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Original Article

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The Relationship Between Serum Angiopoietin-Like Protein-2 Levels and Arterial Stiffness in Hemodialysis Patients

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ABSTRACT

Objective: Arterial stiffness, a predictor of cardiovascular events, is increased in hemodialysis patients than in healthy adults, and also cardiovascular diseases are more common in this population . Angiopoietin-like protein-2 is a glycoprotein which is thought to be associated with arterial stiffness. In this study, our aim is to investigate the relationship between angiopoietin-like protein-2 and arterial stiffness in hemodialysis patients.
Methods: Patients who had been on hemodialysis for at least 3 months, aged 18-80 years, without active infection, and accepted to participate in the study were included in the study. Arterial stiffness was measured with the Mobil-O-Graph device. Angiopoietin-like protein-2 levels were measured by the Enzyme Linked Immuno Sorbent Assay (ELISA) method.
Results: Eighty-five patients were included in the study. The mean angiopoietin-like protein-2 level was 2427 ± 1102 pg/mL, *and a positive correlation between angiopoietin-like protein-2 levels and hemodialysis duration, systolic and diastolic blood pressure was found.* Mean carotid-femoral pulse wave velocity was 8.08 ± 1.9 m/s, and a positive correlation was found between carotid-femoral pulse wave velocity and age, body mass index, systolic blood pressure, creatinine, phosphorus and glucose, and a negative correlation was found with serum albumin level. There was no relationship between angiopoietin-like protein-2 levels and carotid-femoral pulse wave velocity and augmentation index. Age, body mass index, systolic blood pressure, pulse pressure, albumin, glucose, and creatinine levels *were found to be different* when patients were divided into 2 groups based on the median carotid-femoral pulse wave velocity value.
Conclusion: In this cross-sectional study, we found that angiopoietin-like protein-2 levels were not associated with arterial stiffness in hemodialysis patients.
Keywords: Angiopoietin-like proteins, vascular stiffness, dialysis

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INTRODUCTION

The main cause of death in patients with end-stage kidney disease (ESKD) is cardiovascular disease (CV).[1] Arterial stiffness is an important indicator of atherosclerosis and CV events. Increased arterial stiffness causes systolic hypertension, left ventricular hypertrophy, and worsening coronary perfusion, resulting in an increased risk of CV disease.[2] As it is known, arterial stiffness is increased in ESKD patients than in healthy adults.[3] Chronic volume overload, activation of the renin-angiotensin-aldosterone system, arterial calcification, increased lipid oxidation, microinflammation, overactivity of the sympathetic nervous system, and disturbances in the nitric oxide system are thought to cause ESKD patients to have stiffer arteries.[4]

The stiffness of the arteries may be measured by different methods and at different points. Although parameters such as aortic pulse wave velocity (PWV) analysis, augmentation index (AI), arterial compliance, and ambulatory arterial stiffness index are considered in measuring arterial stiffness, aortic pulse wave velocity analysis is

the most commonly used, easy, practical, and reliable method in the clinic. Applanation tonometry (SphygmoCor, Complior) and oscillometric methods (Mobil-O-Graph) are used to measure PWV. Since applanation tonometry measuring devices are quite expensive, oscillometric devices are frequently used today. By the oscillometric devices (Mobil-O-Graph), immediate analysis of PWV as well as a 24-hours recording of PWV and ambulatory blood pressure for 24-hours measurement can be performed.[5]

Angiopoietins are growth factors that have a role in angiogenesis and vasculogenesis. Ang-1 and Ang-2, the best-known angiopoietins, are ligands for the Tie2 receptor tyrosine kinase found on endothelial cells and/or other progenitor cells. In angiogenesis, these growth factors work in harmony with other angiogenic molecules like VEGF.[6]

Angiopoietin-like proteins (ANGPTL) are pro-inflammatory proteins structurally similar to angiopoietins and secreted by endothelial cells, adipocytes, and macrophages.[7] Eight ANGPTL have been identified to date. Some of them have defined effects on inflammation, lipid metabolism, malignant cell invasion, and hematopoietic stem cell activity.[8]

