## Original Article

# The Association of Klotho G-395A Gene Polymorphism with Cardiovascular Complications in Pediatric Patients with End Stage Renal Diseases.

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## ABSTRACT

**INTRODUCTION:** CKD causes irreversible renal function loss and ESRD. Dialysis dominated 20th-century life-sustaining renal replacement therapy. Over 20% of chronic HD children die 30 times faster from cardiovascular disease. Klotho, a kidney-secreted transmembrane protein, controls phosphate elimination and calcitriol synthesis and is anti-aging. Vascular tissue expresses Klotho gene and protein, suggesting it regulates cardiovascular health.

**AIM OF THE STUDY:** To evaluate the association between Klotho G-395A gene polymorphism and cardiovascular complications in pediatric ESRD patients on hemodialysis.

**METHOD:** Two groups were studied: regular hemodialysis and healthy control. Parents gave informed consent, and our Faculty of Medicine's Research Ethics Committee approved the study, which took place at Benha University Hospital's pediatric nephrology unit from May to December 2022.

**RESULTS:** ESRD patients had more GA+AA Klotho G-395A genotypes (55% vs 11%) than healthy controls. Cardiovascular complications were 17-fold higher in GG+AA genotypes than GG genotypes. The "A" allele increased cardiovascular complications 9-fold over the "G" allele. ESRD patients with GA+AA genotypes and “A” allele had higher pre- and post-dialysis blood pressure, left ventricular mass, index, and relative wall thickness than those with GG genotype and “G” allele.

**CONCLUSION:** The study indicates that the Klotho G-395A polymorphism is a significant genetic risk factor for cardiovascular complications in pediatric CKD patients, especially those undergoing hemodialysis. It suggests that this genotype could serve as a biomarker for assessing cardiovascular risk in these patients.

**KEYWORDS:** End stage renal disease (ESRD), Hemodialysis, klotho G-395A polymorphism, cardiovascular complications.

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## INTRODUCTION

Chronic kidney disease (CKD) is defined by an irreversible decline in renal function that progressively advances to end-stage renal disease (ESRD) [1]. Scientific and technological advancements in the latter half of the 20th century established renal replacement therapy as a vital life-sustaining alternative. The most common form of renal replacement therapy for this disease worldwide is hemodialysis HD [2].

Children on hemodialysis have a mortality rate that is 30-fold higher than their age-related healthy peers [3], and cardiovascular disease accounts for over 20% of deaths. Cardiovascular events occur in approximately 25–35% of children on chronic HD and include congestive heart failure, arrhythmias, valvular heart disease, cardiomyopathy, left ventricular hypertrophy and sudden death [4].

Risk factors include chronic hypervolemia, hyperdynamic circulation secondary to arterio-venous fistulas AVFs or anemia, vascular calcification, moreover other factors like chronic inflammation, oxidative stress, endothelial dysfunction and genetic factors may also play significant role [5].

Klotho is Transmembrane protein, primarily secreted in the kidneys, acts as anti-aging protein that regulates cellular responses to oxidative stress, regulates phosphate excretion and calcitriol synthesis through mineral homeostasis and interaction with other hormones (PTH, FGF23, and 1,25-(OH)2 vitamin D3). The Klotho protein is mainly extracellular and consists of two homologous domains (KL1 and KL2). Recent studies have established the expression of the Klotho gene and protein in vascular tissue, indicating that Klotho may play an important role in regulating cardiovascular health, and suggesting a decrease in Klotho may be associated with cardiovascular disease (CVD) [6].

Klotho is located on the large arm of human chromosome 13 and has five exons and four introns spanning 50 kb. The human Klotho gene has over ten SNPs linked to kidney disease, coronary artery disease, stroke, and bone mineral density. These include the Klotho gene promoter G-395A (rs1207568) polymorphism. The wildtype genotype is GG, while the mutant genotype is GA+AA. The G-395A polymorphism mutant allele is A [7].

**AIM OF THE STUDY:** To evaluate the association between Klotho G-395A gene polymorphism and cardiovascular complications in pediatric ESRD patients on hemodialysis.

## METHODS

**Study design:** This case-control study included two groups of children. Group 1 (No 20) had chronic kidney disease (CKD) and regular hemodialysis 3 times a week for at least 6 months. Group 2 (NO 20) is healthy control. The two groups were underage of 18 years. The exclusion criteria were children with ESRD on hemodialysis for less than 6 months and patients who have any chronic systemic non renal conditions. The study was conducted from May to December 2022 at Benha university Hospital's pediatric nephrology unit. Prior to their registration in the trial, the parents gave their informed written consent. It was approved by Benha Faculty of Medicine's Research Ethics Committee (REC).

**Diagnostic criteria of cardiovascular disease:** Echocardiographic imaging was performed on a Vivid 3 Pro machine with

3- and 7-MHz transducers. 2D guided M-mode measurements were taken in a supine position. Echo graphic parameters measured. LVM is calculated using the following equation: LVEDD = end diastole left ventricular diameter, PWT = posterior wall thickness, and IVST = inter-ventricular septum thickness. LVM= 0.8 {1.04 [(IVST + LVEDD + PWT)3 – LVEDD3]} + 0.6. [Nabeshima]. LVM divided by height (meters) yielded left ventricular mass index (LVMI). The National High Blood Pressure Education Program (2004) defined LVH in children with an LVMI limit of 51 g/m2.7 (95 g/$m^2$ for females and 115 g/$m^2$ for males) in their fourth report

RWT means relative wall thickness LVMI and RWT defined abnormal LV geometry as: Concentric remodeling (LVMI < 95 g/$m^2$ in females, 115 g/$m^2$ in males, RWT > 0.42). Concentric hypertrophy (LVMI > 95 g/$m^2$ in females or 115 g/$m^2$ in males, RWT > 0.42). Eccentric hypertrophy (LVMI > 95 g/$m^2$ in females, 115 g/$m^2$ in males, RWT < 0.42)

EF: systolic function marker (E/A: early to late ventricular filling velocities DT: Deceleration time. E/A ratio and DT indicate diastolic function [8].

