Angiopoietin-2 in chronic renal failure patients on hemodialysis: relationship with glomerular filtration rate in the predialysis stages

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Introduction
Cardiovascular disease has increased as a complication of chronic kidney disease even in the absence of diabetes or hypertension. Angiopoietin-1 and 2 are 55 kDa antagonistic nonredundant gatekeepers of endothelial activation and thus are potential important factors in accelerated atherosclerosis.

Aim of the study
The aim of the study was to determine angiopoietin-2 level in patients on hemodialysis (stage 5) and in the predialytic stages (stages 3 and 4) and to find the relationship between angiopoietin-2 levels and glomerular filtration rate in the predialytic stages.

Patient and methods
We prospectively studied 75 patients divided into three groups and 12 healthy controls. Group 1 included 33 patients on maintenance hemodialysis three times a week; group 2 included 21 patients with stage 3 chronic kidney disease; and group 3 included 21 patients with stage 4 chronic kidney disease.

Results
We found highly significant ($P < 0.01$) increase in mean serum angiopoietin-2 levels in all three groups compared with the control. The mean angiopoietin-2 in group 1 was 1669.09 ± 472.64 pg/ml, in group 2 was 1206.91 ± 154.26 pg/ml, in group 3 was 1642.24 ± 113.01 pg/ml, and in control was 476.29 ± 150.37 pg/ml. Furthermore, we found highly significant ($P < 0.01$) increase in group 1 compared with group 2 and group 3, and in group 3 compared with group 2. Our result revealed significant negative correlation of angiopoietin-2 level with estimated glomerular filtration rate in group 2 (r – 0.858, $P < 0.01$) and group 3 (r – 0.825, $P < 0.01$), with hemoglobin in group 1 (r – 0.438, $P < 0.01$), and with BMI (r – 0.468, $P < 0.05$) and cholesterol (r – 0.503, $P<0.05$) in group 3; significant positive correlation was observed with uric acid (r 0.456, $P < 0.05$) in group 3.

Conclusion
Circulating angiopoietin-2 is a putative marker and potential mediator of atherosclerosis, is inversely related to glomerular filtration rate, and is increased with advanced chronic kidney disease. Normolipidemia in chronic kidney disease patients does not prevent atherosclerotic burden; this is because of the presence of other markers such as angiopoietin-2.

Keywords:
chronic kidney disease, end-stage renal disease, glomerular filtration rate, serum angiopoietin-2

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Introduction

The global population with stage 5 chronic kidney disease (CKD) is estimated to have reached about 1.7 million and continues to grow at a significantly higher rate than the world population [1,2]. CKD patients are more likely to develop cardiovascular disease (CVD) and to die from it compared with individuals with normal kidney function [3]. Some new CV risk factors are more powerful, indicating CVD or endothelial dysfunction in CKD patients. Furthermore, some of these new markers [asymmetric dimethyl arginine, neuropeptide Y,visfatin, and angiopoietin-2 (Ang-2)] may also play an important role as mediators of CVD in CKD patients [4].

One of the earliest signs of CVD is endothelial damage and dysfunction, and this has been shown even in children with predialysis CKD. The potential causes of endothelial damage and aberrant repair are disturbances in growth factors involved in the formation of vascular networks [5].

Angiopoietin-1 (Ang-1) is a secreted growth factor that binds and activates Tie-2 receptor tyrosine kinase. The factor enhances endothelial cell survival and capillary morphogenesis and also limits capillary permeability. Ang-2 binds the same receptor but fails to activate it; hence, it is a natural inhibitor of Ang-1. Ang-2 destabilizes capillary integrity, facilitating sprouting when ambient vascular endothelial growth factor (VEGF) levels are high but causing vessel regression when VEGF levels are low. Tie-1 is a Tie-2 homolog

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but its ligand is unknown. Angiopoietin and Tie-1 genes are expressed in the mammalian metanephros, the precursor of the adult kidney, where they may play a role in endothelial precursor growth. During glomerular maturation, podocyte-derived Ang-1 and mesangial-cell-derived Ang-2 may affect the growth of nascent capillaries. After birth, vasa recta acquire their mature configuration and Ang-2 expressed by the descending limbs of the loop of Henle would be well-placed to affect the growth of this medullary microcirculation. Finally, preliminary data implicate angiopoietins in deregulated vessel growth, in Wilms kidney tumors, and in vascular remodeling after nephrotoxicity [6].