ANGPTL-2 is a circulating glycoprotein overexpressed in the heart, kidney, lung, skeletal muscle, and adipose tissue.[8] In mice and humans, circulating ANGPTL-2 levels are related to inflammation, obesity, and insulin resistance.[7] Angiopoietin-like protein-2 is also associated with atherosclerosis, another pathology associated with chronic inflammation and endothelial dysfunction.[9] In addition, ANGPTL-2 levels have been found to increase significantly in many chronic inflammatory diseases, including diabetes, and ANGPTL-2 secretion increases due to abnormal stimulation of cells in conditions such as obesity, diabetes, and metabolic syndrome and even contributes negatively to diabetic complications and especially to the development of diabetic nephropathy.[10] In some studies, it has been found that CV disease risk is similarly increased in patients with increased ANGPTL-2 levels, and ANGPTL-2 levels are one of the determinants of CV-related mortality.[11-13]

ANGPTL-2 is also increased in patients with kidney disease. It was demonstrated that high serum levels of ANGPTL-2 before kidney transplantation decreased after the transplantation. In addition, arterial stiffness in patients after kidney transplantation was found to be associated with ANGPTL-2 levels.[14]

In the literature, there is only 1 study on the contribution of ANGPTL-2 to increased arterial stiffness in hemodialysis (HD) patients, and in this study, ANGPTL-2 level was associated with arterial stiffness.[15] Our study aimed to investigate the relationship between ANGPTL-2 and arterial stiffness in hemodialysis (HD) patients.

MAIN POINTS

- Arterial stiffness is increased in hemodialysis (HD) patients than in healthy adults.
- Arterial stiffness is an important predictor of cardiovascular events.
- Angiopoietin-like protein-2 levels were not associated with arterial stiffness in HD patients.

METHODS

This is a cross-sectional study conducted in the HD unit of Kahramanmaraş Sütçü İmam University Medical Faculty Hospital. Ethics committee approval, dated April 4, 2018, and numbered 2018-07-05, was obtained from the clinical research ethics committee of Kahramanmaraş Sütçü İmam University, Faculty of Medicine. All patients were informed before the study and informed consent forms were signed by them if they agreed to participate in the study. The study was conducted in accordance with the Helsinki Declaration criteria.

Patients under 18 and over 80 years of age with active infections and cardiac arrhythmias were not included in the study. The majority of patients were on dialysis thrice weekly and the last 6 months were taken into account in terms of the average dialysis dose.

Measurement of Arterial Stiffness

A single cuff Arteriograph (Mobil-O-Graph PWA from IEM GmbH) was used. Before the HD session, after resting for at least 30 minutes, the device was attached to the arm of the patient without arteriovenous fistula, data such as age, gender, and blood pressure were entered, 3 measurements were made with an interval of 30 seconds between each measurement, and the average values were obtained. This device was used to measure systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP), heart rate, carotid-femoral PWV (cf-PWV), and AI.

Measurement of Angiopoietin-Like Protein-2

The venous blood was taken by the phlebotomy to beplaced in tubes without anticoagulants. The serum and plasma were obtained by centrifuging the collected blood for 10 minutes at 4000 rpm and stored at −80 °C until the analysis.

Serum Angiopoietin-2 levels were determined using commercial ELISA kit (SEA009Hu; Cloud-Clone Corp., Houston, TX, USA). Specimen absorbance values were determined on Multiskan FC microplate reader (Thermo Fisher Scientific, Waltham, MA, USA) at a wavelength of 450 nm. Values were expressed as pg/mL. The intra-assay coefficient of variance (CV), interassay CV, detection range, and sensitivity of the angiopoietin kit were reported as < 10%, < 12%, and 62.5-4000 pg/mL and 26.3 pg/mL, respectively.

