Benha Journal of Applied Sciences (BJAS) **print: ISSN 2356–9751**
Vol. (9) Issue (2) (2024), (69-76) **online: ISSN 2356–976x**
http://bjas.bu.edu.eg

Klotho G-395A Gene Polymorphism: Impact on Progression of End-Stage Renal Disease and Development of Cardiovascular Complications in Children on Dialysis

Mona M. Alsayed, Ahmed M. Ezzat, Doaa R. Soliman, Inas A. Elsayed, Ahmed S. Abd El Haleem and Wesam E. Afifi
Pediatrics Department, Faculty of Medicine, Benha University
E-Mail: mona990kamal@gmail.com

Abstract

Background: at In recent years, many indicators have been identified that may predict the likelihood of chronic kidney disease in children who already have certain risk factors. But it's still not easy to accurately measure danger. Goal: This research set out to examine the relationship between cardiovascular problems in juvenile patients on hemodialysis for end-stage renal illness and klotho gene polymorphisms (G-395A). Procedures and Materials: Fifty children participated in the case control study; twenty were on maintenance hemodialysis for end-stage renal disease, ten were on conservative therapy for chronic kidney disease, and ten were healthy controls. All forty kids were genotyped for Klotho G-395A and evaluated for clinical and echographic variants. Findings: Compared to control children, children with chronic kidney disease (both conservative and hemodialysis patients) had much higher frequencies of the GA + AA genotype. The frequencies of the G and A alleles were also significantly different. When looking at the two groups side by side, we can see that the GG and GA+AA frequencies were significantly different between the control and hemodialysis groups, but not between the conservative and hemodialysis groups. On the other hand, the AA genotype frequency was significantly different between the conservative and control groups, as well as between the hemodialysis and control groups. In conclusion, CKD risk factors increased the likelihood of development in children carrying the AA or GA genes, which are examples of wild genes.

Keywords: Hemodialysis, Klotho, Klotho G-395A, Chronic Kidney Disease, End-Stage Renal Disease (ESRD) Thesaurus.

1.Introduction

A progressive loss of kidney function that cannot be reversed is the hallmark of chronic kidney disease (CKD), which leads inevitably to end-stage renal disease (ESRD) (ESRD). For the better part of two centuries, CKD has been known to be a major health concern, and up until recently, it was always deadly. It would seem that development to ESRD is inevitable once CKD has occurred. Warady et al. found that factors such as low albumin levels, high blood pressure, abnormal lipid profiles, being male, being anaemia, having dyslipidemia at baseline, having high levels of phosphate in the blood, and having lower glomerular filtration rate (GFR) values at baseline were all indicators of fast progression. [2,3] The high mortality and morbidity rates in patients with end-stage renal disease are both caused by cardiovascular problems. Many specific areas of the heart reflect the cyclical variations in fluid load and the changing metabolism. [4] Infection is the second leading cause of mortality in young children, behind cardiovascular illness. Myocardial ischemia accounted for 14% of the cardiovascular fatalities, stroke for 16%, pulmonary edoema for 12%, hyperkalemia for 11%, and other cardiovascular causes, including arrhythmia, for 22%. Cardiac arrest (cause unclear) accounted for 25% of these deaths. [5] Chronic kidney disease (CKD) is a complex disorder that may include environmental as well as genetic components. Many investigations have shown that CKD is highly heritable (30-75 percent). [6]

Membranes of both the proximal and distal renal tubules are primary sites of klotho gene expression. [7]. The kidney plays a crucial role in appropriate physiological regulation of klotho levels [8]. Nevertheless, klotho levels fall and renal insufficiency is a hallmark of chronic kidney disease (CKD) in both humans and animal models [9]. Experimental evidence suggests a strong correlation between klotho and the pathogenic mechanism of CKD, since the phenotypes of CKD individuals and klotho-deficient mice are identical. [10] Located on the long arm of human chromosome 13, the Klotho gene has over 50 kb of DNA, with five exons and four introns. The human Klotho gene has more than ten single nucleotide polymorphisms (SNPs) that have been associated with kidney disease. One such variation is the G-395A (rs1207568) polymorphism found in the Klotho gene's promoter region. The wildtype genotype is represented by GG, whereas the mutant genotypes are represented by GA + AA. The G-395A polymorphism has a mutant allele denoted by A. [11 and 12]

2.The Aim Of The Work

The purpose of this research was to identify any correlation between klotho gene polymorphisms (G-395A) and cardiovascular problems in hemodialysis-dependent juvenile patients with end-stage renal illness.