**Determination of Klotho G-395A gene polymorphism** The TaqMan allelic discrimination assay (Takara, Dalian, China) was used to identify the KL promoter region G-395A (rs1207568) genotype, as previously [9]. These primers and probes were used:

- Forward primer 5′-TAGGGCCCGGCAGGAT-3′;
- Reverse primer 5′-CCTGGAGCGGCTTCGTC-3′;
- Probe A 5′-FAM CCCCAAGTCGGGAAAAGTTGGTC (TAMRA)-3′;
- Probe G (5′-HEX) CCCCCAAGTCGGGGAAAGTTGGT C (TAMRA)-3′.

**Genotyping via PCR:** 20 µl reaction volume, 10 µl Premix Ex Taq, 1.5 µl each forward and reverse primer, 0.5 µl probe A, 1 µl probe G, 1 µl diluted genomic DNA (10 ng/µl), and 4.25 µl sterile, double-distilled water. Standard procedure: initial denaturation at 95°C for 30 seconds, 40 cycles of denaturation at 95°C for 5 seconds, and annealing at 60°C for 30 seconds. Thermal cycler PTC-100 (Bio-Rad Laboratories Inc., California, USA) held samples. The promoter region KL G-395A genotype was confirmed by randomly selecting 10% of samples for forward and reverse sequencing. We found the results identical to the TaqMan allelic discrimination assay.

## STATISTICAL ANALYSIS

SPSS-20 was used to statistically analyze the data. Frequencies and percentages were used to present the qualitative data, and the Chi-squared test and odds ratio (OR) with a 95% confidence interval (CI) were used to compare intergroup differences. Hardy-Weinberg equilibrium was observed in the genotype frequency distribution for both patients and control subjects. The median and interquartile range were used to represent abnormally distributed quantitative data, and ANOVA was used to compare parametric data between more than two groups.

statistical significance between each of the two groups, the multiple comparison (post-hoc test or least significant difference, LSD) method was also used. For parametric data, a paired t test was used to compare two independent variables across the three groups. For parametric data, a Pearson correlation test is used to examine

potential relationships between alleles for other CVS variables within each group. While the probability of error at 0.05 was deemed significant, it is highly significant at 0.01% and 0.001%. To determine the smallest set of variables that can most effectively distinguish between the presence and absence of cardiovascular problems in the study group, linear regression analysis was performed.

## RESULTS

The clinical and biochemical attributes of the examined groups are presented in **Table 1**. The average age of the children with end-stage renal disease & on hemodialysis (65% males, 35% females) was 8.5 ± 3.5 years, with an average dialysis duration of 4.6 ± 1.6 years., while the mean age of the healthy control children (50% males & 50% females) was 6.2 ± 2.7 years. The predominant cause of end stage renal disease (ESRD) was structural abnormalities, accounting for 45% of cases, the hemodialysis group was significantly associated with older age ($p<0.05$), increased pre-dialysis serum urea, pre-dialysis systolic (SBP) & diastolic (DSP) blood pressure, Higher Serum phosphorus ($p <0.001$) & serum intact parathyroid hormone (PTH) ($p<0.0001$).

Regarding The distribution of Klotho G-395A genotype frequencies, allele frequencies among the study groups **Table 2** hemodialysis group shows statistically significant higher frequencies of genotypes GA (30% vs 10%), AA (25% vs 0%), GA+AA (55% vs 11 %) ($p < 0.01$) than control group. While control group had higher frequency of allele G (95% vs 60%) & GG genotype (90% vs 45%) than hemodialysis group ($p<0.01$).

A comparison of hemodialysis patients with and without complications (according to the type of ECHO used in the study) **Table 3** revealed that those with cardiovascular complications had longer durations of disease and dialysis (3.5±1.2 vs 2.3±0.8 years; 3.2±1.1 vs 1.8±0.5 years, respectively, $p<0.05$). They demonstrated elevated pre-dialysis systolic blood pressure in mmHg (140.7±31 vs 125.8±25 mmHg, $p<0.001$) and diastolic blood pressure (87.1±11.8 vs 76.9±6.2 mmHg, $p<0.05$), increased serum calcium levels (10.1±1.1 vs 8.7±0.8 mg/dL, $p<0.05$), and heightened phosphorus levels (5.3±1.2 vs 3.2±0.5 mg/dL, $p<0.05$).

