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Assessing Vascular Biomarkers and Their Association with Hypertension in Paediatric Chronic Kidney Disease

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Abstract

Objectives: This study aims to examine the correlation between children with chronic kidney disease (CKD) and endothelial dysfunction biomarkers, including asymmetric dimethylarginine (ADMA), angiopoietin-2 (Ang-2), and vascular endothelial growth factor-A (VEGF-A), as well as blood pressure and cardiovascular risk. ***Methods***: A cross-sectional study included 90 children divided into two groups: 45 with CKD with different stages and 45 healthy controls. Blood pressure was taken, and levels of ADMA, Ang-2, and VEGF-A were determined. November 2021 to October 2022 was the time frame of the study. ***Results***: The results showed that children with CKD had significantly higher levels of ADMA, Ang-2, and VEFG-A compared to the control group. Systolic and diastolic blood pressure showed a positive connection with the elevated biomarkers but estimated glomerular filtration rate (eGFR) showed a negative association. ***Conclusion***: Increased cardiovascular risk and antihypertensive have been associated with elevated levels of VEFG-A, Angiopoietin-2, and ADMA in children with chronic kidney disease. These biomarkers might be useful in clinical evaluations to improve therapy and results for children with CKD.

Keywords: Chronic Kidney Disease in Children, Hypertension, Endothelial Dysfunction, Vascular Biomarkers, Cardiovascular Risk

Advances to Knowledge

- Children with chronic kidney disease (CKD) had significantly higher levels of asymmetric dimethylarginine (ADMA), angiopoietin-2 (Ang-2), and vascular endothelial growth factor-A (VEGF-A) compared to healthy controls.
- There was a positive correlation between systolic and diastolic blood pressure and elevated biomarkers (ADMA, Ang-2, and VEGF-A) in children with CKD.
- Estimated glomerular filtration rate (eGFR) was negatively associated with ADMA, Ang-2, and VEGF-A levels.
- Elevated levels of these biomarkers (ADMA, Ang-2, and VEGF-A) were associated with increased cardiovascular risk and hypertension in pediatric CKD patients.
- These biomarkers could be useful tools in clinical evaluations to improve therapy and outcomes for children with CKD.

Application on Patient Care

- Early Detection and Monitoring: Implement routine screening of ADMA, Ang-2, and VEGF-A levels in pediatric CKD patients to identify those at higher risk of developing hypertension and cardiovascular complications early on.
- Personalized Treatment Plans: Use biomarker levels to tailor treatment plans specific to each patient's risk profile, ensuring more targeted and effective interventions.
- Enhanced Blood Pressure Management: Monitor vascular biomarkers alongside traditional blood pressure measurements to provide a more comprehensive approach to managing hypertension in pediatric CKD patients.
- Risk Stratification: Utilize biomarker data to categorize patients into different risk levels, allowing for prioritized and intensive care for those at higher risk of cardiovascular events.
- Improved Prognostic Accuracy: Incorporate these biomarkers into prognostic models to better predict disease progression and potential cardiovascular outcomes, enabling more informed clinical decisions.
- Policy Integration: Develop and update clinical guidelines to include the assessment of ADMA, Ang-2, and VEGF-A, ensuring standardized care practices across healthcare facilities.

- Longitudinal Patient Follow-Up: Establish regular follow-up schedules to monitor changes in biomarker levels over time, adjusting treatment strategies as needed to prevent the progression of CKD and associated cardiovascular risks.

Introduction

Over the last three decades, paediatric CKD care has improved, but a small percentage of children with CKD develop end-stage kidney disease (ESKD) and need Kidney Replacement Therapy (KRT).[1] Hypertension significantly correlates with subclinical cardiovascular diseases (CVD) in CKD children.[2] Hidden hypertension contributes to developing left ventricular hypertrophy (LVH) in this population. [3] Children with CKD need early hypertension identification and treatment to improve renal and cardiovascular outcomes.[3]

An imbalance between reactive oxygen species (ROS) and antioxidant defences causes oxidative stress in children with CKD, which worsens the disease and increases the risk of inflammation, hypertension, and atherosclerosis.[4] An oxidative imbalance reduces nitric oxide (NO) bioavailability, a sign of endothelial dysfunction.[4]

