

Relationship between the Circulating Levels of Angiotensin-2 and Progression of Chronic Kidney Disease.

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Abstract:

Introduction: Chronic kidney disease (CKD) is associated with high overall and cardiovascular (CV) mortality that increases as kidney functions decrease. Angiotensins have been recognized mostly for their involvement in endothelial dysfunction, and inflammation, and it was incriminated in the pathogenesis of atherosclerosis, all these processes are highly prevalent in patients with CKD. High serum angiotensin-2 (Ang-2) was found in acute kidney injury (AKI) and was a strong independent predictor of mortality. Also, Ang-2 was showed to be markedly elevated in patients on maintenance dialysis and was closely associated with the extent of coronary artery disease (CAD) and peripheral arterial disease (PAD). **Aim:** This study was done to investigate the relationship between the circulating levels of angiotensin-2 and renal function throughout all stages of CKD and to assess correlations between serum Ang-2 and known CV risk factors. **Subjects and methods:** Serum levels of angiotensin-2 was measured in 75 CKD patients divided into 5 equal groups representing the five stages of CKD according to K/DOQI guidelines and were compared with angiotensin-2 levels in 15 nonsmokers healthy control matching the patients group as regard, age, sex, and body mass index (BMI). All participants in the study were subjected to full history taking, thorough clinical examination and routine laboratory investigations. Informed written consent was obtained from every subject. **Results:** The circulating Ang-2 was significantly

higher in CKD patients compared to the healthy controls, when we further assessed Ang-2 levels according to CKD stages, Ang-2 levels steadily increased across the following groups: healthy controls, CKD 1, CKD 2, CKD 3, CKD 4, and CKD 5, respectively. However, the Ang-2 levels in patients with CKD stages 1 and 2 were not statistically different from the Ang-2 levels in healthy control but at higher stages a significant increase in circulating Ang-2 compared to healthy controls was found. Next, we performed correlation analyses for Ang-2 and several CV risk factors. A significant direct correlation between Ang-2 and serum phosphorus level was detected. All other tested CV risk factors including patients, age, intact parathyroid hormone (iPTH), mean arterial blood pressure (MAP), serum calcium, cholesterol and triglycerides were not correlated with serum Ang-2 level. Furthermore, there was no detectable influence of erythropoietin or vitamin D treatment on circulating Ang-2 levels. **Conclusions:** circulating Ang-2, a putative marker and potential mediator of accelerated atherosclerosis, is inversely related to GFR and increases with progression of CKD. The finding of the study may serve as a basis for more research to elucidate the mechanisms of release, action of Ang-2 and its interplay with other mediators in patients with CKD.

Keywords: Chronic kidney disease, Endothelial dysfunction, Angiotensin.

Introduction:

Approximately 50% of individuals with end stage renal disease (ESRD) die from CV cause, a CV mortality that is 15 to 30 times higher than the age adjusted CV mortality in the general population.⁽¹⁾ This disparity is present across all ages, but it is most marked in the younger age group (25 to 34 years old), where the CV mortality is 500 fold greater in

ESRD patients compared with age matched controls with normal renal function.⁽²⁾ Studies showed that 40% of patients who have started dialysis treatments have evidence of CAD, and 85% of these patients have abnormal left ventricular structure and function.⁽³⁾ In fact, the majority of patients with stage 3 to 4 CKD die of CV causes rather than progress to ESRD.