The Comparison between ESRD patients with & without Cardiovascular complications and its different types **Table 4** shows that ESRD Patients having GG + AA genotypes demonstrated a 17-fold elevated risk for cardiovascular complications relative to individuals with the GG genotype (OR 17, 95% CI 1.5 - 44, p 0.01). Moreover, individuals with the "A" allele demonstrate a ninefold elevated risk of experiencing cardiovascular complications relative to those with the "G" allele (OR 9, 95% CI 1.2-63, $p<0.05$). ESRD Patients GG + AA genotypes demonstrated a sevenfold elevated risk for left ventricular hypertrophy (LVH) relative to those with the GG genotype (OR 7, 95% CI 0.9-44, $p<0.05$). Additionally, patients with the "A" allele demonstrate an eightfold elevated risk of developing left ventricular hypertrophy (LVH) compared to those with the "G" allele (OR 8, 95% CI 0.5-86, $p<0.05$). ESRD patients having GG + AA genotypes demonstrated an elevenfold heightened risk for Dilated Cardiomyopathy (DCM) relative to those with the GG genotype (OR 11, 95% CI 0.5-65, $p<0.05$). Moreover, individuals with the "A" allele demonstrate a sevenfold

heightened risk of developing DCM relative to those with the "G" allele (OR 7, 95% CI 0.5-64, p<0.05).

The comparison between individuals with GA + AA genotypes and those with GG genotypes **Table 5** indicated that GA + AA individuals demonstrate a significant elevation in pre- dialysis systolic and diastolic blood pressure in mmHg (132.3 ± 3.9, 77.4 ± 5.2 vs 115.8 ± 10.6, 66.8 ± 5.6, $p < 0.01$), as well as post-dialysis systolic and diastolic blood pressure in mmHg (122 ± 12.8, 83 ± 16.8 vs 108 ± 11.2, 61.9 ± 8.9, $p < 0.01$), a significant increase in mean Left Ventricular Mass (LVM – g) (188.1 ± 48.5 vs 87.05 ± 23.4, $p < 0.01$), and mean Left Ventricular Mass Index (LVMI – g/m²) (176.6 ± 29.9 vs 62.5 ± 33.6, $p < 0.01$). Moreover, there was a notable elevation in mean relative wall thickness (RWT) (0.64 ± 0.17 vs 0.41 ± 0.08, $p < 0.01$). No notable difference was detected in ejection fraction (EF), E/A (early to late ventricular filling velocities), and deceleration time (DT).

Pearson's correlation analysis **Table 6** revealed a statistically significant association between A alleles and several cardiovascular complication parameters, including systolic blood pressure ($r = 0.573$, $p = 0.03$), diastolic blood pressure ($r = 0.598$, $p = 0.02$), duration of dialysis ($r = 0.861$, $p < 0.001$), heart rate ($r = 0.457$, $p = 0.019$), left ventricular mass ($r = 0.720$, $p < 0.001$), left ventricular mass index ($r = 0.689$, $p < 0.001$), serum urea ($r = 0.688$, $p < 0.001$), and creatinine ($r = 0.670$, $p < 0.001$). However, no substantial correlation was detected between deceleration time (DT) and hemoglobin (HB).

**TABLE (1)** study groups regarding clinical and biochemical parameters

| | Hemodialysis n=20 | Control n=20 | P value |
|---|---|---|---|
| **Age (years) Mean & SD** | 8.5+3.5 | 6.2+2.7 | <0.05 |
| **Males (%)** | 13(65%) | 10 (50%) | 0.2 |
| **Females (%)** | 7 (35%) | 10 (50%) | 0.3 |
| **Causes of CKD**<br>**Abnormal structures**<br>**Inherited**<br>**Glomerulopathies**<br>**Unknown** | <br>9 (45%)<br>4 (20%)<br>4 (20%)<br>3 (15%) | <br>NA<br>NA<br>NA<br>NA | |
| **Duration of the disease (in years)** | 4.6 ± 1.6 | NA | |
| **Duration of dialysis (in years)** | 3.8+1.2 | NA | |
| **Cardiovascular complications** | 11(55%) | 0(0%) | |
| **LVH** | 7 (35%) | 0 (0%) | |
| **DCM** | 4 (20%) | 0 (0%) | |
| **Pre-dialysis urea** | 119.1 ± 58.34 | 24.5 ± 3 | <0.001 |
| **Post – dialysis urea** | 53.88 ± 29.2 | NA | |
| **Kt/v** | 1.6+0.3 | NA | |
| **Pre dialysis SBP** | 131.5 ± 13.06 | 95 ± 9.98 | <0.001 |
| **Pre-dialysis DBP** | 74.9±7.57 | 55 ± 6.13 | <0.001 |
| **Post-dialysis SBP** | 125+10.7 | NA | |
| **Post dialysis DBP** | 65+7.6 | NA | |
| **Serum calcium(mg/dl)** | 9.1 ± 2.1 | 9.09 ± 0.5 | 0.1 |
| **Serum phosphorus (mg/dl)** | 4.8+1.6 | 3.5+1.2 | <0.001 |
| **Serum intact PTH (pmol/l)** | 164+23.2 | 5.6+1.4 | <0.0001 |

**Table 2:** The distribution of Klotho G-395A genotype frequencies, allele frequencies between hemodialysis and control groups

| Parameters | | | Hemodialysis (n=20) | Control (n=20) | P value |
|---|---|---|---|---|---|
| Genotypes | GG | Count | 9 | 18 | <0.01* |
| | | % | 45 % | 90 % | |
| | GA | Count | 6 | 2 | <0.01* |
| | | % | 30 % | 10% | |
| | AA | Count | 5 | 0 | <0.01* |
| | | % | 25 % | 0% | |
| | GA + AA | Count | 11 | 2 | <0.01* |
| | | % | 55 % | 10 % | |
| | | | | | |
| Allele | G | Count | 12 | 19 | <0.01* |
| | | % | 60 % | 95 % | |
| | A | Count | 11 | 3 | <0.01* |
| | | % | 55 % | 15% | |

