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# Soluble Klotho Levels in Prediction of Premature Vascular Aging in Children with Chronic Kidney Disease

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## ABSTRACT

**Background:** In pediatrics, chronic kidney disease (CKD) and its accompanying cardiovascular (CV) adverse events are frequently predominant. The degree of vascular calcification could be utilized as a measure of biologic vascular age, suggesting that cases with kidney disorders could display an accelerated form of vascular ageing. CKD is a pathological condition characterized by Klotho deficiency as it is a primary source of circulating Klotho.

**Aim:** This study aimed to correlate soluble klotho (s-klotho) as a new biochemical marker to premature vascular aging to achieve better outcomes in children with CKD.

**Methods:** This prospective observational study included 90 children and divided into three groups, group 1 included 30 cases of pre-dialysis patients, group 2 included 30 cases of dialysis patients and group 3 included 30 healthy children of matched age and sex. Measurement of serum klotho level was done by ELISA. Ultrasound assessment of intimal thickness in the right and left common carotids was done.

**Results:** The mean SBP, mean DBP and stage of kidney disease were statistically significantly lower in the CKD group compared to the dialysis group. There was insignificant difference between the three groups regarding serum klotho level and carotid intima-media thickness of right common carotid (CC), left CC, minimum diameter and maximum dimeter at baseline and at follow up.

**Conclusion:** There was no correlation between serum klotho level with carotid artery intima media thickness (CIMT) either at baseline or during follow up and no relationship between it and CKD stage. Lack of significant correlation could be due to small included sample size.

**Keyword:** Klotho, Vascular aging, CKD.

## INTRODUCTION

Chronic kidney disease (CKD) is a global health issue causing increased mortality and morbidity, particularly among the aging global population [1]. It presents by several renal abnormalities, comprising diminished glomerular filtration rate, a decreased renal mass, and renal fibrosis [2]. In pediatrics CKD and its accompanying cardiovascular (CV) complications are frequently predominant [3].

Aging is a well-known risk factor for mortality in a group of "burden of lifestyle diseases", which include CKD, that is considered as a prototype disease leading to premature vascular aging [4]. Premature aging in CKD have become more critical health troubles in present societies. In CKD, cases exhibit cellular changes distinctive of raised inflammation. Further etiologies of the premature vascular aging noticed in CKD involve uremia, hemodialysis (HD), and increased phosphorus pools and angiotensin II [4, 5].

The pathogenesis of premature vascular ageing in CKD is complex, although humoral factors in specific are believed to have an important role, which involve phosphorus, hormones, which include parathyroid hormone (PTH), angiotensin II, and uremic toxins [6].

Uremic toxins including FGF-23–klotho pathway axis and the transcription factor NRF2, all are pathways that are considered as potential etiologies for premature aging process in CKD and are associated with hallmarks of the aging process [7]. In addition, cases with CKD develop calcified intimal vessel together with atherosclerosis, which could happen concurrently with medial calcification. On the other hand, studies recommend that mortality is related to arterial stiffness, a result of medial calcification. Essentially, the degree of calcified vessel could be utilized as a tool to assess the biologic vascular age, suggesting that cases with kidney diseases could display a progressive form of vascular ageing [8, 9].

Klotho is an aging suppressor gene [10]. Klotho was first described as a novel protein by Kuro-O**.** It is expressed in numerous tissues. The highest values of Klotho are determined in the cerebral and renal tissues. In addition, this protein is also expressed in the myocardium, but to minor degree [11]. It isn't surprising that CKD is a condition of Klotho deficits as the kidneys are the main source of plasma Klotho. Diminished plasma values and inhibited renal Klotho expression might or mightn't be accompanied by negative outcomes in CKD cases [12] . Therefore, this study aimed to correlate s-klotho as a new biochemical marker to premature vascular aging to achieve better outcomes in children with CKD.

## PATIENTS AND METHODS

This prospective observational study was held at Mansoura University Children Hospital (MUCH), Mansoura, Egypt, (from June 2021 to June 2022). The study included 90 children younger than 18 years old and divided into three groups, group 1 included 30 cases of pre-dialysis patients (from stage 1- stage 4 according to Kidney dysfunction), group 2 included 30 cases of dialysis patients (stage 5 according to kidney



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dysfunction) and group 3 (control group) included 30 healthy children of matched age and sex (control group) from the General Outpatient Clinic of MUCH with no comorbidities and came for routine follow up.

**Inclusion criteria:** All stages of CKD (stages I to V) were classified by a glomerular filtration rate (GFR) and were eligible for the study regardless of renal injury and kidney failure treated by dialysis [13].
**Exclusion criteria:** Patients with acute kidney diseases, renal transplantation, leukemia, rheumatoid arthritis or other collagen diseases and diabetic patients.

**Methods:** All children incorporated in this study were subjected to **full history taking** comprising personal history, present history about primary cause of CKD and whether they were referred by a pediatric nephrologist or accidently discovered, past history (admission to hospital, developmental history, nutritional history, vaccination history and perinatal history), and family history (parent's consanguinity and history of similar condition in family members).

**Clinical examination** included vital signs, and anthropometric measurement [weight (kg), height ($m^2$) & BMI ($kg/m^2$)]. Local examinations included local abdominal examinations and local cardiac examinations.

**Laboratory investigations** included calcium (Ca), phosphorus (P), serum creatinine (Ser Cr) and GFR calculation using the revised schwarts formula as following: GFR (ml/min/1.73 $m^2$) = 0.413 x height (Ht) (cm)/serum Cr [14]. Measurement of serum klotho level was done by sandwich ELISA technology.