Age, gender, duration of HD, additional diseases such as diabetes mellitus and coronary artery disease, medications used, demographic data such as hepatitis serology, monthly biochemical laboratory tests, and calculations such as Kt/V and urea reduction ratio (URR), were obtained from the patient files. Body mass index (BMI) was measured as "body weight/height^2" after dialysis and BMI ≥ 25 was accepted as obesity, SBP ≥ 140 mmHg or DBP ≥ 90 mmHg was accepted as hypertension (being on antihypertensive medication is also considered as having hypertension). Kt/V ≥ 1.20 and URR ≥ 60% were considered as normal. We also considered high or low above the specified reference values for C-reactive protein (CRP) (reference range: 0-5 mg/L), low-density lipoprotein (LDL)-cholesterol (reference range: 50-130 mg/dL), high-density lipoprotein (HDL)-cholesterol (reference range: 10-40 mg/dL), triglycerides (reference range: 50-150 mg/dL), albumin (reference range: 2.5-4.0 g/L), glucose (reference range: 60-200 mg/dL), creatinine (reference range: 0.4-1.2 mg/dL for men and 0.3-1 mg/dL for women), calcium (reference range: 8.4-10.4 mg/dL), phosphorus (reference range: 3.5-5.5 mg/dL), uric acid (reference range: 3.5-7 mg/dL for men, 2.5-6.0 mg/dL for women) parathormone (reference range: 12-65 ng/L).

Statistical Analysis

SPSS version 22.0 (IBM Corp., Armonk, NY, USA) package was used for the statistical analysis. The data obtained by measurement were expressed as mean ± SD, and the data obtained by counting as numbers or ratios. Kolmogorov–Smirnov and/or Shapiro–Wilk tests were used for the normal distribution analysis of continuous data. Student-*t* or Mann–Whitney *U*-tests were used when comparing continuous variables depending on whether the data conformed to the normal distribution. Any *P*-value smaller than .05 was considered statistically significant.

RESULTS

Eighty-five patients were included in the study between May 2018 and December 2018. The mean age of the patients was 51.5 ± 13.2 (24-76) years, and 48 (56.5%) were male. The mean duration of HD was 54 ± 44 months. Thirty-three (38.8%) patients had diabetes, and 25 (29.4%) patients had a history of coronary artery disease. While hepatitis serology was negative in 80 patients, 2 patients had hepatitis B, 3 patients had hepatitis C. Fifty-seven (67.1%) patients received antihypertensive medication and 7 patients received statin therapy. Table 1 shows the patients' laboratory data.

Since the European Society of Cardiology report accepted 7.7 mm/s as the threshold value for PWV as a cardiovascular risk factor, the patients were divided into 2 groups as below and >=7.7.[16]

The mean ANGPTL-2 level was 2427 ± 1102 pg/mL, and a positive correlation was found between ANGPTL-2 and HD duration, SBP, and DBP. Mean cf-PWV was 8.08 ± 1.9 mm/s, and a positive correlation was found between cf-PWV and age (*P* = .0000), BMI (*P* = .000), SBP (*P* = .0000), creatinine (*P* = .002), phosphorus (*P* = .01), and glucose (*P* = .001), and a negative correlation was found with serum albumin level (*P* = .002). There was no relationship between ANGPTL-2 levels and cf-PWV and AI. Age, BMI, SBP, pulse pressure, albumin, glucose, and creatinine levels were found to be *not similar* when patients were divided into 2 groups based on the median cf-PWV value (Table 2). Diabetic patients had higher PWW than non-diabetic groups.

Table 1. General Data of Patients

Variables	Mean ± SD (ranges)
BMI (kg/m^2)	26.73 ± 5.72 (17.02-42.90)
SBP (mm Hg)	142.87 ± 24.55 (84.00-193.00)
DBP (mm Hg)	87.62 ± 16.60 (45.00-132.00)
Kt/V	1.56 ± 0.21 (1.01-2.26)
Urea reduction ratio (URR, %)	72.06 ± 6.36 (57.00-94.00)
cf-PWV (m/s)	8.08 ± 1.90 (1.33-12.00)
Augmentation index (%)	25.38 ± 12.33 (2.00-58.30)
ANGPTL-2 (pg/mL)	2427.92 ± 1102.65 (62.50-4000.00)
Glucose (mg/dL)	144.15 ± 86.51 (63.00-508.00)
Creatinine (mg/dL)	8.59 ± 2.31 (3.70-13.40)
Calcium (mg/dL)	8.59 ± 0.66 (6.20-10.50)
Phosphorus (mg/dL)	4.74 ± 1.08 (2.60-7.90)
Albumin (g/L)	4.26 ± 0.36 (2.91-4.73)
Uric acid (mg/dL)	6.43 ± 1.36 (3.60-10.00)
Parathormone (PTH) (ng/L)	485.34 ± 458.38 (6.00-2083.00)
LDL cholesterol (mg/dL)	92.96 ± 29.07 (25.00-179.00)
Triglyceride (mg/dL)	200.13 ± 134.87 (47.00-772.00)
HDL cholesterol (mg/dL)	35.07 ± 11.24 (12.00-81.00)
CRP (mg/L)	19.33 ± 24.62 (3.00-125.00)