Concomitant occurrence of Ang-2 and other stimuli, such as tumor necrosis factor-α and angiogenic VEGF, will promote endothelial proliferation, facilitate angiogenesis, and induce inflammation. In the absence of VEGF, the endothelium switches back to the resting state, resulting in endothelial cell apoptosis and vascular regression. Elevated plasma Ang-2 has been shown in diseases with systemic inflammation, including diabetes mellitus, hypertension, congestive heart failure, acute coronary syndrome, peripheral arterial disease, critical illness, CKD, and end stage renal disease (ESRD) [7].

There are several potential mechanisms for increase in circulating Ang-2 in patients with CKD. The increase in Ang-2 may be the direct consequence of elevated blood pressure [8].

Korff and colleagues demonstrated that hypertension (HTN) in mice led to release of stored Ang-2 from Weibel–Palade bodies. There is also evidence that mediators of vascular tone, such as angiotensin II, can directly alter Ang-2 expression. A lack of endothelial nitric oxide may also predispose to a release of Weibel–Palade bodies, which would theoretically increase Ang-2 levels. One potential factor that could bring these various mechanisms together is uric acid. Urate is retained in CKD and found to correlate with the Ang-2 levels in dialysis patients [9].

Estimated glomerular filtration rate (eGFR) is calculated from the formula that adjusts the creatinine for age, sex, and race. The most widely used is the modification of diet in renal disease (MDRD) equation, as it appears to be most reliable and reproducible in individual patients. Normal glomerular filtration rate (GFR) is 100 ml/min/1.73 m^2; hence, eGFR roughly gives a percentage kidney function. The CKD stages 1–5 are based on eGFR [10].

Aim of this study

This study was designed to determine serum Ang-2 level in patients on hemodialysis and to find its relationship with glomerular filtration rate in the predialysis stages (stages 3 and 4 of CKD).

Patients and methods

A total of 75 patients (31 female and 44 male) were collected from Al-Zahraa university hospital and divided into three groups along with 12 controls (10 female and two male). Group 1 included 33 patients (12 female and 21 male) with ESRD (stage 5 CKD) on maintenance hemodialysis, group 2 included 21 patients (10 female and 11 male) with stage 3 CKD, and group 3 included 21 patients (nine female and 12 male) with stage 4 CKD. Patients with infection, malignancy, systemic lupus erythematosus (SLE), vasculitis, and peripheral arterial disease (PAD) were excluded. All patients were matched with respect to age, sex, and BMI. Detailed history taking and thorough physical examination, including ECG, were carried out to exclude patients with coronary heart disease. All patients were informed about the procedure and verbal consent was taken. Approval of the ethical committee was also obtained.

A volume of 5 ml of fasting (12–16 h) venous blood samples were drawn from each individual participating in the study and divided into two parts: the first part (2 ml) was added to a tube containing EDTA for hemoglobin determination using a Coulter Counter T 890 (Coulter Counter, Harpenden, UK) and the second part was transferred to a plain tube and left to clot. The serum was separated by centrifugation at 3000*g* (it is the force calculated on the basis of the given rotor speed and given rotor radius of a centrifuge. It is reported as *g*) for 5 min, and fasting blood glucose was immediately determined by colorimetric technique using Hitachi 912 autoanalyzer (Roche Diagnostics, Mannheim, Germany). The rest of the serum was stored at −20°C for determination of the following Urea, creatinine, calcium, phosphorous, uric acid, total cholesterol, and triglyceride levels were determined by colorimetric techniques using Hitachi 912 autoanalyzer (Roche Diagnostics), sodium and potassium levels were determined by ion selective electrodes using Hitachi 912 autoanalyzer (Roche Diagnostics), and Ang-2 levels were also determined.

For determination of HDL-cholesterol, phosphotungstic acid and magnesium ions were used for precipitating all lipoproteins except HDL fraction that was present in the supernatant and measured using Hitachi 912 autoanalyzer. LDL-cholesterol was measured by the Friedewald formula [11].