3.Materials And Methods

Study design

Children admitted to the paediatric nephrology unit at Benha University Hospital with ESKD between May and December 2022 were the subjects of this case-control research. Forty youngsters, of both sexes, were divided into three categories for this study: (a) Eleven disease-free youngsters of the same age and gender as the patients made up Group I (Control). (a) Ten

juvenile patients dealing with chronic kidney disease who were under conservative treatment and had never been on hemodialysis were part of Group II, the Conservative group. (c) Twenty paediatric patients undergoing hemodialysis for chronic renal disease, with or without cardiovascular problems, made up Group III (Hemodialysis group). Parents gave their written agreement before their children could participate in the research, and the Research Ethics Committee (REC) at the Benha Faculty of Medicine gave their blessing.

Designing operations:

All of the patient's vitals, medical history, and results from standard tests were entered onto a standardised data sheet. The Vivid 3 Pro, which has transducers that operate at 3 and 7 MHz, was used to do the echocardiographic imaging. In a supine posture, two-dimensional (2D) guided M-mode measurements were taken. After collecting echographic measurements such as left ventricular end diastolic diameter (LVEDD), posterior wall thickness (PWT), and inter-ventricular septum thickness (IVST), the following equation was used to calculate left ventricular mass (LVM): The formula for left ventricular mass index (LVMI) is LVM divided by height, and the variables IVST, LVEDD, PWT, and LVEDD3 were inputted into the formula LVM=0.8 + 0.6[13]. (meters). [14]. Ejection fraction (EF), deceleration time (DT), and E/A ratio were also recorded as functional echographic parameters.

The dialysis treatment began with the collection of blood samples. Following the manufacturer's procedure, DNA was extracted from the blood samples using the Gene JET Whole Blood Genomic DNA purification Mini Kit (Thermo Fisher Scientific, Germany) [15]. A genetic analysis was conducted on the Klotho G-395A gene SNP (rs1207568) utilising the TaqMan SNP Genotyping kits (Applied Biosystems, USA). The Step One Plus Real Time PCR apparatus was used for the PCR amplification (Applied Biosystems, USA). [16]

Data analysis using statistics

The quantitative parametric measurements were presented as Mean ± SD, whereas the classified data were presented as either number or percentage.

The following statistical analyses were conducted: 1. Analysis of Variance was used to compare more than two patient groups' parametric data (ANOVA). 2. Using the Paired t test, compare two dependent groups using parametric data. Third, the odds ratio (OR) quantifies the strength of the correlation between a set of variables (gene allele) and a result.

4.Results

A- ***Patient baseline characteristics***

Table 1 summarises the demographic and biochemical characteristics of the groups that were investigated. The 20 children who were part of the study had an average age of 166.6 ± 40.54 months and an average length of 52.15 ± 62.04 months of dialysis. There were 13 boys and 7 girls in the group. The average age of the ten conservative youngsters, which included five boys and five girls, was 90 ± 38.73 months.

Table (1) Personal, medical, and laboratory data.

Demographic data			**Group I**	**Group II**	**Group III**
Residence	Rural	Count	7	7	13
		%	70%	70%	65%
	Urban	Count	3	3	7
		%	30%	30%	35%
Sex	female	Count	5	5	7
		%	50%	50%	35%
	male	Count	5	5	13
		%	50%	50%	65%
Age	Min- Max		25-125	30-150	30 -216
(months)	Mean ± SD		74.9 ± 31.81	90 ± 38.73	166.6 ± 40.54
	P- value		< .00001**		
Cause of CKD			Group II		Group III
congenital renal hypoplasia/aplasia		Count	2		4
		%	20%		20 %
Glomerulonephritis		Count	1		3
		%	10%		15 %
nephrotic		Count	2		3
		%	20%		15 %
Obstructive uropathy		Count	2		4
		%	20%		20.0%
Unkown		Count	3		6
		%	30%		30.0%
Onset and duration			Group II		Group III