ANGPTL-2, angiopoietin-like protein-2; BMI, body mass index; cf-PWV, carotid-femoral pulse wave velocity; CRP, C-reactive protein; DBP, diastolic blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein; kt/V, clearance × dialysis time/volume of distribution of urea; SBP, systolic blood pressure.

In multivariate regression analysis, age and systolic blood pressure predicted c-f PWV (model included; age, SBP, BMI, albumin, CRP, and ANGPTL-2) and HD time and systolic blood pressure predicted ANGPTL-2 (model included; age, HD time, SBP, CRP, calcium).

DISCUSSION

There are very few studies in the literature examining the relationship between ANGPTL-2 levels and arterial stiffness in kidney patients. In a study conducted on HD patients and 1 kidney transplant patient, ANGPTL-2 levels were shown to be associated with arterial stiffness. This cross-sectional study found

Table 2. Comparison of cf-PWV < 7.7 mm/s and cf-PWV > 7.7 mm/s According to Median cf-PWV Value

Variables	cf-PWV < 7.7 mm/s	cf-PWV >=7.7	*P*
Age (years) (mean ± SD)	41.80 ± 8.23	61.09 ± 9.96	**.000**
HD period (months) (mean ± SD)	55.24 ± 44.97	52.97 ± 43.85	.810
BMI (kg/m^2) (mean ± SD)	24.43 ± 4.91	28.97 ± 5.62	**.000**
SBP (mm Hg) (mean ± SD)	136.28 ± 23.88	149.30 ± 23.72	**.010**
DBP (mm Hg) (mean ± SD)	89.21 ± 18.89	86.06 ± 14.07	.380
Kt/V (mean ± SD)	1.57 ± 0.23	1.55 ± 0.19	.680
URR, % (mean ± SD)	72.53 ± 6.97	71.59 ± 5.75	.500
ANGPTL-2 (pg/mL) (mean ± SD)	2480.66 ± 1193.89	2376.40 ± 1017.23	.660
Glucose (mg/dL) (mean ± SD)	120.91 ± 74.63	166.83 ± 92.02	**.010**
Creatinine (mg/dL) (mean ± SD)	9.26 ± 2.60	7.78 ± 1.72	**.003**
Albumin (g/L) (mean ± SD)	4.1 ± 0.3	3.9 ± 0.1	**.010**
LDL cholesterol (mg/dL) (mean ± SD)	91.75 ± 32.80	94.14 ± 25.26	.710
Triglyceride (mg/dL) (mean ± SD)	181.51 ± 101.11	218.30 ± 160.37	.210
HDL cholesterol (mg/dL) (mean ± SD)	35.53 ± 11.43	34.61 ± 11.17	.710
CRP (mg/L) (mean ± SD)	21.56 ± 31.80	17.16 ± 14.69	.410

ANGPTL-2, angiopoietin-like protein-2; BMI, body mass index; cf-PWV, carotid-femoral pulse wave velocity; CRP, C-reactive protein; DBP, diastolic blood pressure; HD, hemodialysis; HDL, high-density lipoprotein; kt/V, clearance × dialysis time/volume of distribution of urea; LDL, low-density lipoprotein; SBP, systolic blood pressure; URR, urea reduction ratio.
Statistically significant values are signed in bold.

that ANGPTL-2 levels, an atherogenic molecule, were not associated with arterial stiffness in HD patients.