Two hours after meal, 2 ml of blood was drawn from each participant of the study and added to a tube containing fluoride for determination of post prandial blood glucose (PPBG) by colorimetric kits using Hitachi 912 autoanalyzer.

The determination of serum Ang-2 was performed using quantitative sandwich enzyme immunoassay technique [12]. The ELISA kit was supplied from R&D Systems (Minneapolis, Minnesota, USA).

Estimated glomerular filtration rate using the modification of diet in renal disease formula

The most recently advocated formula for calculating GFR is the one that was developed by the Modification of Diet in Renal Disease Study Group. Most laboratories in Australia and UK now calculate and report the MDRD estimated GFR along with creatinine measurements, and this forms the basis of CKD and staging. The adoption of the automatic reporting of MDRD-eGFR has been widely criticized [13].

The most commonly used formula is the '4-variable MDRD,' which estimates GFR using four variables: serum creatinine, age, race, and sex. The original MDRD used six variables with the additional variables being the blood urea nitrogen (BUN) and albumin levels. The equations have been validated in patients with CKD; however, both versions underestimate the GFR in healthy patients with GFRs over 60 ml/min. The equations have not been validated in acute renal failure [14].

For creatinine in mg/dl:

eGFR = 186 × serum creatinine $^{-1.154}$ × age $^{-0.203}$×) 1.212 (if black) × (0.742 if female).

Creatinine levels in μmol/l can be converted to mg/dl by dividing them by 88.4. The 32 788 number above is equal to 186 × $88.4^{1.154}$.

A more elaborate version of the MDRD equation also includes serum albumin and BUN levels:

eGFR = 170 × serum creatinine$^{-0.999}$× age $^{-0.176}$ × (0.762 if female) × (1.180 if black) BUN$^{-0.170}$ × albumin$^{+0.170}$

where the creatinine and BUN concentrations are both in mg/dl. The albumin concentration is in g/dl.

These MDRD equations are to be used only if the laboratory has not calibrated its serum creatinine measurements to isotope dilution mass spectrometry. When isotope dilution mass spectrometry-calibrated serum creatinine is used (which is about 6% lower), the above equations should be multiplied by 175/186 or by 0.94086. As these formulae do not adjust for body mass, they (relative to the Cockcroft–Gault formula) underestimate eGFR for heavy individuals and overestimate it for underweight individuals [15].

Statistical analysis

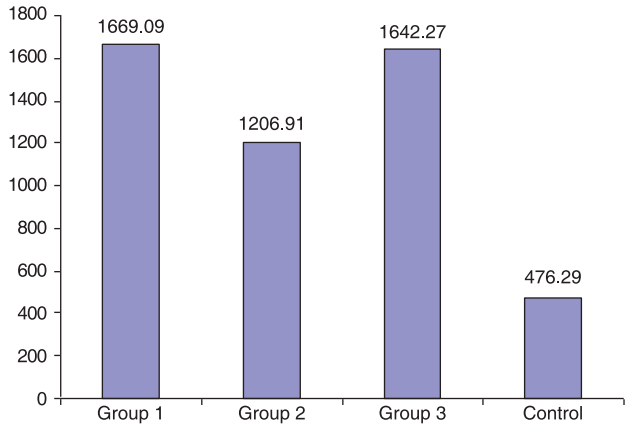
Data were analyzed by Microsoft Office 2003 (excel) and statistical package for social science, version 16 (SPSS Inc., 233 South Wacker). Parametric data were expressed as mean ± SD and nonparametric data were expressed as number and percentage of the total. Comparison of mean ± SD of two groups was carried out using unpaired Student's *t*-test. Measurement of the mutual correspondence between two values was carried out using correlation coefficient.

P value greater than 0.05 was considered nonsignificant, *P* value less than 0.05 was considered significant, and *P* value less than 0.01 was considered highly significant.

Results

In our study, we found highly significant increase in serum Ang-2 level in all three groups compared with control ($P < 0.01$) and highly significant increase in serum Ang-2 level in group 1 compared with group 2 ($P < 0.01$). In addition, we found highly significant increase in serum Ang-2 in group 3 (stage 4 CKD) compared with group 2 (stage 3 CKD) ($P < 0.01$) and highly significant increase in group 1 (stage 5 CKD) compared with group 3 (stage 4 CKD) ($P < 0.01$) (Table 1 and Fig. 1).