Table (1) Continue

Onset of disease (month of onset)	Min- Max	27-194	6-213
	Mean ± SD	89.2 ± 49.71	114.85 ± 56.03
	F - ratio	1.499	
	p- value	0 .2309	
Duration of disease (months)	Min- Max	1-12	3-210
	Mean ± SD	5.3 ± 3.53	54.85 ± 63.7
	F - ratio	5.94	
	p- value	0.021*	
Duration of hemodialysis	Min- Max	0	3 -208
	Mean ± SD	0	52.15 ± 62.04
Frequency of hemodialysis sessions/week		0	3
Duration of each hemodialysis session (hours)	Min- Max	0	3– 4
	Mean ± SD		4 ± 0
Biochemical data	Group I	Group II	Group III
Urea (mg/dl)	24.5 ± 3	51.2 ± 25.2	119.1 ± 58.34
Cratinine (mg/dl)	0.56 ± 0.16	2.94 ± 0.68	7.71 ± 2.14
Na level (meq/l)	140.6 ± 5.6	146.1 ± 10.1	140.15 ± 5.2
K level (meq/l)	3.53 ± 0.51	4.86 ± 0.4	5.02 ± 1.22
Ca level (mg/dl)	9.09 ± 0.5	9.64 ± 0.94	4.88 ± 2

When relevant, values are shown as percentages, means ± SD, or median (interquartile range). Statistical significance is indicated by a P value less than 0.05.

B- ECHOGRAPHIC DATA

Table (2) Patient distribution according to echo results.

Parameters				**Group I**	**Group II**	**Group III**	**F ratio**	**P value**
Normal			Count	10	7	2	25.97	>0.001**
			%	100%	70%	10 %		
Abnormal	Total		Count	0	3	13	8.21	0.001**
			%	0%	30 %	65%		
	Left ventricle structure	total	Count	0	1	11	8.06	0.001**
			%	0%	10 %	55 %		
		CH	Count	0	1	9	5.22	0.01*
			%	0%	10 %	45 %		
		EH	Count	0	0	2	1.03	0.37
			%	0%	0%	10 %		
	CR		Count	0	2	2	1.09	0.35
			%	0%	20%	10%		
	Left ventricle function	Total	Count	0	0	5	3.08	0.057
			%	0%	0%	25%		
		Systolic dysfunction	reduced	Count	0	0	2	1.03	0.37
				%	0%	0%	10%		
			HD	Count	0	0	3	1.63	0.21
				%	0%	0%	15%		
		Total		Count	0	0	9	7.57	0.0017**
				%	0%	0%	45 %		
		Diastolic dysfunction	RF	Count	0	0	4	2.3	0.11
				%	0%	0%	20%		
			AR	Count	0	0	5	3.08	0.058
				%	0%	0%	25 %		

After the study ended, the geometric ECHO pattern of the left ventricle was analysed to determine the distribution of patients with various conditions. LVH, CH, EH, CR, HD, RF, and AR all stand for left ventricular hypertrophy, eccentric hypertrophy, restrictive filling, hyperdynamic heart disease, and abnormal relaxation, respectively. As left

ventricular hypertrophy and diastolic dysfunction were the most common and statistically significant, the proportion of patients in the group whose ECHO results were normal decreased significantly. The outcome is meaningful (*) when p <.05 and very significant (**) when P < 0.01.

C- Genotyping DATA

Table (3) we can see how individuals with and without cardiovascular (CVS) problems are distributed in terms of the Klotho G-395A genotype and allele frequencies.

Gene + alleles			Patients Without CVS complications (n= 9) (30%)	Patients WithCVS complications (n= 21) (70%)					
				HTN (n=17) (56.7%)	LV structure		LV dysfunction		
					LVH (n=12) (40%)	CR (n=4) (13.3%)	SR (n=2) (6.7%)	HD (n=3) (10%)	RF (n=4) (13.3%)	AR (n=5) (16.7)
Genotypes	GG	Count	8	3	2	1	0	1	2	2
		%	88.9%	17.6%	16.6%	25%	0 %	33.4%	50%	40%
	GA	Count	1	9	5	3	1	1	0	2
		%	11.1%	52.9%	41.7%	75%	50 %	33.3%	0 %	40%
	AA	Count	0	5	5	0	1	1	2	1
		%	0%	29.5%	41.7%	0%	50 %	33.3%	50 %	20%
	GA + AA	Count	1	14	10	3	2	2	2	3
		%	11.1%	82.4%	83.4%	75%	100 %	66.6%	50 %	60%
Allele	G	Count	17	15	9	5	1	3	4	6
		%	94.4%	44.1%	37.5%	62.5	25 %	50%	50 %	60%
	A	Count	1	19	15	3	3	3	4	4
		%	5.6%	55.9%	62.5%	37.5%	75 %	50%	50%	40%