It is known that arterial stiffness is increased in HD patients than in healthy adults. Studies have shown that this increase in arterial stiffness may be due to factors such as age, blood pressure, obesity, uremia, hyperphosphatemia, hypoalbuminemia, and hyperlipidemia. Similar to the literature, our study showed a relationship between age, BMI, SBP, creatinine, phosphorus and glucose, and arterial stiffness.

In most previous studies, ANGPTL-2 levels were found to be increased in ESKD patients than in healthy adults.[14,15] In the study by Desjardins et al.[14] ANGPTL-2 levels were found to be close to normal in ESKD patients after kidney transplantation. The same study emphasized that ANGPTL-2 could be a marker of CV disease due to ESKD because of a positive correlation between arterial stiffness and ANGPTL-2 levels and mortality.

In their study, Ashokachakkaravarthy et al[17] emphasized that endothelial dysfunction and inflammatory markers such as asymmetric dimethylarginine and ANGPTL-2 are increased in ESKD patients than in the healthy population; these levels are correlated with disease severity and duration. Similarly, our study found a correlation between ANGPTL-2 level and HD duration.

The study by Fukami et al[15] concluded that the ANGPTL-2 level is a better marker of arterial stiffness in uremic patients than high-sensitivity C-reactive protein (hs-CRP) and can be a unique marker of vascular aging progression. In addition, there was a much more robust association between ANGPTL-2 levels and vascular aging in patients with pre-HD high MAP, high pulse pressure, or increased body weight. Thus, it was found that ANGPTL-2 levels can be used as a marker to assess the risk of CV disease and early death in HD patients. When our study was compared with this study, the difference in results might have been obtained due to the small sample size and the difference in our arterial stiffness measurement method.

In a study conducted by Gellen et al[11] on 1353 type 2 diabetic patients, it was shown that patients with high ANGPTL-2 levels were at an increased risk of developing CV disease. In other studies, serum ANGPTL-2 levels have been found to be one of the determinants of CV and all-cause mortality.[12,13]

ANGPTL2 secreted from endothelial cells activates nuclear factor kappa B (NFκB), which significantly inhibits endothelial NO release by reducing eNOS activity. Moreover, ANGPTL-2 derived from endothelial cells has been shown to increase the expression of adhesion molecules in an autocrine or paracrine manner. Macrophages that access arterial walls secrete high levels of ANGPTL-2 and stimulate the secretion of tumor necrosis factor (TNF), interleukin (IL)-1β and IL-6. Angiopoietin-like protein-2 has important functions in the regulation of bone cell matrix and maturation of bone and cartilage. Taken together, it is thought that chronic inflammation due to ANGPTL-2 may cause arterial stiffness and vascular calcification in uremic conditions.[15]

The study by Erdan A et al[18] concluded that arterial stiffness was associated with both peripheral and aortic blood pressure. In the study conducted by Guzel et al.[19] it was stated that there is a relationship between arterial stiffness and age and SBP. And

also in the study conducted by Heleniak Z et al[20] reported that obesity is a factor which is affecting arterial stiffness. Similarly, we observed a positive correlation between arterial stiffness and SBP, age, and BMI in our study.

The relatively small number of patients is one of our study's limitations. It should also be kept in mind that antihypertensive drugs, commonly used in HD patients, may affect ANGPTL-2 levels. And also interdialytic weight gain might affect the arterial stiffness measurements which was not considered in this study.

CONCLUSION

In conclusion, in contrast to the limited number of studies, we discovered no relationship between ANGPTL-2 levels and arterial stiffness in HD patients.

Ethics Committee Approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed Consent: Written informed consent was obtained from the patient who agreed to take part in the study.

Peer-review: Externally peer-reviewed.