Figure 1



Highly significant increase in mean serum Ang-2 level in all three groups compared with the control, in G1 compared with G2, and in G3 compared with G2 ($P < 0.01$). Ang-2, angiopoietin-2; G1, group 1; G2, group 2; G3, group 3.

Correlation of Ang-2 levels with different clinical and laboratory parameters in the three patient groups was determined, revealing the following. In group 1, there was highly significant negative correlation of Ang-2 with Hb level (r – 0.438, P < 0.01) (Tables 2 and 3 and Fig. 2). Group 2 correlation revealed highly & (Fig. 3).

In group 3, there was significant negative correlation of Ang-2 level with BMI (r – 0.468, P < 0.05) and cholesterol (r – 0.503, P < 0.05) (Table 3 and Fig. 4) and positive correlation with serum uric acid (r 0.456, P < 0.01) (Table 3 and Fig. 5). In addition, there was highly significant negative correlation with eGFR (r – 0.825, P < 0.01) (Table 3 and Fig. 6).

Table 1 The mean ± SD of serum angiopoietin-2 level (pg/ml) in the three patient groups compared with the control group and in-between the groups

Parameters	Groups				
	Group 1	Group 2	Group 3	Control	*P*
Angiopoietin (pg/ml)	1669.09 ± 472.64	1206.91 ± 154.26	1642.27 ± 113.01	476.29 ± 150	P_1<0.01P_2<0.01P_3<0.01P_4<0.01

P > 0.5 is statistically nonsignificant; P < 0.05 is statistically significant; P < 0.01 is statistically highly significant; P_1 = patient groups versus control; P_2 = group 1 versus group 2; P_3 = group 3 versus group 2; P_4 = group 1 versus group 3.

Table 2 Clinical and laboratory findings of the three patient groups and the control group

Parameters	Group 1	Group 2	Group 3	Control
Number of patients	33	22	22	15
Sex (M/F)	21/12	11/10	12/9	12/2
Age (years)	51.24 ± 11.64	46.45 ± 11.99	50.14 ± 11.36	45.23 ± 11.35
Duration of dialysis (years)	5.59 ± 3.57	–	–	–
Hemoglobin (gm/dl)	10.05 ± 1.93	9.91 ± 0.97	9.81 ± 0.65	13.23 ± 0.81
BMI (kg/m^2)	24.37 ± 2.96	23.73 ± 2.73	23.02 ± 2.96	23.54 ± 3.32
Blood urea (mg/dl)	143.2 ± s26.91	116.77 ± 39.99	163.64 ± 27.08	28.00 ± 7.19
Serum creatinine (mg/dl)	8.94 ± 2.97	6.26 ± 1.14	8.30 ± 2.80	1.01 ± 0.13
Na (mEq/l)	138.68 ± 4.30	129.45 ± 2.58	131.00 ± 3.82	146.67 ± 3.70
K (mEq/l)	6.03 ± 1.29	6.52 ± 0.71	6.81 ± 0.50	4.02 ± 0.39
Ca (mg/dl)	8.17 ± 1.03	7.82 ± 0.88	7.72 ± 0.79	7.72 ± 0.79
P (mg/dl)	5.52 ± 1.43	6.64 ± 0.94	6.95 ± 0.82	2.91 ± 0.15
Cholesterol (mg/dl)	155.9 ± 39.31	169.7 ± 15.96	160.59 ± 21.88	112.47 ± 28.76
TG (mg/dl)	173.18 ± 89.26	161.95 ± 30.44	140.09 ± 17.00	76.87 ± 16.66
HDL (mg/dl)	66.68 ± 16.13	59.22 ± 12.43	53.87 ± 8.09	72.032 ± 15.21
LDL (mg/dl)	87.85 ± 29.67	90.46 ± 19.36	97.23 ± 21.32	44.07 ± 5.74
Albumin (g/dl)	3.36 ± 0.35	3.2 ± 0.33	3.0 ± 0.30	4.41 ± 0.47
Serum uric acid	7.03 ± 1.37	7.42 ± 0.88	8.98 ± 0.94	4.24 ± 0.57
eGFR (ml/mi/1.73 m^2)	–	45.8 ± 7.72	22.93 ± 3.81	–