Below is a table displaying the distribution of patients based on their geometric ECHO pattern after the study concluded. The acronyms stand for various conditions such as hypertension (HTN), left ventricular hypertrophy (LVH), concentric remodelling (CR), systolic reduction (SR), hyperdynamic (HD), restrictive filling (RF), and abnormal relaxation (AR). The outcome is meaningful (*) when p <.05 and very significant (**) when P < 0.01.

D- Risk association of the different Klotho G-395A allele genotypes with CKD among the study groups:

Table (4) Klotho G395A genotype and allele risk relationship between control and CKD (conservative plus hemodialysis) groups.

Parameters	Control group(n=10)	CKD group (n=30)	OR (95% CI)	P value
GG	9 (90 %)	15 (50%)	9 (1 to 80.1)	0.048 *
GA + AA	1 (10%)	15 (50%)		
G allele	19 (95 %)	40 (66.7 %)	9.5 (1.2 to 76.1)	0.03*
A allele	1 (5 %)	20 (33.3%)		

Ratio of odds (OR) was used. The research found that children with the GA + AA genotype had a 9-fold increased risk of developing CKD compared to those with the GG genotype (P =0.04), when looking at the risk relation between the various Klotho G-395A allele genotypes and ESRD across the study groups. The outcome is meaningful (*) when p <.05 and very significant (**) when P < 0.01.

5.Discussion

Among the patients in our research, only 2% on dialysis and 0% on conservative therapy had hypotension. While hypertension is the most prevalent BP change in CKD patients, hypotension may manifest as either intradialytic hypotension or simply a fluctuation in blood pressure. Blood pressure fluctuations in HD patients are linked to harm to target organs, cardiovascular events, and death in the short, medium, and long terms (within 24 hours, day-to-day, and visit-to-visit). [17]

By eliminating the need for venipuncture, ABPM greatly improves the accuracy of blood pressure assessments and greatly lessens the impact of white coat phenomena, pre-HD fluid overload, and dialysis ultrafiltration. When evaluating blood pressure in children undergoing dialysis, ABPM need to be thought of as the benchmark. Home blood pressure monitoring is more accurate than casual measures taken before and after hemodialysis for gauging volume load and BP regulation. [18]

The children with end-stage renal disease (ESRD) who were on dialysis were compared to the children

with chronic kidney disease (CKD) who were on conservative therapy (CT) using echocardiographic data. Abnormalities in left ventricular geometry and function were far less common in children who had CT:

A substantial increase in left ventricular mass, index of left ventricular mass, and relative wall thickness was seen in 60% of dialysis patients compared to 30% of CT patients, indicating an aberrant LV geometric pattern. Left ventricular hypertrophy was more common in children on dialysis (55% of patients) than in adults on dialysis (10 percent of patients).

Some research indicated LVH in around one-third of children with CKD, while others discovered more than 70%; the types and incidence of aberrant LV geometry have also varied across investigations. referenced in [19, 20]

Stage and length of renal illness, type of dialysis, proper regulation of blood pressure, indexation of left ventricular mass (LVM), and other variables may affect the frequency of LVH in children with chronic kidney disease (CKD). [21]

Our investigation found that almost 10% of children on CT had an aberrant LV geometric pattern; nevertheless, a prior publication from a worldwide registry of children with CKD on PD indicated a prevalence of 48%. [22]

Hypertension and dialysis were shown to be related with LVH when all patients with CKD were taken into account. More severe illness is often present in dialysis patients, putting them at risk for LVH due to chronic volume overload and other variables ([23]).

Hypertension was the only risk factor for LVH in children undergoing dialysis for chronic kidney disease. While earlier research failed to find a correlation between blood pressure and left ventricular hypertrophy (LVH) in children with chronic kidney disease (CKD), our findings are in line with those of other bigger and more recent studies that have shown AH to be a predictor of LVH [23, 24].