**Table 3:** A comparison of hemodialysis patients with and without cardiovascular complications regarding clinical and biochemical parameters

| Parameters | Hemodialysis Patients with CVS complications (n=11) | Hemodialysis Patients Without CVS complications (n=9) | P value |
|---|---|---|---|
| Age | 8.1+3.4 | 7.8+2.1 | 0.3 |
| Sex (males/females) | 7(63%) / 4(37%) | 6(67%)/3(33%) | 0.4 |
| Duration of disease (in years) | 3.5+1.2 | 2.3+ 0.8 | <0.05* |
| Duration of dialysis (in years) | 3.2+1.1 | 1.8+0.5 | <0.05* |
| Kt/v | 1.2+0.7 | 1.6+0.9 | <0.05* |
| Pre dialysis SBP | 140.7+31 | 125.8+25 | <0.01* |
| Pre-dialysis DBP | 87.1+11.8 | 76.9+6.2 | <0.05* |
| Post-dialysis SBP | 129.8+23.7 | 112.9+10.6 | <0.01* |
| Post dialysis DBP | 75.9+6.1 | 70.1+5.2 | 0.3 |
| Pre-dialysis urea | 121.6+12.1 | 101.1+6.3 | 0.007* |
| Post – dialysis urea | 38.9+3.9 | 22.2+2.1 | <0.05* |
| Serum calcium | 10.1+1.1 | 8.7+0.8 | <0.05* |
| Serum phosphorus | 5.3+1.2 | 3.2+0.5 | <0.05* |

**Table 4:** Frequency distribution of KlothoG-395Agenotypes and alleles among the end-stage renal disease (ESRD) patients regarding different cardiovascular complications.

| Parameters | Hemodialysis Patients With CVS complications (n=11) | Hemodialysis Patients Without CVS complications(n=9) | OR (95% CI) | P value |
|---|---|---|---|---|
| GG | 4 (36%) | 7 (78%) | 17 (1.5-44) | <0.01* |
| GA + AA | 10 (91%) | 1 (11%) | | |
| G allele | 4(36%) | 8(89%) | 9 (1.2-63) | <0.05* |
| A allele | 9 (81%) | 2 (22%) | | |
| **Parameters** | **Hemodialysis Patients with LVH (n=7)** | **Hemodialysis Patients Without LVH (n= 13)** | **OR (95% CI)** | **P value** |
| GG | 2 (28%) | 7 (54 %) | 7 (0.9-44) | <0.05* |
| GA + AA | 6 (86%) | 5 (38%) | | |
| G allele | 1(14%) | 4(31%) | 8 (0.5-86) | <0.05* |
| A allele | 7 (100%) | 4 (31%) | | |
| **Parameters** | **Hemodialysis Patients with DCM (n=4)** | **Hemodialysis Patients Without DCM (n= 16)** | **OR (95% CI)** | **P value** |
| GG | 0 (0%) | 9 (56%) | 11 (0.5-56) | <0.05* |
| GA + AA | 4 (100%) | 7 (43%) | | |
| G allele | 0 (0%) | 5 (31%) | 7 (0.2-64) | <0.05* |
| A allele | 4 (100%) | 7 (43%) | | |

**Table 5:** Comparison of Klotho G395A genotypes carrying persons regarding to clinical and Echographic findings of CVS complications of chronic kidney disease.

| characteristics | GG persons (n=9) | GA+AA persons (n=11) | P value |
|---|---|---|---|
| Heart rate (beat/min) | 94 ± 17 | 105 ± 20 | 0.076 |
| Pre dialysis -SBP | 115.8 ±10.6 | 132.3 ± 3.9 | <0.01* |
| Predialysis -DBP | 66.8 ± 5.6 | 77.4 ± 5.2 | <0.01* |
| Post-dialysis SBP | 108+11.2 | 122+12.8 | <0.01* |
| Post dialysis DBP | 61.9+8.9 | 83+16.8 | <0.01* |
| Serum calcium (mg/dl) | 8.4+1.3 | 10.2+1.7 | <0.01* |
| Serum phosphorus (mg/dl ) | 3.6+0.6 | 6.1+1.8 | <0.01* |
| LVM (g) | 87.05 ± 23.4 | 188.1± 48.5 | <0.01* |
| LVMI ($g/m^2$) | 62.5 ± 33.6 | 176.6 ± 29.9 | <0.01* |
| RWT | 0.41 ± 0.08 | 0.64 ± 0.17 | < 0.01* |
| EF (%) | 67.8 ± 5.7 | 67 ± 12.2 | 0.46 |
| E/A ratio | 1.8 ± 0.6 | 1.3 ± 0.4 | 0.9 |
| DT (msec) | 133.2 ± 28.8 | 145 ± 30.7 | 0.75 |

**Table 6:** Outcomes of Pearson's correlation analysis between the Aalleles and the other potential risk factors for development of cardiovascular complications.