Endothelial dysfunction is an early marker of atherosclerosis, detectable in initial CKD stages and worsening as the disease progresses to ESKD. The progressive decline in endothelial function is linked to heightened cardiovascular mortality.[5]

ADMA is an intrinsic nitric oxide synthase inhibitor. It causes endothelial dysfunction and vasoconstriction, which accelerates CKD by inhibiting NO production and increases cardiovascular risk and death.[6]

VEGF-A functions as a crucial regulator of angiogenesis and plays a role in the pathophysiology of microangiopathic processes in CKD**.** [7] Also, angiopoietin- (Ang-2) increases endothelial cell permeability and contributes to the destabilization of vascular structures by interacting with activated integrin β1.[8]

Numerous biomarkers in CKD provide insight into both kidney pathology and CV risk. These biomarkers facilitate non-invasive assessments of vascular function and serve as surrogate indicators of cardiovascular (CV) outcomes. However, only a select few have

been investigated in paediatric CKD populations, particularly concerning hypertension and CV risk.

Our study aimed to explore the association between specific biomarkers indicative of endothelial dysfunction, renal injury, inflammation, oxidative stress, and their pathophysiological roles in cardiovascular disease, as evidenced by systemic hypertension, in pediatric chronic kidney disease.

Methods

This is a case-controlled study that included 90 children. The study included 45 pediatric patients, aged 8 to 18 years, diagnosed with chronic kidney disease (CKD). Among these patients, 25 were receiving regular hemodialysis, and none were on peritoneal dialysis, while the remaining participants were at various stages of CKD. The control group consisted of 45 age- and sex-matched healthy children.

A stratified random sampling method was employed to ensure a representative distribution across different CKD stages. The study was conducted over a period from November 2021 to October 2022

For sample size calculation, the power analysis was performed using G power software using the correlation between VEGF and blood pressure, the criteria for significance were set at 0.05(type I error), and total power of 80%, the correlation coefficient between VEGF and blood pressure was 0.28 and effect size was set at 0.28 the total sample size required was 90.[9]

Ethical Consideration:

The "Faculty of Medicine, local Research Ethical Committee (FMREC), Minia University" approved the study. All study participants obtained written consent for the use of their data. The study followed the principles of the Helsinki Declaration.

Inclusion criteria:

All children with different causes and stages of CKD.

Exclusion criteria:

Children identified with hypertension, whether primary or due to secondary causes other than renal origins.

Pediatric evaluation:

Cases and controls arose from the outpatient clinics of pediatric nephrology in the hospitals of El-Minia University and the general outpatient pediatric clinic at Minia University, Egypt. All of them underwent detailed history taking and general physical examination to exclude the presence of any systemic diseases and to confirm their eligibility for the study.

Blood pressure (BP) measurement:

Blood pressure (BP) measurements were taken using either mercury sphygmomanometers or aneroid auscultatory devices, with a maximum error margin of ±1 mmHg. To guarantee accuracy, these devices were periodically calibrated against a reference standard device. The study included only devices that met validation criteria, including a mean blood pressure difference of ≤5 mmHg and a standard deviation (SD) of ≤8 mmHg.[10]

Blood pressure was measured in a quiet environment after at least 5 minutes of rest for the child. The measurements were obtained with the child seated, back supported, feet flat on the ground, and arm supported at heart level. The proper cuff size was chosen based on the child's mid-upper arm circumference, ensuring that the cuff covered 75-100% of the arm circumference with a width of 37-50% of the circumference to achieve reliable readings.[10]

Three successive blood pressure readings were recorded at 1–2-minute intervals. The average of these three readings was used to calculate the final BP for each visit. All measures were obtained from the right arm, with repeated readings taken from the same arm to ensure consistency.[10]

Systolic (SBP) and diastolic (DBP) blood pressures were normalized using age, gender, and height percentiles as per the Fourth Report on the Diagnosis, Evaluation, and Treatment of High Blood Pressure in Children and Adolescents.[11] Normalized blood

pressure results were provided as z-scores and percentiles to help identify hypertensive children who may need antihypertensive medication.