A growing number of studies have demonstrated that the relationship between renal dysfunction and increased CV morbidity and mortality extends across the spectrum of renal dysfunction to encompass the mildest degrees of renal impairment.⁽⁴⁾

Both traditional and non-traditional risk factors have been implicated in the development of CVD in CKD.⁽⁵⁾ Traditional risk factors are those defined in the Framingham heart study and used to predict CAD outcomes in the general population. It includes DM, Hypertension, smoking, dyslipidemia, physical inactivity, and left ventricular hypertrophy.⁽⁶⁾ Non-traditional risk factors are uremia related factors that increase in prevalence or severity as kidney function declines and may contribute to the excess risk of CVD seen in CKD. Non-traditional risk factors include, anemia, albuminuria, abnormal calcium and phosphorus metabolism, sympathetic over activity, homocysteine, lipoprotein a, and chronic inflammation.⁽⁷⁾

Endothelial dysfunction and accelerated atherosclerosis are almost universal in CKD; impairment of kidney function is responsible for creation of atherogenic milieu. Retention of toxic substances and/or metabolic changes leads to increased oxidative stress and subclinical low grade inflammatory state. These changes result in endothelial dysfunction.⁽⁸⁾

Reduced nitric oxide synthesis due to the accumulation of the endogenous inhibitor of NOS, asymmetric dimethylarginine (ADMA) has been accused of accelerating progression of endothelial dysfunction in CKD.⁽⁹⁾ High levels of ADMA were reported in CKD and were associated with higher intima-media thickness and CV events.⁽¹⁰⁾

Angiotensins are protein growth factors that promote angiogenesis, there are four identified angiotensins: Ang-1, Ang-2, Ang-3, and Ang-4. Of them, Ang-1 and Ang-2 are the most studied. These ligands bind to the transmembrane receptor Tie 2 and possibly

Tie1, members of a family of receptor tyrosine kinases expressed primarily in vascular endothelium.⁽¹¹⁾ The angiotensins have been recognized mostly for their involvement in endothelial activation, angiogenesis and inflammation, the major processes which lie at the core of atherogenesis,⁽¹²⁾ and so; the angiotensins/Tie2 system has been identified as a potential new player in the pathogenesis of CKD associated atherosclerosis.⁽¹³⁾

Ang-2 was discovered by sequence homology to Ang-1, it is secreted by endothelial cells as it is stored in endothelial Weibel-Palade bodies from where it can be rapidly released upon stimulation. A broad range of factors has been reported to modulate Ang-2 expression, including hypoxia, vascular endothelial growth factor (VEGF), angiotensin II, and leptin.⁽¹⁴⁾ In contrast to the widespread expression of Ang-1, Ang-2 expression occurs in areas of endothelial activation and angiogenesis, for example, in ovaries and tumor vessel endothelia, where it coincides with vessel destabilization during angiogenesis.^(14,15)

Ang-1 and Ang-2 have opposing effects on receptor activation, while Ang-1 stimulates Tie2; Ang-2 is capable of antagonizing this effect. Genetic evidence verified that Ang-2 can counteract Ang-1 activity as Ang-2 over expression in transgenic mice leads to embryonic death with a phenotype similar to Ang-1 or Tie2 deletion.⁽¹⁴⁾ Signaling through Tie2 appears to depend on the balance between Ang-1 and Ang-2. Ang-1 has powerful vascular protective effects; it suppresses plasma leakage, inhibits vascular inflammation, and prevents endothelial death. In studies in which Ang-1 is directly administered or overexpressed, it leads to marked improvements in vascular integrity in both growing and adult mice. There is now strong evidence to support the concept that Ang-1 provides a paracrine constitutive tonic signal to promote quiescence of the

endothelium, and this is modified by the more actively regulated autocrine antagonistic signals from Ang-2.⁽¹⁶⁾

Ang-2 has been shown to be a key regulator of vascular inflammation. Ang2 is required for TNF- α induced monocyte adhesion to cultured human vascular endothelial cells and expression of ICAM-1 and VCAM-1. Mice deficient in Ang-2 have a markedly attenuated inflammatory response to staphylococcus aureus, and other stimuli and administration of Ang-2 reverses this.⁽¹³⁾

High serum Ang-2 was found in acute AKI and was a strong independent predictor of mortality.⁽¹⁷⁾ David S et al. showed that circulating Ang-2 is also markedly elevated in patients on maintenance dialysis and that Ang-2 is closely associated with the extent of CAD and PAD.⁽¹⁸⁾