| Predictive factor | Risk factors | R value | P value |
|---|---|---|---|
| A allele | Period of hemodialysis | 0.861 | <0.001* |
| | Systolic blood pressure | 0.573 | 0.03* |
| | Diastolic blood pressure | 0.598 | 0.02* |
| | Heart rate | 0.457 | 0.019* |
| | LVM (g) | 0.720 | <0.001* |
| | LVMI ($g/m^2$) | 0.689 | <0.001* |
| | DT (msec) | -0.829 | <0.001* |
| | Hb | -0.346 | 0.62 |
| | urea | 0.688 | <0.001* |
| | Creatinine | 0.670 | <0.001* |
| | Ca | 0.106 | 0.323 |

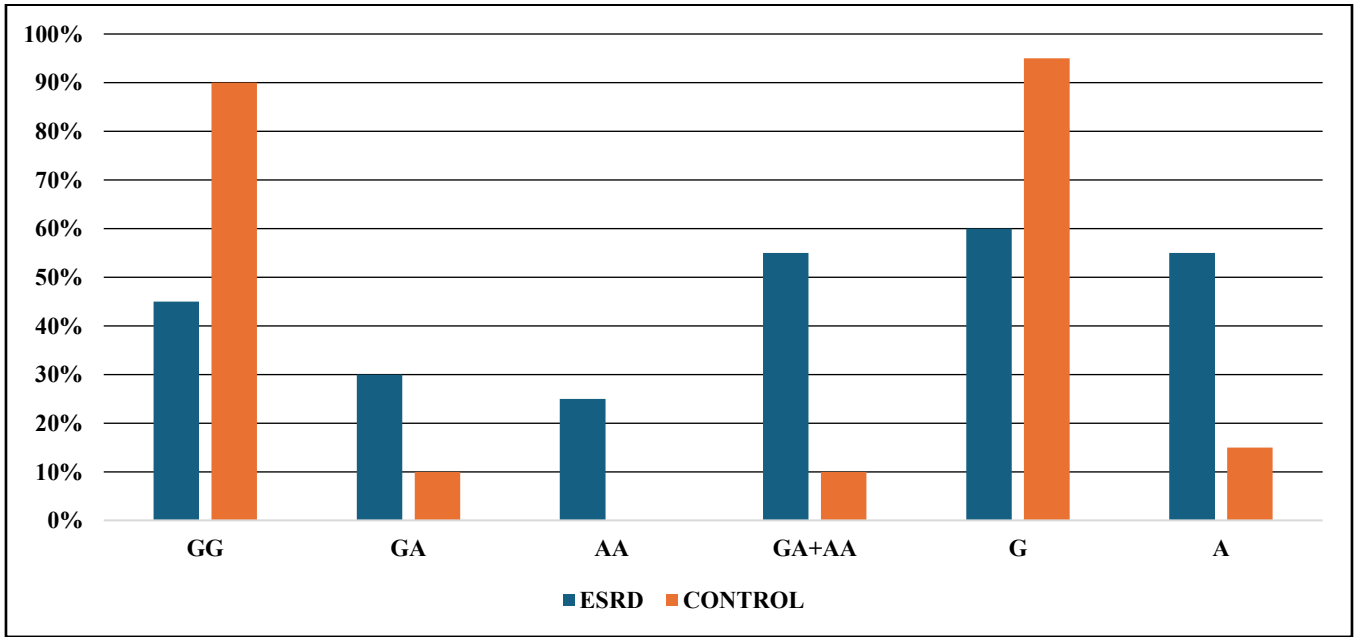


**Figure 1:** The distribution of Klotho G-395A genotype frequencies, allele frequencies between hemodialysis and control groups.

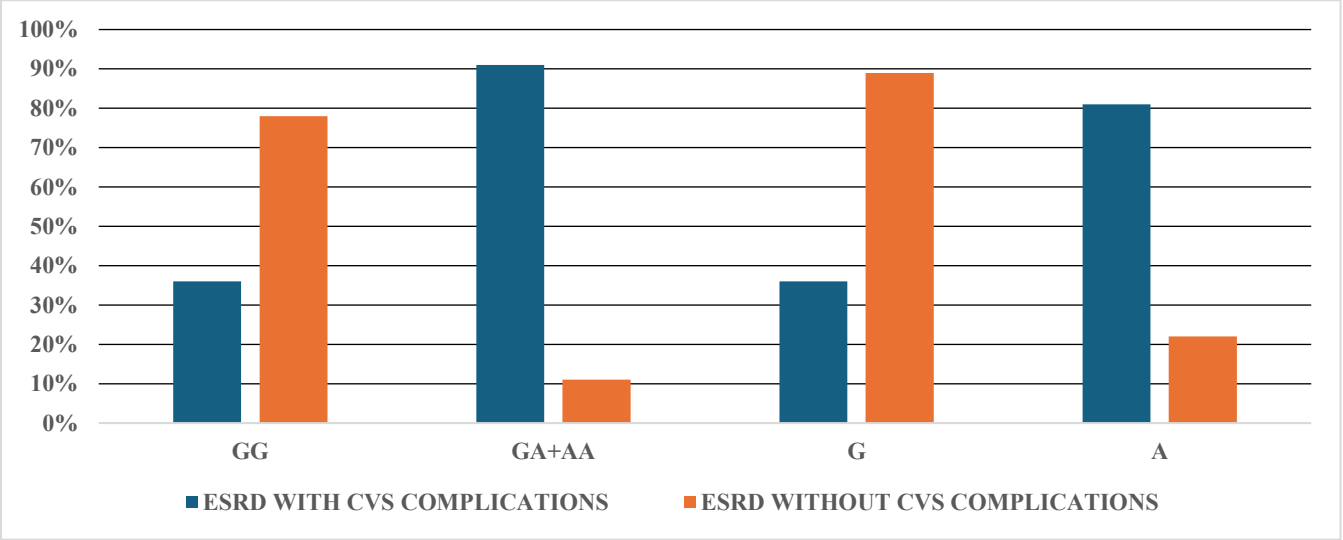


**Figure 2:** Frequency distribution of KlothoG-395Agenotypes and alleles among the end-stage renal disease (ESRD) patients regarding differ.