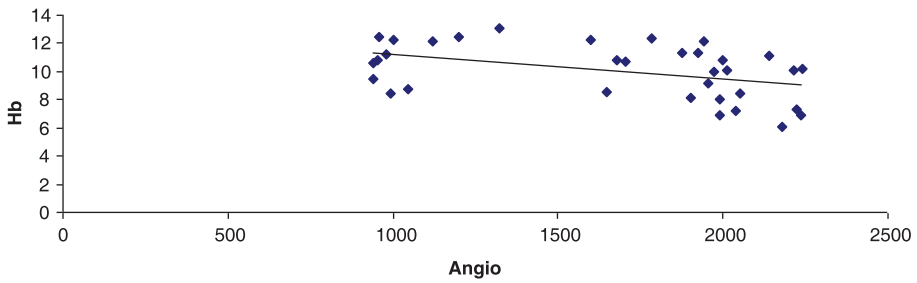
eGFR, estimated glomerular filtration rate; F, female; M, male; TG, triglycerides.

Table 3 Correlation of serum angiopoietin-2 level with different clinical and laboratory parameters in different patient groups

	Group 1		Group 2		Group 3	
	r value	*P* value	*r* value	*P* value	*r* value	*P* value
Age	0.209	>0.05	–0.077	>0.05	0.268	>0.05
BMI	0.124	>0.05	0.111	>0.05	–0.468	<0.05
Hemoglobin	–0.438	<0.01	–0.094	>0.05	–0.098	>0.05
Blood urea	0.020	>0.05	–0.220	>0.05	–0.264	>0.05
Serum creatinine	0.073	>0.05	–0.348	>0.05	–0.133	>0.05
Albumin	–0.267	>0.05	–0.129	>0.05	0.232	>0.05
TG	0.237	>0.05	–0.169	>0.05	–0.261	>0.05
Cholesterol	–0.242	>0.05	0.051	>0.05	–0.503	<0.05
Ca	–0.001	>0.05	–0.171	>0.05	0.122	>0.05
P	0.155	>0.05	0.251	>0.05	0.113	>0.05
Na	–0.255	>0.05	–0.306	>0.05	0.131	>0.05
K	0.214	>0.05	0.207	>0.05	–0.046	>0.05
UA	0.310	>0.05	0.078	>0.05	0.456	<0.05
eGFR	–	–	–0.858	<0.01	–0.825	<0.01

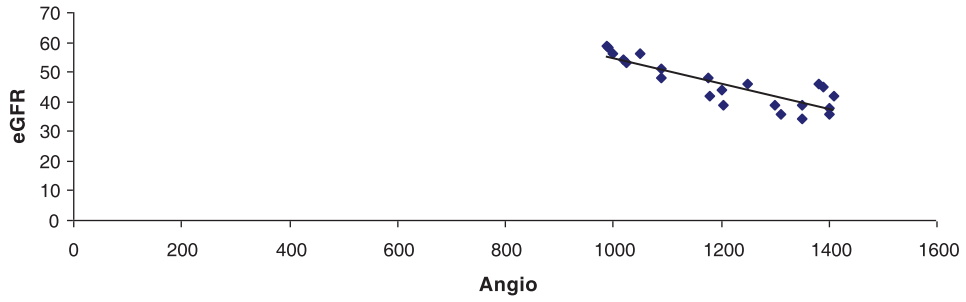
eGFR, estimated glomerular filtration rate; TG, triglycerides.

Figure 2



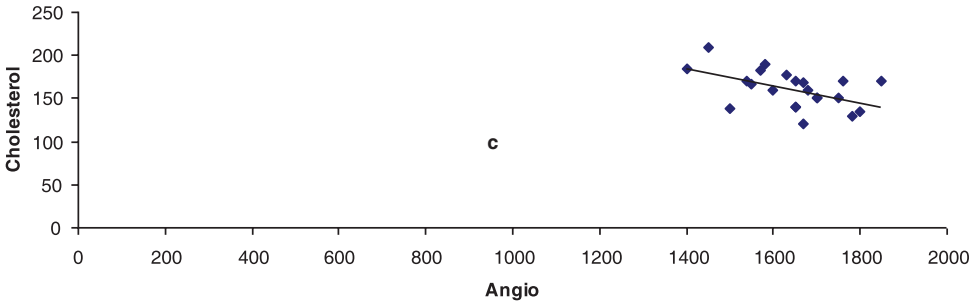
Negative correlation of serum Ang-2 level with hemoglobin level in group 1 patients (on hemodialysis) (r – 0.438, P < 0.01). Ang-2, angiopoietin-2.

