonary hypertension. *J Physiol Pharmacol*. 2007;58:591-602.

28. Lourenco AP, Fontoura D, Henriques-Coelho T, Leite-Moreira AF. Current pathophysiological concepts and management of pulmonary hypertension. *Int J Cardiol.* 2012;155:350-61.
29. Chun HJ, Ali ZA, Kojima Y, et al. Apelin signaling antagonizes Ang II effects in mouse models of atherosclerosis. *J Clin Invest.* 2008;118:3343-5.

Correspondence to:
Aboozar Mojbian, MD
Institute of Basic and Clinical Physiology Sciences, Kerman
University of Medical Sciences, Kerman, Iran
Tel: +98 913 249 2517
E-mail: dr.a.mojbian@gmail.com

Received December 2017

Revised April 2017

Accepted April 2017

Archive of SID