## DISCUSSION

CKD is defined as the presence of structural or functional kidney damage that persists over a minimum period of three months. Functional damage is characterized by sustained reduction of estimated GFR, persistent elevation of urinary protein excretion, or both. [10]. Based on this definition, clinical practice guidelines from Kidney Disease: Improving Global Outcomes (KDIGO in 2012) published criteria for diagnosis and staging of pediatric CKD. The KDIGO diagnosis criteria and staging classification are the standard used in clinical practice, research, and public health in the care of children with CKD. [11]. *Jankowski et al.* (2021) claims that cardiovascular diseases (CVD) are known to be the most important causes of morbidity and mortality in children with chronic kidney disease (CKD), particularly in those undergoing hemodialysis (HD) [12]. The main cardiovascular diseases (CVD) in children with CKD are arterial dysfunction, atherosclerosis, myocardial ischemia and alteration of the left ventricular (LV) geometric pattern, including left ventricular hypertrophy (LVH), which may lead to systolic and diastolic dysfunction [13].

This study examined how klotho gene polymorphisms (G-395A) affect cardiovascular complications in pediatric chronic kidney disease patients. Twenty children on chronic hemodialysis (group I), and twenty healthy children (group II) were evaluated after the study.

In this study, the hemodialysis group had a mean pre-dialysis systolic blood pressure (SBP) of 131.5 ± 13 mmHg and a Pre-dialysis diastolic blood pressure (DBP) of 74.9 ± 7.57 mmHg. Between 30% and 95% of pediatric dialysis patients have hypertension. Multifaceted problem. Sodium (Na) retention and volume overload cause most hypertension (HTN) in CKD children, according to multiple pediatric studies. Before and after hemodialysis, home or Ambulatory blood pressure monitoring ABPM improves BP assessment [14][15][16].

The study gathered echocardiograms, revealing that 11 patients (55%) from the ESRD cohort

exhibited abnormal findings. Seven patients (35%) exhibited left ventricular hypertrophy (LVH). Four patients (20%) exhibited dilated cardiomyopathy (DCM). The results corroborate recent, larger studies indicating that dialysis patients exhibit more advanced disease, characterized by persistent volume overload and other factors, such as arterial hypertension, that may contribute to left ventricular hypertrophy (LVH), which is a predictor of LVH in children with chronic kidney disease.[17] [18]. Research has identified no correlation between blood pressure and left ventricular hypertrophy [19][20]. dysfunction. Other studies found left ventricular hypertrophy in over 70% of children with chronic kidney disease [21][22][23]. LVH prevalence in children with CKD may depend on renal disease stage and duration, dialysis modality, blood pressure control, and left ventricular mass index [23]. Due to the statistically insignificant difference in LV ejection fraction, these children cannot be diagnosed with LV systolic dysfunction. Left ventricular ejection fractions were lower than the control group in some authors [20][24][25][26]. Our study examined diastolic function. We found significant changes in E/A ratio and deceleration time across groups. Some studies on diastolic dysfunction in pediatric CKD patients found no E/A ratio below 1.0 [25][27][28]. Child diastolic function is difficult to interpret, with little consensus [29].

Compared to the GG genotype, the frequencies of the KlothoG-395A GA+ AA genotype were substantially higher among children on hemodialysis, in this study. It has been suggested that the A allele, which is more common in hemodialysis patients than in healthy controls, may be an allelic variant that puts children with chronic kidney disease at an increased risk of developing end-stage renal disease (ESRD). One possible explanation is that the G/A substitution weakens protein binding to the Klotho promoter. Another possibility is that the A allele forms fewer DNA-protein complexes compared to the G allele, leading to decreased Klotho production.

Additionally, we found that the control group had a much higher frequency of the GG genotype and G alleles, suggesting that the wild-type G allele protects against end-stage renal disease (ESRD) by fighting renal insults through increased Klotho production. Our findings aligned with the 2018 study by *El-ghoroury et al*., which indicated elevated frequencies of the GA + AA genotypes in ESRD patients compared to controls, whereas GG genotype frequencies were more prevalent in the control group. Moreover, patients with end-stage renal disease exhibited elevated frequencies of the A allele, while controls demonstrated increased frequencies of the G allele [30].

Additionally, our results corresponded with those of other studies. The study by *Zeng et al.* (2019) revealed notable disparities in G and A allele frequencies, as well as GG, GA, and AA genotypes, between the two groups [31]. A 2013 study by *Ko et al*. established a correlation between the Klotho A-allele and increased mortality risk in Korean hemodialysis patients [32]. Conversely, *Tawfik et al's* 2022 study examining the alternative Klotho promoter region G-395A (rs9536314) revealed that 64% of patients exhibited the TT genotype, 34% the TG genotype, and 2% the GG genotype. The genotypic results of the control group exhibited 62%, 38%, and 0%

gene polymorphism for the TT, TG, and GG variants, respectively [33].