Patients and methods:

This study was conducted on 90 subjects; they were subdivided into two groups: group A: included 75 patients from Alexandria Main University Hospital, they subdivided into five equal groups representing the five stages of CKD according to the K/DOQI guidelines (group AI, AII, AIII, AIV, and AV) and group B: included 15 apparently healthy nonsmoking subjects. They were matched with respect to age, gender, and body mass index (BMI) to group A. Smokers, patients having diabetes mellitus, CAD, PAD, systemic lupus erythematosus, vasculitis, and patients positive for hepatitis B surface antigen (HBsAg) or hepatitis C virus antibodies (HCV Abs) were excluded from the study. The study was conducted in accordance with ethical guidelines of the declaration of Helsinki and an informed consent was obtained from every subject.

All subjects were interviewed and subjected to thorough history taking, complete physical examination, and routine laboratory investigation, in the form of CBC, blood urea

and serum creatinine and estimated GFR using MDRD formula, liver functions tests, fasting and postprandial blood glucose, serum cholesterol and triglyceride, and serum calcium, phosphorus and intact parathyroid hormone

Determination of serum levels of angiotensin-2 was done using commercially available enzyme linked immunosorbent assay (ELISA) kit purchased from R&D systems (Quantikine, R&D Systems Inc., Minneapolis, USA; catalog number (Dang20)) following the manufacturer's instructions.

Ultrasound examination of abdomen and pelvis was done to assess kidney shape and size and any abnormalities consistent with kidney disease and to exclude presence of liver cirrhosis or fibrosis.

ECG and assessment of ankle: brachial pressure index (ABPI): was done for all participants in the study to detect signs of myocardial ischemia and to exclude PAD respectively.

Results:

The mean of age were 39.87 ± 10.46 , 37.00 ± 13.55 , 38.20 ± 9.99 , 41.67 ± 13.25 , 40.20 ± 12.65 and 38.20 ± 13.07 years for Group AI, II, III, IV, V and group B respectively, there were no statistical significant differences between the studied groups regarding age. ($P=0.919$).

There were no statistical significant differences between the studied groups regarding sex. ($P=0.197$).

The mean of BMI was 27.13 ± 1.74 , 26.97 ± 0.61 , 26.22 ± 1.21 , 26.31 ± 1.90 , 25.39 ± 2.12 and 26.15 ± 1.86 kg/m² for Group AI, II, III, IV, V and group B respectively, there were no statistical significant differences between the studied groups regarding BMI. ($P=0.068$).

Angiotensin-2 ranged between 290-660 pg/ml and 361-3222 pg/ml with the mean of

447.67111.63 pg/ml and 1510.9676.275 pg/ml for group B and group A respectively, patients group had values statistically higher than control group. (P=0.0001).

Angiotensin-2 ranged between 361-616, 490-867, 423-3184, 373-2542, 809-3222 and 290-660 pg/ml with the mean of 494.4771.85, 543.5361.57, 1722.33910.46, 1838.67744.33, 1939.07988.74 and 447.67111.63 pg/ml for group AI, II, III, IV, V and group B respectively, there were statistical significant differences between the studied groups regarding angiotensin-2 (P=0.001). Group AIII, AIV and AV had values statistically higher than group B

Table I showed the relation between angiotensin-2 levels and drugs received, it illustrated that: In those who were receiving vitamin D, serum angiotensin-2 ranged between 361 and 3200 pg/ml with a mean of 1468.2 701.3 pg/ml and in those who were not receiving vitamin D, it ranged between 380 and 3222 pg/ml with a mean of 1605.3 608.3 pg/ml, with no statistically significant difference between them and in those who were receiving erythropoietin, serum angiotensin-2 ranged between 366 and 3222

pg/ml with a mean of 1506.2 586.9 pg/ml and in those who were not receiving erythropoietin, it ranged between 361 and 3205 pg/ml with a mean of 1525.6 652.3 pg/ml, with no statistically significant difference between them.