Laboratory investigation:

Routine investigations were: Renal function tests**,** Lipid profile**,** Albumin/creatine ratio in urine**,** and Serum albumin**.** They were all assessed by a fully automated autoanalyzer system **(Selectra pro-XI 16-8361, Eli Tech Group Systems, Germany).** Also, Serum vitamin D was measured by enzyme-linked immunosorbent assay using an available commercial kit (Pelobiotech GmbH, Planegg, Germany)**.**

Special investigations were Angiopoietin 2, Asymmetric dimethyl arginine (ADMA), and Vascular Endothelial Growth Factor -A (VEGF-A). ***Kits supplied by BT LAB bioassay technology, assessment by EIA using Humareader 451572, Germany***

The formula used for the calculation of e-GFR (Original Schwartz Equation):

eGFR = k x (height in cm) ÷ serum Cr

Statistical analysis: Data were analyzed through IBM SPSS (Statistical Package for Social Science) software, version 26.0 with the help of IBM, which is based in Armonk, New York, USA. Data were expressed in mean±SD, min, and max for quantitative, in addition to number and percentage for categorized data. The comparison of the mean values for two independent groups was done by the t-test, in the case of parametric data, or the Mann-Whitney U test in the case of non-parametric data. The test was chosen between the Chi-square test or Fisher's exact test for comparing categorical variables. The analysis of correlations between the parameters was performed using the Pearson correlation analysis. Significance was considered at p value<0.05.

Results

The study compared 45 pediatric patients with chronic kidney disease (CKD) to 45 healthy children, examining various demographic and clinical parameters. Among the CKD patients, 30% had Congenital Anomalies of the Kidney and Urinary Tract (CAKUT), while 70% had glomerulopathies (Table 1).

The two groups were of similar age, with no significant difference ($p = 0.85$). However, the CKD group had significantly lower height, weight, and BMI compared to the controls (all $p < 0.001$). Blood pressure was higher in the CKD group, with both systolic and diastolic values significantly elevated compared to the control group ($p = 0.04$ and $p = 0.03$, respectively) (Table 1).

Laboratory parameters showed that CKD patients had lower serum albumin levels and higher urea, and creatinine levels compared to the control group (all $p < 0.001$). The estimated Glomerular Filtration Rate (e-GFR) was significantly lower in the CKD group ($p < 0.001$), with most CKD patients in advanced stages of kidney disease, while all control subjects were in the early stage ($p < 0.001$) (Table 2).

Vitamin D levels were significantly lower in the CKD group, with more patients showing deficiency or insufficiency compared to the controls ($p = 0.002$). Also, Vascular markers, including ADMA, VEGF, and Angiopoietin2, were significantly elevated in CKD patients (all $p < 0.001$) (Table 2).

There were strong inverse correlations between e-GFR and these vascular markers in CKD patients ($r = -0.81$ to -0.82, $p < 0.001$), while correlations were weak and not significant in the control group (Table 3).

Additionally, strong positive correlations were found between vascular markers and blood pressure in CKD patients ($r = 0.74$ to 0.88, $p < 0.001$), which were weaker or absent in the control group (Table 4).

Discussion

Children with CKD, particularly those receiving dialysis, have considerable cardiovascular risks and fatality rates according to recent studies,.[12]

Regarding anthropometric measures, our study found a statistically significant reduction in height, weight, and BMI in children with CKD compared to healthy children, which is consistent with previous research highlighting similar growth deficits in this population.[13] Additionally, we observed that children with CKD had significantly higher blood pressure readings than their healthy peers, as previously explained.[14]

Our study identified several standard laboratory findings commonly observed in children with CKD. We found that serum albumin levels were significantly lower in children with CKD, aligning with the findings of Alves et al.15, who noted that hypoalbuminemia frequently accompanies CKD and is linked to systemic inflammation, which negatively impacts survival rates. [15] Additionally, our study observed that blood vitamin D levels were notably lower in children with CKD, consistent with the research of Kari et al., which demonstrated a higher likelihood of vitamin D insufficiency or deficiency in this population compared to healthy children.[16]

The main focus of our work was to investigate the correlation between vascular biomarkers and the progression of CKD with increased cardiovascular risks including hypertension. Our study revealed a notable increase in ADMA levels among children with CKD compared to healthy controls. This finding aligns with the results reported by Abd El-Salam et al., who also observed significantly higher ADMA levels in children with ESRD on hemodialysis, especially those with hypertension, compared to healthy populations. Also reported that ADMA levels were positively correlated with increasing LV systolic diameter. Furthermore, a cutoff value of >35 ng/mL for ADMA was associated with 92.5% sensitivity and specificity for detecting early LV dysfunction.[17]