Figure 3



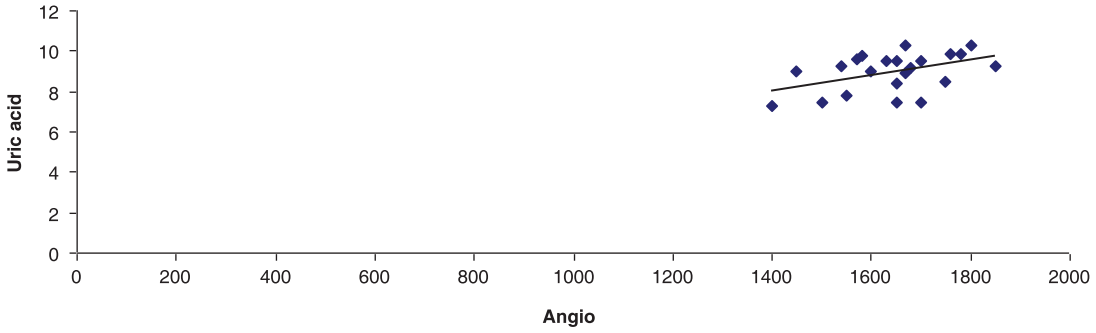
Negative correlation of serum Ang-2 level with eGFR in group 2 patients (stage 3 CKD) (r – 0.858, P < 0.01). Ang-2, angiopoietin-2; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate.

Figure 4



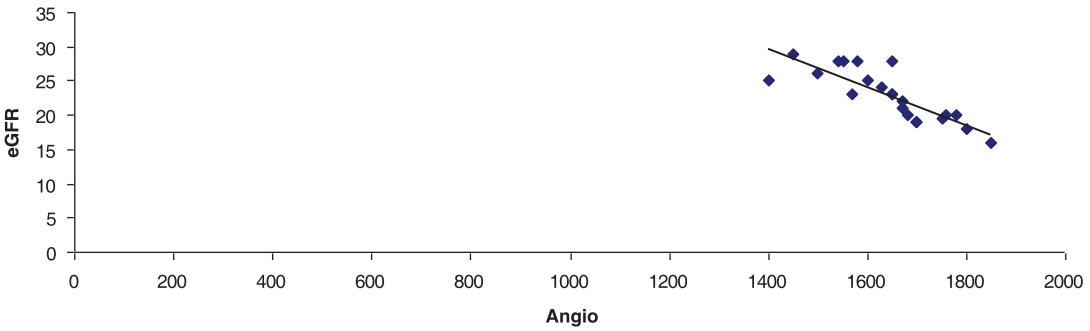
Significant negative correlation of serum Ang-2 level with cholesterol in group 3 patients (stage 4 CKD) (r – 0.503, P < 0.05). Ang-2, angiopoietin-2; CKD, chronic kidney disease.

Figure 5



Significant positive correlation of serum Ang-2 level with serum uric acid in group 3 patients (stage 4 CKD) (r 0.456, P < 0.01). Ang-2, angiopoietin-2; CKD, chronic kidney disease.

Figure 6



Highly significant negative correlation of serum Ang-2 level with eGFR in group 3 patients (stage 4 CKD) (r 0.825, P < 0.01). Ang-2, angiopoietin-2; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate.

Discussion

Our study demonstrated that circulating serum Ang-2 levels were markedly elevated in dialysis patients compared with healthy controls and predialysis CKD individuals. The observation that circulating Ang-2 is also elevated in children on dialysis suggests that uremic environment may directly influence vascular growth factor expression; this is because children do not have many of the cardiovascular comorbidities that are commonly seen in adults. In addition, the pathophysiology of CVD in children may be different to that found in adults [16].