Correlation analysis between serum levels of angiotensin-2 and different CV risk factors, demonstrate that serum angiotensin-2 levels have a statistically significant positive correlation with serum creatinine levels ($r = 0.54$, $p = 0.011$) and with serum phosphorus levels ($r = 0.490$, $p = 0.006$) while, they showed a statistically significant negative correlation with estimated GFR levels ($r = -0.632$, $p = 0.002$) as shown in figures III, IV, V respectively.

There was statistically insignificant positive correlation between Ang-2 and age, mean arterial pressure, intact parathyroid hormone, calcium phosphorus product, serum cholesterol a triglyceride. ($r = .112$, $P = .377$), ($r = .091$, $P = .496$), ($r = .131$, $P = .655$), ($r = .141$, $P = .624$), ($r = .030$, $P = .876$), and ($r = .012$, $P = .949$) respectively.

There was statistically insignificant negative correlation between Ang-2 and serum calcium ($r = -.168$, $P = .357$).

Table I: The relation between angiotensin-2 levels and drugs received.

	With treatment	Without treatment	p
Vit. D intake			
n	10	35	0.109(NS)
Range	361-3200	380 – 3222	
Mean	1468.2	1605.3	
S.D.	701.3	608.3	
Erythropoietin			
n	11	34	0.526(NS)
Range	366 – 3222	361 – 3205	
Mean	1506.2	1525.6	
S.D.	586.9	652.3	

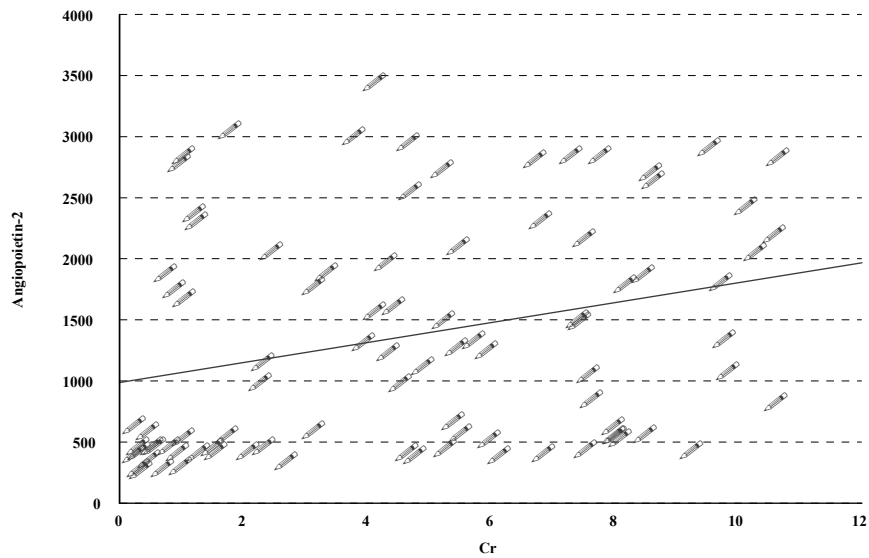


Figure (1): Correlation between serum Ang-2(pg/ml) and serum creatinine mg/dl.