Although there was a statistically non-significant difference in the left ventricular ejection fraction across the groups, it cannot be concluded that these youngsters had left ventricular systolic dysfunction. Though some writers failed to note LV ejection fractions that were much lower than control, others did note that they were within the usual range. [23]

Despite the discovery of a maintained ejection fraction, exercise echocardiography or strain employing speckle tracking may reveal impaired systolic function in children on dialysis for chronic kidney disease. [26]

In addition, we measured diastolic function. The E/A ratio and the time it took to decelerate varied significantly between the categories. When comparing children with chronic kidney disease (CKD) to a control group, several studies found diastolic dysfunction based on tissue doppler parameters and mitral inflow. However, some of these studies did not include kids whose E/A ratio was less than 1.0. Nevertheless, there is little consensus among observers when it comes to understanding diastolic function in children. (27, 28)

A membrane protein called α-Klotho acts as the receptor for the hormone fibroblast growth factor 23, which circulates in the blood (FGF23). The kidneys express klotho at high levels, mostly in the distal tubules but also in the proximal tubules to a lesser degree. Animals and people suffering from chronic kidney disease (CKD) due to various causes, such as glomerular and tubulointerstitial illnesses, exhibit a drop in the concentration of soluble Klotho in their blood and urine. (29, 30)

Hyperphosphatemia, hypermethylation, or deacetylation of the Klotho gene promoter by inflammatory cytokines or uremic toxins such indoxyl sulphate are further potential causes of decreased Klotho expression in CKD, in addition to loss of viable tissue. [31]

One such variation is the G-395A (rs1207568) polymorphism found in the Klotho gene's promoter region. The wildtype genotype is represented by GG, whereas the mutant genotypes are represented by GA + AA. The G-395A polymorphism has a mutant allele denoted by A. [32]

Those with chronic kidney disease (both those on conservative treatment and those on hemodialysis) had substantially higher frequencies of the GA + AA genotype compared to children without CKD. The frequencies of the G and A alleles were also significantly different. When looking at the two groups side by side, we can see that the GG and GA+AA frequencies were significantly different between the control and hemodialysis groups, but not between the conservative and hemodialysis groups. On the other hand, the AA genotype frequency was significantly different between the conservative and control groups, as well as between the hemodialysis and control groups. There was a statistically significant difference in the frequencies of G and A alleles between the control and hemodialysis groups, but no such difference between the conservative and control groups or the conservative and hemodialysis groups. The fact that the control group had a much greater frequency of the GG genotype and G alleles suggests that the wild-type G allele protects against end-stage renal disease (ESRD) by fighting renal insults via increased Klotho synthesis.

Elghoroury et al. (2018) used the same Klotho promoter region G-395A (rs1207568) as our study and interviewed 55 children with late CKD (stage 5). The participants were drawn from the hemodialysis unit of the Center of Pediatric Nephrology and Transplantation at Children's Hospital, Cairo University, and the research took place between 2013 and 2016. The research showed that ESRD patients had higher GA + AA genotype rates than the control group, whereas the control participants had higher GG genotype frequencies than ESRD patients (54.5 vs. 7.1 percent and 92.9 vs. 45.5%, respectively, $P < 0.001$).

The frequency of the A allele was greater in the group of patients with end-stage renal disease (ESRD), whereas the frequency of the G allele was higher in the group of healthy controls (86.4 vs. 69.1%, P = 0.001). [33]

The second research was a cross-sectional analysis of 100 paediatric patients seen at Menoufia University Hospital's paediatric department between 2020 and 2021 (Tawfik et al., 2022). In the patient group, the investigation indicated that about 32 (64% of the total) children had TT, 17 (34% of the total) had TG, and one (2% of the total) had GG using the other Klotho promoter region G-395A (rs9536314). On the other hand, genotypic data revealed that TT variations accounted for 62%, TG variants for 38%, and GG variants for 0% of the gene polymorphism in the control group. [34]

6.Conclusion

Finally, our results suggest that the A allele of the G-395A Klotho gene polymorphism is more common in children with chronic kidney disease (CKD) and may serve as a predictor of end-stage renal disease (ESRD). For children with end-stage renal disease (ESRD) and cardiovascular problems, as well as those presenting with left ventricular hypertrophy (LVH), it is much greater and may

7.Recommendations

- Here are some recommendations based on the study's findings:
- When it comes to children on maintenance hemodialysis, G-395A (rs1207568) gentyping should be employed for risk evaluation.
- Further research on the usefulness of G-395A (rs1207568) genotyping for identifying individuals at risk of developing CKD problems is warranted.
- The sample size was small since the research only included one hospital. We don't have enough information to say whether or not the study's findings will hold water for other age groups of children.