In addition, we have observed a significant inverse relationship between ADMA levels and e-GFR, which is consistent with the results reported by Vo T et al., who showed an established negative correlation between ADMA and eGFR. Their investigation highlighted that increased levels of ADMA could reliably predict a decrease in eGFR value below 60 mL/min/1.73m² with a high degree of sensitivity and specificity. [18] This suggests that as CKD progresses and renal function deteriorates, ADMA accumulates in the bloodstream due to reduced clearance by the kidneys. [19]

Our study also explored the role of VEGF-A as a vascular biomarker in children with CKD, an area that has not been extensively studied in previous research. We observed that VEGF-A levels were significantly elevated in children with CKD compared to healthy controls, suggesting its potential involvement in the pathophysiology of CKD. This observation aligns with the findings of Driianska et al., who reported that serum VEGF levels were markedly higher in patients undergoing dialysis than in healthy

individuals.[20] However, our findings contrast with those of Shroff et al., who found lower VEGF-A levels in dialysis patients compared to pre-dialysis CKD patients, possibly indicating an anti-angiogenic environment in the dialysis cohort. [21]

The primary cause of the rise in VEGF-A levels in CKD patients is thought to be a response to renal hypoxia and tissue damage. According to Morozova et al., hypoxia-inducible factors (HIFs) in the kidney upregulate VEGF, which is a critical regulator of angiogenesis, particularly in conditions of reduced oxygen supply—a common occurrence in CKD. This compensatory mechanism may help maintain and repair the renal microvasculature. However, elevated VEGF-A levels might also contribute to increased vascular permeability and inflammation, potentially exacerbating cardiovascular risks in these patients. [22]

In our study, Angiopoietin-2 (Ang-2) levels were significantly higher in children with CKD than in healthy controls, and we observed an inverse correlation between Ang-2 levels and estimated glomerular filtration rate (e-GFR). This aligns with findings from Abdel-Salam et al., who reported elevated Ang-2 levels in children with CKD on hemodialysis, correlating with reduced GFR due to impaired renal clearance.[23]

The increase in Ang-2 levels in children with CKD is primarily due to endothelial dysfunction and the associated inflammatory state [23] Ang-2 contributes to vascular instability and endothelial permeability, responding to chronic inflammation and tissue hypoxia in CKD.[8] This change exacerbates cardiovascular risks and complications, highlighting Ang-2's role as a key biomarker in CKD progression.[8]

In evaluating the mentioned vascular biomarkers as potential indicators of hypertension risk, a well-known cardiovascular complication, we identified a significant correlation between ADMA levels and both systolic and diastolic blood pressure. This is in line with the study by Hsu et al., which observed a higher ADMA-to-SDMA ratio in hypertensive children with CKD in stages G1–G4 (eGFR $\geq$ 15 mL/min/1.73 m²) compared to normotensive children, suggesting a specific link between elevated ADMA levels and hypertension. [24] These findings indicate that ADMA may contribute to the increased susceptibility to cardiovascular complications associated with elevated blood pressure in children with CKD.

Furthermore, our findings are supported by Chien et al., who observed a positive correlation between ADMA levels and systolic blood pressure in children with CKD stages 1–3. [25] Similarly, a study by Mihout et al. demonstrated the effects of elevated ADMA levels on renal structure and function in an experimental model; ADMA administration (60 mg/kg per day) in uni-nephrectomized mice over 8 weeks resulted in significantly elevated blood pressure levels compared to controls.[26] These studies further reinforce the role of ADMA in contributing to hypertension and its associated cardiovascular complications in CKD.

Regarding VEGF-A, we found a significant direct correlation between VEGF-A levels and both systolic and diastolic blood pressure in children with CKD. Although few studies directly address this correlation, our findings are partially supported by Morozova et al., who identified a strong positive relationship between urinary VEGF levels and markers of renal hypoxia, such as macrophage infiltration density and capillary network density.[22] Similarly, a study by Anderson et al., in adults demonstrated that median VEGF-A levels were higher in CKD patients than in controls, suggesting an association with CKD, which is often accompanied by hypertension and other cardiovascular risk factors[27].