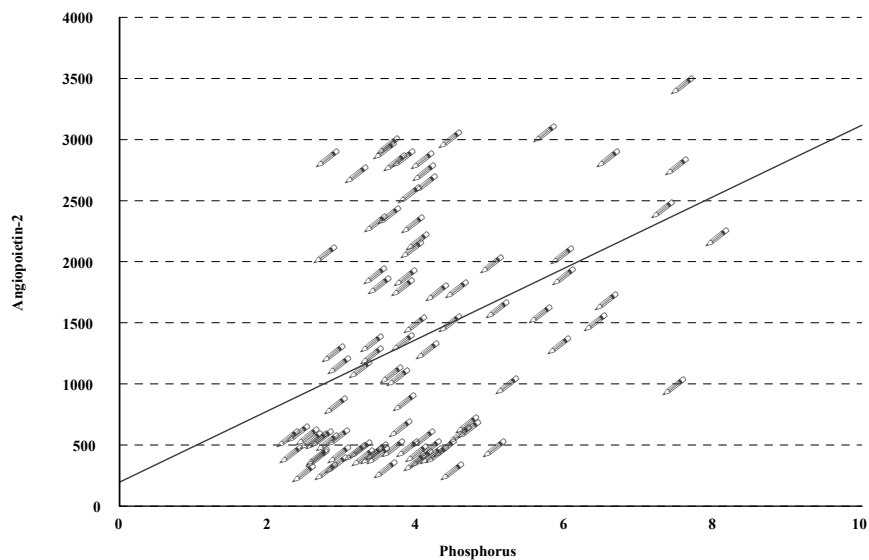


Figure (2): Correlation between circulating angiopoietin-2(pg/ml) levels and serum phosphorus (mg/dl).

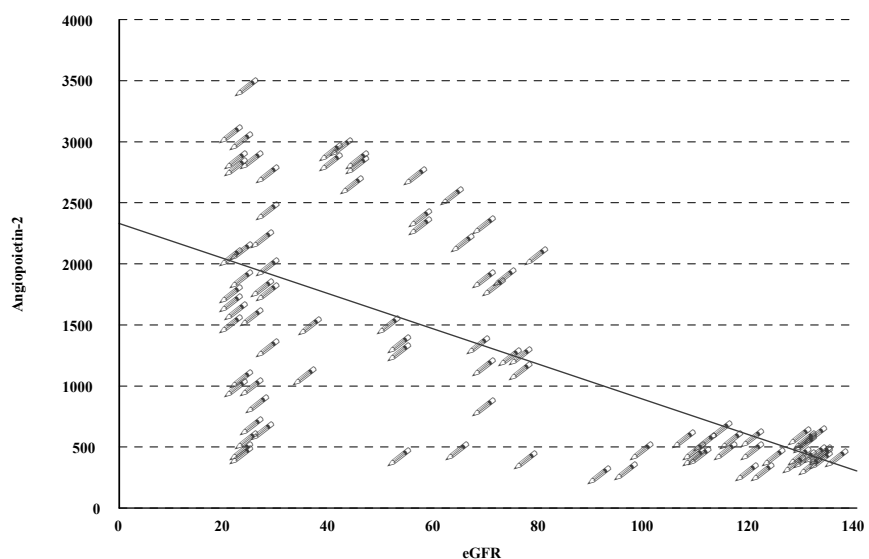


Figure (3):- Correlation between circulating angiopoietin-2 (pg/ml)levels and eGFR (ml/min).

Discussion:

Endothelial dysfunction and accelerated atherosclerosis are almost universal in CKD. Impairment of kidney function is responsible for creation of atherogenic milieu. Retention of toxic substances and/or metabolic changes leads to increased oxidative stress and subclinical low grade inflammatory state, these changes result in endothelial dysfunction.⁽⁸⁾

Angiotensins have been recognized mostly for their involvement in endothelial activation, angiogenesis and inflammation, the major processes which lie at the core of atherogenesis,⁽¹²⁾ and so the angiotensins/Tie2 system has been identified as a potential new player in the pathogenesis of CKD associated atherosclerosis.⁽¹³⁾