Our study found that GA + AA Klotho G-395A allele genotypes increased CKD risk compared to GG genotypes ($P < 0.01$), while those with mutant A allele had higher risk than those with wild-type G allele. ESRD risk was 15.6 times higher in GA + AA genotype children than in GG genotype children, according to *El-ghoroury et al.* (2018). People with the mutant A allele had a 2.8-fold higher risk of ESRD than those with the wild-type G allele [30].

Our research found that children with end-stage renal disease (ESRD) undergoing hemodialysis and experiencing cardiovascular complications exhibited a significantly higher prevalence of the GA + AA genotype (91% vs. 11%, $p < 0.01$) With increasing risk 17 folds (OR 17, 95% CI 1.5– 44) compared to those without cardiovascular complications.

The A allele exhibits a higher prevalence in children with end-stage renal disease (ESRD) undergoing hemodialysis and experiencing cardiovascular complications (81% compared to 22%, $p < 0.05$) with 9 folds increased risk compared to the control group. A notable elevation in the GG gene and G allele was observed in patients without cardiovascular complications (78% compared to 36% and 89% compared to 43%, respectively; $p < 0.01$). The findings align with *El-ghoroury et al.* (2018), which determined that ESRD patients with cardiovascular complications exhibited increased frequencies of the GA + AA genotype, whereas those without such complications showed higher frequencies of the GG genotype. Patients with end-stage renal disease (ESRD) exhibiting left ventricular hypertrophy (LVH) & dilated cardiomyopathy (DCM) demonstrated increased frequencies of the GA + AA genotype and the A allele. Patients with ESRD lacking LVH exhibited increased frequencies of the GG genotype and the G allele [30]. The presence of the GA + AA genotype and the A allele may serve as predictors for cardiovascular complications in children with ESRD. Two studies found that A allele carriers have a 161-fold higher risk of cardiovascular disease compared to non-carriers ($P < 0.001$). A 2017 meta-analysis found that the Klotho G395A polymorphism is significantly associated with cardiovascular disease (CVD), with the 395A allele having a higher risk than the 395G allele [30][31].

This study analyzes patient variables among GG, GA, and AA gene carriers to assess the impact of the Klotho G-395A gene polymorphism on them. Chronic kidney disease patients with GA + AA genotypes exhibited elevated pre- and post-dialysis systolic and diastolic blood pressure, as well as increased left ventricular geometric measures, in comparison to those with GG genotypes, along with higher serum calcium and phosphorus levels. The findings indicated that A-allele carriers adversely impact renal and cardiovascular functions in patients with chronic kidney disease, influencing disease severity and mortality rates. Our results align with those of *El-ghoroury et al.,* who examined the clinical and biochemical characteristics of A allele carriers (GA + AA genotypes) versus noncarriers (GG genotype) in patients with end-stage renal disease. Carriers of the "A" allele exhibited elevated systolic blood pressure (SBP) and diastolic blood pressure (DBP) both prior to and following dialysis compared to non-carriers, along with increased serum calcium and

phosphorus levels [30]. Numerous studies have linked Klotho G395A gene polymorphism to hypertension. In 2006, *Rhee et al*. found that A allele carriers had significantly higher SBP levels [34]. According to *Wang et al.,* the G-395A polymorphism may be linked to essential hypertension [35]. *Friedman et al*. discovered that only the Klotho rs577912 polymorphism correlated with modified clinical and biochemical variables, as well as an elevated mortality risk in chronic HD patients [36].

We also investigated the impact of the "A"-allele on cardiovascular disease parameters. Cardiovascular disease is associated with the "A"-allele in patients with chronic kidney disease. The presence of the "A"-allele is significantly correlated with an elevation in cardiovascular risk factors such as SBP, DBP, and hemodialysis duration. Regression analysis was employed to ascertain the impact of parameters on the increase of LVMI. Diastolic blood pressure and the A allele in GA or AA genotypes significantly elevated left ventricular mass index (LVMI) and may forecast cardiovascular complications in chronic kidney disease. *El-ghoroury et al*. identified systolic blood pressure (SBP) and diastolic blood pressure (DBP) as risk factors for cardiovascular disease through regression analysis. This analysis identifies statistically significant predictors of cardiovascular disease. The frequency of DBP and A allele is positively correlated with the incidence of cardiovascular disease following dialysis, whereas SBP exhibits an inverse correlation. LVMI was associated with cardiovascular disease [30].

## LIMITATIONS OF THE STUDY

A small sample of twenty hemodialysis patients and twenty healthy control children was studied. This limits the applicability of the findings to pediatric ESRD patients. The study was conducted at Benha University Hospital, one center. This may limit generalizability and introduce prejudice related to that center's patient population or medical procedures.

The study is cross-sectional, so it cannot prove causality without longitudinal data. It only shows Klotho polymorphism-cardiovascular problems at one time. The gene polymorphism's temporal relationship to cardiovascular complications must be determined by longitudinal research.

Age, blood pressure, and biochemical markers are considered in the study, but other genetic, environmental, or lifestyle factors may affect cardiovascular complications in this population.

The study did not examine these factors, but chronic inflammation, oxidative stress, endothelial dysfunction, and genetic factors may play a major role in cardiovascular disease in hemodialysis children.