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REFERENCES

1. Collins AJ, Li S, Ma JZ, Herzog C. Cardiovascular disease in end-stage renal disease patients. *Am J Kidney Dis*. 2001;38(4)(suppl 1):S26-S29. [CrossRef]
2. Kato A. Arterial stiffening and clinical outcomes in dialysis patients. *Pulse (Basel)*. 2015;3(2):89-97. [CrossRef]
3. Covic A, Gusbeth-Tatomir P, Goldsmith DJ. Arterial stiffness in renal patients: an update. *Am J Kidney Dis*. 2005;45(6):965-977. [CrossRef]
4. Georgianos PI, Sarafidis PA, Lasaridis AN. Arterial stiffness: a novel cardiovascular risk factor in kidney disease patients. *Curr Vasc Pharmacol*. 2015;13(2):229-238. [CrossRef]
5. Gungor O, Toz H. Factor affecting arterial stiffness on renal transplant patients. *Turk Neph Dial Transpl*. 2012;21(2):107-110. [CrossRef]
6. Fam NP, Verma S, Kutryk M, Stewart DJ. Clinician guide to angiogenesis. *Circulation*. 2003;108(21):2613-2618. [CrossRef]
7. Tabata M, Kadomatsu T, Fukuhara S, et al. Angiopoietin-like protein 2 promotes chronic adipose tissue inflammation and obesity-related systemic insulin resistance. *Cell Metab*. 2009;10(3):178-188. [CrossRef]
8. Santulli G. Angiopoietin-like proteins: a comprehensive look. *Front Endocrinol*. 2014;5:4. [CrossRef]
9. Farhat N, Thorin-Trescases N, Mamarbachi M, et al. Angiopoietin-like 2 promotes atherogenesis in mice. *J Am Heart Assoc*. 2013;2(3):e000201. [CrossRef]
10. Ishii T, Furuya F, Takahashi K, et al. Angiopoietin-like protein 2 promotes the progression of diabetic kidney disease. *J Clin Endocrinol Metab*. 2019;104(1):172-180. [CrossRef]
11. Gellen B, Thorin-Trescases N, Sosner P, et al. ANGPTL2 is associated with an increased risk of cardiovascular events and death in diabetic patients. *Diabetologia*. 2016;59(11):2321-2330. [CrossRef]
12. Hata J, Mukai N, Nagata M, et al. Serum angiopoietin–like Protein 2 is a novel risk factor for cardiovascular disease in the community: the Hisayama study. *Arterioscler Thromb Vasc Biol*. 2016;36(8):1686-1691. [CrossRef]
13. Huang CL, Wu YW, Wu CC, Hwang JJ, Yang WS. Serum angiopoietin-like protein 2 concentrations are independently associated with heart failure. *PLOS ONE*. 2015;10(9):e0138678. [CrossRef]
14. Desjardins MP, Thorin-Trescases N, Sidibé A, et al. Levels of angiopoietin-like-2 are positively associated with aortic stiffness and mortality after kidney transplantation. *Am J Hypertens*. 2017;30(4):409-416. [CrossRef]
15. Fukami H, Morinaga J, Okadome Y, et al. Circulating angiopoietin-like protein 2 levels and arterial stiffness in patients receiving maintenance hemodialysis: a cross-sectional study. *Atherosclerosis*. 2020;315:18-23. [CrossRef]
16. Reference Values for Arterial Stiffness' Collaboration. The Reference Values for arterial Stiffness' Collaboration, Determinants of pulse wave velocity in healthy people and in the presence of cardiovascular risk factors: 'establishing normal and reference values'. *Eur Heart J*. 2010;31(19):2338-2350. [CrossRef]
17. Ashokachakkaravarthy K, Rajappa M, Parameswaran S, et al. Asymmetric dimethylarginine and angiopoietin-like protein-2 are independent predictors of cardiovascular risk in pre-dialysis non-diabetic chronic kidney disease patients. *Int Urol Nephrol*. 2020;52(7):1321-1328. [CrossRef]
18. Erdan A, Ozkok A, Alpay N, Akkaya V, Yildiz A. Volume status and arterial blood pressures are associated with arterial stiffness in hemodialysis patients. *Int J Artif Organs*. 2018;41(7):378-384. [CrossRef]
19. Guzel FB, Ozturk I, Gisi K, et al. The relationship between hepatic fibrosis and arterial stiffness in hemodialysis patients. *Semin Dial*. 2021;8:1-6. [CrossRef]
20. Heleniak Z, Illersperger S, Brakemeier S, Debska-Slizien A, Budde K, Halleck F. P0821Obesity, fat tissue, cell membrane integrity parameters and arterial stiffness in renal transplant recipients. *Nephrol Dial Transplant*. 2020;35(Supplement_3):6. [CrossRef]