Shroff *et al.* [8] found that elevation in circulating serum or plasma Ang-2 levels was similar immediately before and after hemodialysis session. Both Ang-1 and Ang-2 form multimeric structures composed of 55 kDa, and therefore are unlikely to be affected by dialysis clearance [17].

As previously mentioned, we found an elevation in serum Ang-2 level in predialysis CKD patients compared with healthy controls. At this point, our study does not agree with the study by Shroff *et al.* [8] who did not detect different serum or plasma Ang-2 levels in predialysis children compared with healthy controls. One explanation for this discrepancy could be that the children under study had not been exposed to diabetes mellitus (DM) and that dyslipidemia and hypertension were less common in children than in adult with CKD. Indeed, each of these factors has been shown to be associated with elevated Ang-2 [18]; however, children with predialysis CKD had decreased circulating Ang-1 compared with healthy controls. This loss of Ang-1 in predialysis CKD children may decrease the blood vessels stability and could be an early sign of the endothelial dysfunction, which occurs in these patients [19].

In our study, we found negative correlation between eGFR and serum Ang-2 levels in stages 3 and 4 CKD (predialysis); this was in agreement with the study by Chang *et al.* [20] who found an inverse correlation between eGFR and serum Ang-2 levels in moderate to severe CKD patients. Interestingly, the Ang-2 elevation first became evident in patients with a GFR less than 60 ml/min/1.73 m^2 and was normalized after successful kidney transplantation [21].

There are three theoretical possibilities for how the Ang-2 homeostasis could be influenced by the kidney.

First possibility is reduced excretion of Ang-2 by the kidney (Ang-2 exists mainly as a multimeric protein *in vivo*); thus its excretion is rather unlikely. Ang-2 is neither detectable in urine of apparently healthy individuals (unpublished data) nor cleared by dialysis. These observations argue against glomerular filtration or tubular secretion as physiologic routes for Ang-2 clearance from the circulation [22].

Second possibility is Ang-2 release by the impaired kidney. The kidney endothelium itself has been identified as a rich source of Ang-2; hence, chronic organ impairment might directly result in increased Ang-2 release from the kidney [23].

Third possibility is CKD-related indirect release of systemic endothelial Ang-2. CKD and the associated uremia might trigger the release of Ang-2 from distant systemic endothelium through circulating uremic toxins. It is conceivable to assume that elevated Ang-2 levels in CKD patients might reflect excess WPB exocytosis as a consequence of decreased nitric oxide bioavailability in the presence of high asymmetric dimethyl arginine (NO synthase inhibitors) levels [22].

In our study, Ang-2 levels positively correlated with urate levels in predialysis patients (stage 4 CKD); this was in agreement with the study by Shroff *et al.* [8], who hypothesized that elevated urate might increase

Ang-2 expression by releasing it from endothelial and/or vascular smooth muscle cells.

Elevated Ang-2 levels in dialysis patients compared with predialysis CKD patients were also associated with an antiangiogenic and proinflammatory (high urate, E-selectin) milieu. Serum urate correlated with Ang-2 levels in dialysis patients, and addition of uric acid was able to induce rapid release of Ang-2 from the cultured endothelial cells. Thus, Ang-2 is a marker for CVD in children on chronic dialysis and may act as antiangiogenic and proinflammatory effector in this context. The possibility that the release of Ang-2 from the endothelial cells is mediated by urates should be confirmed [24].

Kuo and colleagues showed that uric acid could directly induce the release of Ang-2 with corresponding decrease in mRNA abundance within the cells. In addition, there is an increasing evidence that urate may have a role in hypertension effects, which include inducing endothelial dysfunction, oxidative stress, and the production of angiotensin II. These findings might account for how urate can contribute to cardiovascular complications. In conclusion, Ang-2 acts as antiangiogenic and proinflammatory effector in this context [25].

Conclusion

Circulating Ang-2 is a putative marker and potential mediator of atherosclerosis, is inversely related to GFR, and is increased with advanced CKD. Normolipidemia in CKD patients does not prevent atherosclerotic burden; this is because of the presence of another markers such as Ang-2.

Acknowledgements

Conflicts of interest

There are no conflicts of interest.

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