Elevated VEGF levels in CKD patients have been linked to renal dysfunction, highlighting VEGF's role in the progression of the disease.[28]Additionally, the inhibition of VEGF signaling can result in increased blood pressure and renal dysfunction due to endothelial cell damage and reduced nitric oxide production. This underscores the critical role of VEGF in maintaining vascular and renal homeostasis and its involvement in the development of hypertension when these pathways are disrupted. [27]

Our results showed a significant direct correlation between Ang-2 and both systolic and diastolic blood pressure, this agreed with Shroff et al., who reported that Ang-2 levels were significantly elevated in children with CKD on dialysis compared to both healthy controls and children with pre-dialysis CKD and reported a strong positive correlation with systolic blood pressure in dialysis patients but not in pre-dialysis CKD. Furthermore, Shroff et al., also found that Ang-2 levels were positively correlated with

carotid artery intima-media thickness (cIMT) in dialysis patients, indicating an association with early cardiovascular disease.[21]

Similarly, a study by Abdel-Salam et al., found that children on regular hemodialysis exhibited significantly higher levels of Ang-2 compared to healthy controls. These elevated levels were positively correlated with increased cardiovascular risk factors, such as intima-media thickness and systolic velocities, suggesting a direct correlation with hypertension and cardiovascular disease.[23] However, another study by David et al. reported no significant correlation between Ang-2 levels and blood pressure readings, which contradicts our findings.[29]

One limitation of this study is the variation in grading methods used across different studies for assessing vascular biomarkers and cardiovascular risks in pediatric CKD patients. While our findings align with previous research in demonstrating elevated ADMA, VEGF-A, and Ang-2 levels in CKD patients, differences in measurement techniques and definitions of hypertension and CKD stages may impact the comparability of results. Future studies with standardized methodologies across populations would be beneficial in validating and expanding upon these findings.

The study proposes several future recommendations for advancing research and clinical practice related to pediatric CKD patients. Longitudinal investigations of pediatric CKD patients' biomarkers are needed to establish causal linkages and assess the predictive value of these biomarkers for cardiovascular problems. Interventional trials are necessary to evaluate therapies targeting ADMA, Angiopoietin-2, and VEGF-A modulation, which may involve pharmacological or behavioural interventions to alleviate endothelial dysfunction and oxidative stress. Additionally, expanding biomarker panels is crucial to further understanding molecular processes, potentially improving risk categorization and personalized treatment. Furthermore, creating and updating clinical practice guidelines to incorporate biomarker suggestions based on current research will standardize care and expedite the clinical translation of new studies.

Conclusion

Children with CKD have significantly higher levels of vascular biomarkers including ADMA, VEGF-A, and Ang-2 compared to healthy controls. These elevated biomarkers

correlate positively with higher systolic and diastolic blood pressure and negatively with eGFR, suggesting a role in endothelial dysfunction and increased cardiovascular risk in pediatric CKD patients. The monitoring of these biomarkers in clinical practice may enhance early diagnosis, risk stratification, and the development of tailored treatments for hypertension and related cardiovascular complications in CKD children."

Conflict of Interest

The authors declare no conflicts of interest.

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Authors' Contribution

SZS was responsible for the oversight of the study design and critical revision of the manuscript. ERM did the conceptualization of the study, data collection, analysis and manuscript preparation. MTAM contributed to the review process. LHA conducted the laboratory biomarker analysis and contributed to interpreting results. MHM supervised the pediatric evaluations and assisted with the study design. All authors approved the final version of the manuscript.