Elevated levels of Ang-2 have been found in arteriosclerosis,⁽¹⁹⁾ acute coronary syndrome,⁽²⁰⁾ PAD⁽²¹⁾ as well as acute and chronic CHF.⁽²²⁾ Patients with hypertension had higher concentrations of plasma Ang-2 that were predictive of MI.⁽²³⁾ In a study by Freestone B et al. they demonstrated that in patients with atrial fibrillation, the levels of plasma Ang-2 correlate with vWF, this finding points to the link between endothelial damage/dysfunction and Ang-2.⁽²⁴⁾ Increased concentrations of plasma Ang-2 were detected in patients with diabetes. Patients who received intensified diabetes management had a reduction of plasma Ang-2 if CVD was absent but not in the presence of CVD.⁽²⁵⁾ Atherosclerotic plaques with high microvessel density show higher concentrations of Ang-2 than Ang-1 and plaque Ang-2 levels are associated with higher concentrations of matrix metalloproteases (MMPs) which promote extracellular matrix degradation,⁽²⁶⁾ a phenotype associated with unstable and rupture prone plaques.⁽²⁷⁾

In the present study, serum levels of Ang-2 were significantly higher in patients with CKD compared to healthy controls. Indeed, Ang-2 levels steadily increase with the progression of CKD. The Ang-2 levels in patients with low

CKD stages (i.e. 1 and 2) were not statistically different from the Ang-2 levels in healthy controls. However, at higher stages (i.e. CKD 3–5), there was a significant increase in circulating Ang-2 compared to healthy controls. There was a strong negative correlation between the eGFR and the circulating Ang-2 levels and a strong positive correlation between serum creatinine and the circulating Ang-2 levels.

These findings are supported by previous results by Futrakul N et al. who found that the circulating levels of Ang-2 were increased in patients with mildly and moderately impaired renal function.⁽²⁸⁾

David S et al. studied the angiotensins levels in patients treated by means of dialysis and kidney transplantation, the association of altered angiotensins levels with atherosclerosis, and the changes in altered levels after renal transplantation. They found that, circulating Ang-2 level was increased in patients treated with dialysis and its level correlated significantly with scores of CAD and PAD. Indeed, Ang-2 levels have been normalized 3 months after kidney transplantation.⁽¹⁸⁾

In a succeeding study by David S et al. they showed that, serum Ang-2 levels steadily increase with progression of CKD as shown by significant positive and negative correlations with serum creatinine and GFR respectively. They further added power to the relation between CKD and Ang-2 by investigating the effect of sudden loss of GFR after unilateral nephrectomy for kidney donation on the serum Ang-2 levels. They detected a close inverse correlation between the mean changes (0–72 h) in Ang-2 level and the decrease in GFR.⁽²⁹⁾

In another study conducted by David S et al. who investigated the impact of Ang-2 level on the outcome in CKD, they prospectively studied 128 CKD patients [43 CKD stage 4, 85 CKD stage 5 (57 hemodialysis,

28 peritoneal dialysis)] over a follow-up period of 4 years. They found that, Ang-2 values were significantly higher in CKD patients than in controls. Furthermore, Ang-2 was significantly higher in dialysis than in stage 4 CKD patients and correlated with markers of vascular disease [cholesterol, high sensitivity CRP (hsCRP), osteoprotegerin (OPG)]. Moreover, Ang-2 was an independent predictor of mortality in both unadjusted and models adjusted for age and vascular calcification.⁽³⁰⁾

The relation between Ang-2 and AKI was studied by Kumpers P et al.; they measured circulating Ang-2 by ELISA in 117 critically ill patients with AKI at inception of renal replacement therapy (RRT) in the ICU. Mortality, length of stay and renal recovery were prospectively assessed during a study period of 28 days. They found that, the circulating Ang-2 levels were significantly higher in AKI patients with RIFLE category-Injury or Failure, compared to patients with RIFLE category-Risk. Elevated levels of circulating Ang-2 correlated with impaired oxygenation, low mean arterial pressure and vasopressor dose. Ang-2 concentrations were significantly higher in non-survivors than in survivors at day 0 and day 14 after initiation of RRT. There was a strong independent prognostic impact of elevated Ang-2 on patients 28 day survival.⁽¹⁷⁾

El-Banawy HS et al. showed that the Ang-2 levels were significantly increased in SLE patients than controls, and it was significantly higher in patients with lupus nephritis than in patients without. Ang-2 was significantly positively correlated with proteinuria and histological activity index, and was negatively correlated with C3, eGFR and FMD.⁽³¹⁾