The study focuses on the Klotho G-395A polymorphism but notes that other Klotho gene polymorphisms are linked to kidney disease. It ignores other Klotho gene variants or genes that may affect cardiovascular disease in this patient population. Another study examined Klotho promoter region G-395A (rs9536), according to the paper. Limited Diastolic Function Knowledge: The study finds diastolic function difficult to understand in children. More diastolic dysfunction testing could provide a more complete cardiovascular picture. The study found

significant E/A ratio and deceleration time changes in children, but opinions vary.

The study excludes patients with other chronic systemic non-renal diseases and those on hemodialysis for less than six months, suggesting selection bias. Although inclusion and exclusion rules are crucial, this decision may bias research.

## FUTURE RESEARCH DIRECTIONS

To understand how the Klotho G-395A polymorphism affects cardiovascular disease progression, longitudinal studies are needed. It would prove causality, not correlation. Future studies should use larger sample sizes and multi-center studies to generalize results to more pediatric ESRD patients. This would overcome the limitations of the single-center, small sample study.

In this patient population, future studies should examine genetic polymorphisms linked to the Klotho gene and other genes that may affect cardiovascular disease. Although this study focuses on the Klotho G-395A polymorphism, other Klotho gene polymorphisms may also worsen cardiovascular issues. Oxidative stress, chronic inflammation, and endothelial dysfunction may also be important, and future studies could examine them. Detailed Diastolic Function Evaluation: In chronic kidney disease children, diastolic function should be examined more thoroughly. Diastolic function is difficult to understand, so future research could use more consistent methods to measure and analyze it in children. Future research should examine how interventions affect cardiovascular outcomes in ESRD children with different Klotho G-395A genotypes. This may include testing dialysis, blood pressure control, and other therapies in this population.

## CLINICAL APPLICATIONS

For clinical practice, the study suggests using the Klotho G-395A polymorphism as a biomarker for pediatric ESRD patients at higher risk for cardiovascular complications. Genetic screening for this polymorphism can help stratify these patients' risks. Customized Treatment: Young ESRD patients' genetic profiles may inspire more customized treatment plans. A allele or GA+AA genotype patients may benefit from more vigorous blood pressure and cardiovascular risk factor management. The study may help develop targeted cardiovascular health interventions to mitigate the Klotho G-395A polymorphism. To boost Klotho expression or activity, investigate therapies. The study found that the A allele increases cardiovascular risk factors like systolic, diastolic, and hemodialysis duration. This helps chronic kidney disease children predict cardiovascular risk. Diastolic blood pressure and the A allele in GA or AA genotypes predict cardiovascular complications, the study found. Monitoring Cardiovascular Parameters: Children with chronic kidney disease with GA+AA genotypes had higher pre- and post-dialysis blood pressure, left ventricular mass, and relative wall thickness, indicating the need for more cautious monitoring. Genetic factors are generally important in the development of cardiovascular complications in young ESRD patients. Based on these findings, future research should better understand the Klotho G-395A polymorphism and develop better cardiovascular disease

prevention and control strategies for this susceptible population.

## CONCLUSION

This study provides evidence that the Klotho G-395A polymorphism is a significant genetic risk factor for the development of cardiovascular complications in pediatric CKD patients, particularly those on hemodialysis. The findings suggest the potential utility of the Klotho G-395A genotype as a biomarker for cardiovascular risk assessment in this patient population.

## ABBREVIATIONS

| | | | |
|---|---|---|---|
| **ABPM** | Ambulatory blood pressure monitoring | **LSD** | Least significant difference |
| **ANOVA** | Analysis of variance | **LVEDD** | Left ventricular end-diastolic diameter |
| **CKD** | Chronic kidney disease | **LVH** | Left ventricular hypertrophy |
| **CVD** | Cardiovascular disease | **LVMI** | Left ventricular mass index |
| **DBP** | Diastolic blood pressure | **LVM** | Left ventricular mass |
| **DCM** | Dilated cardiomyopathy | **PCR** | Polymerase chain reaction |
| **DT** | Deceleration time | **PTH** | Parathyroid hormone |
| **EF** | Ejection fraction | **PWT** | Posterior wall thickness |
| **E/A** | Early to late ventricular filling velocities | **RWT** | Relative wall thickness |
| **ESRD** | End-stage renal disease | **SBP** | Systolic blood pressure |
| **HD** | Hemodialysis | **SNP** | Single nucleotide polymorphism |
| **IVST** | Interventricular septum thickness | | |

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## AUTHORS' CONTRIBUTIONS

**The submitted manuscript is the work of the author & co-author.**
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**Approval of the version of the manuscript to be published:** All authors**.**

## STATEMENTS

**Consent for publication and exclusive submission:** The contents and material of the manuscript have not been previously reported at any length or being considered for publishing elsewhere.
**Ethics approval and consent to participate:**
This study protocol and the consents were approved and deemed sufficient by the Ethical Committee of Benha university hospital (No MS 22-3-2022) and informed written consent was obtained in every case from their legal guardians.
**Availability of data and material:**
The data and material are factual and genuine.
**Conflict of interest:**
The authors declare no conflict of interest.
**Funding:**
The authors declare that this research work did not receive any funds.
**Acknowledgement:**
Authors would like to thank all patients and their family members for their valuable contributions to the study.

**Submitted: 30/04/2025**
**Accepted: 20/05/2025**
**Published online: 30/06/2025**