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Table 1: Comparison of Demographic and Clinical Characteristics Between Pediatric CKD Patients and Healthy Controls

	CKD group (N=45)	**Control (N=45)**	**p-value**
Age (years) Mean ±SD (Range)	12.3 ±3 8:18	12.5±2.7 8:18	0.85
Sex Male Female	27(60%) 18(40%)	21(46.7%) 24(53.3%)	0.20
Height (cm) Mean ±SD (Range)	129.5±13.5 (96-158)	136.8±11.6 (114-158)	<0.001
Weight (kg) Mean ±SD (Range)	32±8.4 (12-55)	39.6±8.9 (20-55.5)	<0.001*
BMI Mean ±SD (Range)	 18.9±3 13-30.3	 23.3±3.4 16-30	<0.001*
Systolic blood pressure Mean ±SD (Range)	 121.4±15.3 90:150	 114.6±8.6 90:130	0.04*

Diastolic blood pressure Mean ±SD (Range)	 80±12.3 60:100	 74.8±7.5 60:85	0.03*
Dialysis No Yes	 20(44.4%) 25(55.6%)	 ---	 -----
Type of dialysis (n=25) Hemodialysis Peritoneal dialysis	 25(100%) 0(0%)	 ---	 ----
Non dialysis CKD origin (n=20) Glomerulopathy CAKUT	 14(70%) 6(30%)	 ---	 ----

** Significant*
SD: standard deviation
BMI: body mass index
CKD: Chronic kidney disease

Table 2: Comparison of laboratory data between Pediatric CKD Patients and Healthy Controls

	CKD group (N=45)	**Control (N=45)**	**p-value**
Serum albumin (g\dl) Mean ±SD (Range)	 3.1±0.1.1 (1.1-5.0)	 4.1±0.69 (3.1-5.5)	 <0.001*
Urea (mg/dL) Mean ±SD (Range)	 101±64 (7-267)	 23±10.1 (10-40)	 <0.001*
Creatinine (mg/dl) Mean ±SD (Range)	 3.8±3.2 (0.4-9.3)	 0.7±0.18 (0.4-1.1)	 <0.001*
GFR (ml/min) Mean ±SD (Range)	 39.7±38.5 5:118	 100.6±6.9 90:105	 <0.001*
GFR grading G1 G2 G3/G4 G5	 9(20%) 6(13.3%) 5(11.1%) 25(55.6%)	 45(100%) 0(0%) 0(0%) 0(0%)	 <0.001*
Serum vit D (ng\dl) Mean ±SD (Range)	 14.5±9.8 (3-33.6)	 25.3±7.1 (10-35)	 <0.001*
Vit. D level Deficiency Insufficiency Sufficiency	 15 (33.3%) 20 (44.4%) 10(22.2%)	 5 (11.1%) 15 (33.3%) 25 (55.5%)	 0.002*
ADMA (ng\dl) Mean ±SD (Range)	 1203±577 (250-2537)	 186±55 (105-294)	 <0.001*

VEGF (ng\dl) Mean ±SD (Range)	833±507 (312-2346)	128±51 (39-210)	<0.001*
Angiopioten2 (ng\dl) Mean ±SD (Range)	334.4±147 (127-608)	83±19.5 (44-115)	<0.001*

** Significant*
SD: standard deviation
e-GFR: estimated glomerular filtration rate.
ADMA: Asymmetric-dimethylarginine
VEGF-A: vascular endothelial growth factor-A

Table 3: Correlation between e-GFR and different vascular markers

	e-GFR			
	CKD group		**Control**	
	r	**p**	**r**	**P**
ADMA (ng\dl)	-0.81	<0.001*	0.15	0.32
VEGF (ng\dl)	-0.81	<0.001*	-0.11	0.46
Angiopioten2 (ng\dl)	-0.82	<0.001*	-0.19	0.19

_* *Significant*
e-GFR: estimated glomerular filtration rate.
ADMA: Asymmetric-dimethylarginine
VEGF-A: vascular endothelial growth factor-A

Table 4: Correlation between systolic blood pressure and different vascular markers

	Systolic blood pressure				**Diastolic blood pressure**			
	CKD group		**Control**		**CKD group**		**Control**	
	r	**P**	**r**	**P**	**r**	**P**	**r**	**P**
ADMA (ng\dl)	0.88	<0.001*	0.20	0.17	0.82	<0.001*	0.20	0.16
VEGF (ng\dl)	0.75	<0.001*	0.15	0.31	0.79	<0.001*	0.07	0.61
Angiopioten2 (ng\dl)	0.74	<0.001*	0.01	0.93	0.79	<0.001*	0.01	0.92

** Significant*
ADMA: Asymmetric-dimethylarginine
VEGF-A: vascular endothelial growth factor-A