Acknowledgment

We would like to express our sincere appreciation to everyone who worked in the Pediatrics and Biochemistry departments at Benha University's Faculty of Medicine for their assistance and collaboration.

References

[1] **Lee, J. (2021).** Children with Kidney Disease: An Overview of Pediatric Primary Nephrotic Syndrome. Pediatric Nursing, 47(3).

[2] **Warady BA, Abraham AG, Schwartz GJ, et al. (2015).** Predictors of rapid progression of glomerular and nonglomerular kidney disease in children and adolescents: the Chronic Kidney Disease in Children (CKiD) cohort. *Am J Kidney Dis*. 2015 Jun. 65 (6):878-88.

[3] **KDIGO 2012. (2013).** Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease. Kidney Int Suppl 2013; 3:136.

[4] **Agarwal R, Andersen MJ, Pratt JH. (2008).** On the importance of pedal edema in hemodialysis patients. Clin J Am Soc Nephrol. 3(1):153-158.

[5] **Jankowski, J., Floege, J., Fliser, D., et al. (2021).** Cardiovascular disease in chronic kidney disease: pathophysiological insights and therapeutic options. *Circulation*, *143*(11), 1157-1172.

[6] **Mencarelli, F., Fabi, M., Corazzi, V., et al. (2014).** Left ventricular mass and cardiac function in a population of children with chronic kidney disease. *Pediatric Nephrology*, *29*, 893-900.

[7] **Sgambat, K., Clauss, S., & Moudgil, A. (2023).** Alterations in diastolic function and cardiac geometry in children: a longitudinal study across the spectrum of dialysis and transplant. *Pediatric Nephrology*, *38*(6), 1887-1896.

[8] **Sreedharan, R., & Pan, C. G. (2019).** Chronic Kidney Disease: Conservative Management. *IAP Specialty Series on Pediatric Nephrology*, *39*(2 Suppl 1), 227.

[9] **Becherucci, F., Roperto, R. M., Materassi, M., et al. (2016).** Chronic kidney disease in children. *Clinical kidney journal*, *9*(4), 583-591.

[10] **Safouh, H., Fadel, F., Essam, R., Salah, A., & Bekhet, A. (2015).** Causes of chronic kidney disease in Egyptian children. *Saudi Journal of Kidney Diseases and Transplantation*, *26*(4), 806.

[11] **Ghonemy, T. A., Farag, S. E., Soliman, S. A., et al. (2016).** Epidemiology and risk factors of chronic kidney disease in the El-Sharkia Governorate, Egypt. *Saudi Journal of Kidney Diseases and Transplantation*, *27*(1), 111.

[12] **El-Gamasy, M. A., El-Shehaby, W. A., & Mabrouk, M. M. (2019).** Early predictors of cardiac dysfunction in Egyptian children with chronic kidney disease. *Annals of pediatric cardiology*, *12*(1), 10.

[13] **Darwish, M. M., Hassan, S. H., Taha, S. F., et al. (2021).** Health-related quality of life in children with chronic kidney disease in Assiut, Egypt. *Egyptian Journal of Community Medicine*, *39*(2).

[14] **Goldstein SL (2004):** Adequacy of dialysis in children: does small solute clearance really matter?. Pediatric Nephrol; 19:1–5.

[15] **Slinin Y, Greer N, Ishani A *et al*., (2015):** Timing of dialysis initiation, duration and frequency of hemodialysis sessions, and membrane flux: a systematic review for a KDOQI clinical practice guideline. *American Journal of Kidney Diseases*; *66*(5): 823-836.

[16] **Maalej, B., Louati, H., Guirat, R., et al. (2018).** Childhood Chronic Kidney Disease: Experience of a Pediatric Department. *J Nephrol Urol*, *2*(1), 1.

[17] **Cañadas-Garre, M., Anderson, K., Cappa, et al. (2019).** Genetic susceptibility to chronic kidney disease–some more pieces for the heritability puzzle. *Frontiers in Genetics*, *10*, 453.