Similarly, Kumpers P et al. showed that, Ang-2 levels were increased and Ang-1 levels were decreased in patients with active SLE compared to healthy controls and that, this tendency still present in inactive SLE, although

less pronounced. Ang-2 concentrations correlated well with SLE disease activity index (SLEDAI) score, proteinuria, double-stranded DNA (dsDNA) titre and soluble vascular cell adhesion molecule-1 (sVCAM-1). In this study, renal involvement was the only independent predictor for elevated Ang-2 level and serum Ang-2 was identified as a strong predictor for disease activity. Additionally, Protein expression of Ang-2 was upregulated in glomeruli of patients with lupus nephritis.⁽³²⁾

There are three theoretical possibilities for how Ang-2 levels increased in parallel to loss of renal function. First, this may occur due to reduced renal excretion of Ang-2 either due to reduced GFR or diminished tubular secretion with gradual accumulation of Ang-2 in the circulation but there are several observations that argue against this theory, purified recombinant Ang-2 protein exhibits predominant single bands of a molecular mass of 62 kDa. Furthermore, in vivo Ang-2 exists mainly as a multimeric protein; so, its glomerular excretion is rather unlikely.⁽³³⁾ Indeed, Ang-2 is neither detectable in urine from apparently healthy subjects⁽³⁴⁾ nor cleared by dialysis.^(17,35)

A second possibility is that, the damaged glomerular endothelium may over secrete Ang-2 into the circulation. This theory is supported by the finding that, in normal mature glomeruli, Ang-2 levels are low or undetectable^(36,37) but reported to be upregulated in certain disease models, including diabetic nephropathy⁽³⁸⁾ and glomerulonephritis.⁽³⁷⁾ Although such a scenario is more likely to occur in AKI, it would not also explain Ang-2 level raising after the unilateral nephrectomy without inducing kidney disease in the remaining healthy kidney described by David S et al.⁽²⁹⁾

A third possibility is that, CKD and the associated uraemic environment might trigger the release of Ang-2 from distant systemic endothelium via circulating uraemic toxins.

This theory may be supported by the decrease in serum Ang-2 level observed after correction of uremic environment by kidney transplantation in a study done by David S et al. ⁽¹⁸⁾

David S et al. found that in their cohort of CKD patients, that ADMA correlated significantly with circulating Ang-2. Also ADMA remained a significant predictor of Ang-2 after adjusting for GFR, serum phosphate and homocysteine in a multiple linear regression model.⁽²⁹⁾ ADMA is the most potent endogenous inhibitor of NO which starts to increase at the earlier CKD stages.⁽³⁹⁾ ADMA has been accused of accelerating progression of endothelial dysfunction in CKD.⁽⁹⁾ High levels of ADMA were associated with higher intima-media thickness and CV events in CKD.⁽¹⁰⁾ So, it is conceivable to assume that, the elevated Ang-2 levels in CKD patients might reflect excess exocytosis from Weibel palade body in which it is stored. This may be due to decreased NO bioavailability in the presence of high ADMA levels, as NO inhibit Ang-2 exocytosis from WPB.

In the present study, there was significant positive correlation between serum Ang-2 and serum phosphorus level, one of the non-traditional CV risk factors known to increase with loss of renal function due to secondary hyperparathyroidism, reduced GFR or hypocalcemia, this correlation was also supported by David S et al. ⁽²⁹⁾

In the present study, there was no significant correlation between Ang-2 level and any of the other tested CV risk factors including patients' age, iPTH, MAP, serum calcium, cholesterol or triglycerides. This may be explained by the effect of drugs that control or nearly control patients' blood pressure, elevate serum calcium to near normal values and malnutrition that may accompany CKD with associated negative impact on serum lipid. Furthermore, there was no detectable influence of erythropoietin or vitamin D treatment on circulating Ang-2 levels.

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