[18] **Rene'G, V., Barletta, G. M., Chand, D. H., et al. (2007):** Blood pressure control in pediatric hemodialysis: the Midwest Pediatric Nephrology Consortium Study. Pediatric Nephrology, 22(4), 547-553.

[19] **Halbach, S. M., Martz, K., Mattoo, T., et al. (2012):** Predictors of blood pressure and its control in pediatric patients receiving dialysis. The Journal of pediatrics, 160(4), 621-625.

[20] **Paglialonga, F., Consolo, S., Edefonti, A., et al. (2018):** Blood pressure management in children on dialysis. Pediatric Nephrology, 33(2), 239-250.

[21] **Raina, R., Lam, S., Raheja, H., et al. (2019).** Pediatric intradialytic hypotension: recommendations from the pediatric continuous renal replacement therapy (PCRRT) workgroup. *Pediatric Nephrology*, *34*, 925-941.

[22] **Agarwal, R., Flynn, J., Pogue, V., et al. (2014).** Assessment and management of hypertension in patients on dialysis. *Journal of the American Society of Nephrology*, *25*(8), 1630-1646.

[23] **Flythe, J. E., Chang, T. I., Gallagher, M. P., et al. (2020).** Blood pressure and volume management in dialysis: conclusions from a Kidney Disease: Improving Global Outcomes (KDIGO) Controversies Conference. *Kidney international*, *97*(5), 861-876.

[24] **Bircan Z, Duzova A, Cakar N, et al. (2010).** Predictors of left ventricular hypertrophy in children on chronic peritoneal dialysis. Pediatr Nephrol 2010;25:1311–1318. 17.

[25] **Bakkaloglu SA, Borzych D, Soo Ha I, et al. (2011):** International Pediatric Peritoneal Dialysis Network (IPPN) registry. Cardiac geometry in children receiving chronic peritoneal dialysis. Findings from the International Pediatric Peritoneal Dialysis Network (IPPN) registry. Clin J Am Soc Nephrol 2011;6:1926–1933.

[26] **Mitsnefes M, Flynn J, Cohn S, et al. (2010):** Masked hypertension associates with left ventricular hypertrophy in children with CKD. J Am Soc Nephrol 2010;21(1):137–144.

[27] **Sinha MD, Tibby SM, Rasmussen P, et al. (2011):** Blood pressure control and left ventricular mass in children with chronic kidney disease. Clin J Am Soc Nephrol 2011;6:543–551.

[28] **Simpson JM, Savis A, Rawlins D, et al. (2010):** Incidence of left ventricular hypertrophy in children with kidney disease: Impact of method of indexation of left ventricular mass. Eur J Echocardiogr 2010;11:271–277.

[29] **Sethna, C. B., Merchant, K., & Reyes, A. (2018).** Cardiovascular disease risk in children with kidney disease. In *Seminars in Nephrology* (Vol. 38, No. 3, pp. 298-313). WB Saunders.

[30] **Haroun, M. R., Eldin, A. A., EL-Khouly, S. M., & Mostafa, I. A. (2020).** The Protective Effect of Ascorbic Acid and Curcumin on the Chronic Toxicity of Meloxicam on the Testis of Adult Albino Rats. *Benha Journal of Applied Sciences*, *5*(5 part (2)), 189-207.

[31] **Mitsnefes, M. M. (2021).** Cardiovascular disease risk factors in chronic kidney disease in children. In *Seminars in nephrology* (Vol. 41, No. 5, pp. 434-438). WB Saunders.

[32] **Mitsnefes, M. M., Xu, Y., Ng, D. K., et al. (2021).** Diastolic function and ambulatory hypertension in children with chronic kidney disease. *Hypertension*, *78*(5), 1347-1354.

[33] **Elghoroury, E. A., Fadel, F. I., Elshamaa, M. F., et al. (2018).** Klotho G-395A gene polymorphism: impact on progression of end-stage renal disease and development of cardiovascular complications in children on dialysis. *Pediatric Nephrology*, *33*, 1019-1027.

[34] **Tawfik, M. A., Elharoun, M. S., & Radwan, F. A. (2022).** Study of klotho polymorphism in Egyptian children with chronic kidney disease on regular hemodialysis: single-center study. *Menoufia Medical Journal*, *35*(3), 1274.