Ultrasound assessment of intimal thickness in the right and left common carotids (CCA) was done by a single skilled vascular sonographer, blinding to the data of the subjects. The images were captured by utilizing a General Electrical medical US machine equipped with 7.5–10MHz linear-array transducer. Carotid arteries imaging was conducted with the child resting in the supine position with extended neck, and the head turned 45° toward the opposite side.

A longitudinal section of the CCA one cm superior to the carotid bulb was captured to accomplish reliable area of measurement. Three maximal intima-media thickness (IMT) measurements of the far wall of the artery at three-millimeter intervals were acquired beginning at one cm superior to the bulb and moving superiorly. The recorded IMT was the mean of 3 measurements and the recorded IMT for each case was the mean of the 6 measurements (on both sides). Magnified vessel wall permits simple recognition of the IMC, described by the margin between the echo-lucent vessel lumen and the echogenic intima and the border between the echo-lucent media and echogenic adventitia [15, 16]. Follow up was conducted following one year by US evaluation of carotid IMT.

**Ethical consideration: The study design was approved by the IRB of Mansoura Faculty of Medicine. (MS.21.06.1536). Confidentiality was respected. A written informed consent was acquired from all the legal guardian of the cases before the study. The study followed The Declaration of Helsinki through its execution.**

**Statistical Analysis**

Data were analysed using the SPSS program. The one-sample Kolmogorov-Smirnov test was used to evaluate the data's normality. Numbers and percentages were used to describe the qualitative data. To evaluate the association between categorical variables, the Chi-square test was employed. When the anticipated cell count was fewer than five, the Monte Carlo and Fisher exact tests were employed. The continuous variables were displayed as median for non-normal data and mean ± SD for properly distributed data. The independent t test (parametric) and the U test (nonparametric) were utilized to compare the two groups and the paired t test was utilized to compare the paired groups. The one-way ANOVA test was utilized to compare more than two groups. Continuous data were correlated using both non-parametric Spearman correlation and parametric Pearson correlation. The level of significance was set at 5% level (p-value).

**RESULTS**

Table **(1)** displayed that there was insignificant difference between the three studied groups regarding the age, sex distribution and anthropometric measurements including weight, height and BMI. The mean SBP was significantly lower in the CKD group compared to the remaining two groups (**p<0.001**). In contrast, there was insignificant difference between the dialysis and control group regarding the SBP (p =0.254). The mean DBP was significantly lower in the CKD group compared to the dialysis group (**p<0.001**) and control group (**p<0.001**). Additionally, there was insignificant difference between the dialysis and control groups regarding the DBP (p =0.254). The mean pulse was significantly increased in the HD group compared to the CKD group (**p<0.001**) and the controls (**p<0.001**). Additionally, there was insignificant difference between the CKD and control group regarding the pulse rate (p =0.259). The serum klotho value was 4231.13± 955.5, 4175.87± 552.42 and 4338.77± 555.6 in the CKD group, dialysis group and control group respectively. The serum klotho level demonstrated insignificant differences between the three groups.

**Table (1):** Comparison of demographic data, Anthropometric measurements, vital data and serum klotho level among the studied groups

| **Demographic data** | **CKD group (no=30)** | **Dialysis group (no=30)** | **Control group (no=30)** | **Test of significance** | | |
|---|---|---|---|---|---|---|
| | | | | **P1** | **P2** | **P3** |
| **Age (Years)** Mean ± SD | **9.48±4.36 2-18** | **11.38±3.61 4-18** | **9.70±4.29 2-18** | **t=0.194 P=0.847** | **t=1.64 P=0.106** | **t=1.83 P=0.071** |
| **Sex** Male Female | 17 (56.7%) 13 (43.3%) | 17 (56.7%) 13 (43.3%) | 17 (56.7%) 13 (43.3%) | $\chi^2$=0 P=1 | $\chi^2$=0 P=1 | $\chi^2$=0 P=1 |
| **Weight (kg)** Mean ± SD | 30.65±17.91 | 28.50±11.94 | 35.71±17.79 | t=1.09 P=0.276 | t=1.84 P=0.07 | t=0.547 P=0.587 |
| **Height (cm)** Mean ± SD | 122.43±27.14 | 124.37±17.1 | 134.73±24.96 | t=1.82 P=0.073 | t=1.87 P=0.066 | t=0.330 P=0.742 |
| **BMI (kg/m$^2$)** Mean ± SD | 18.83±5.27 | 17.73±3.92 | 18.49±2.91 | t=0.306 P=0.761 | t=0.851 P=0.398 | t=0.914 P=0.365 |
| **SBP (mmHg)** Mean ± SD | 104.50±7.69 | 118.0±15.84 | 114.33±7.27 | t=5.08 P≤0.001* | t=1.15 P=0.254 | t=4.198 P≤0.001* |
| **DBP (mmHg)** Mean ± SD | 66.00±7.23 | 76.67±10.93 | 75.67±5.68 | t=5.75 P≤0.001* | t=0.444 P=0.658 | t=4.45 P≤0.001* |
| **Pulse (beat/min)** Mean ± SD | 74.00±6.21 | 84.67±11.36 | 72.17±4.49 | t=1.13 P=0.259 | t=5.60 P≤0.001* | t=4.51 P≤0.001* |
| **Serum klotho level** Mean ± SD | 4231.13± 955.5 | 4175.87± 552.42 | 4338.77± 555.6 | t=0.533 P=0.596 | t=1.14 P=0.259 | t=0.274 P=0.785 |

**t:** Independent t- test, **$\chi^2$:** Chi square test, **P1:** CKD vs. control, **p2:** Dialysis vs. control, **p3:** CKD vs. dialysis groups.

Table **(2)** displayed that accidental discovery of the condition was reported in 20% and 16.7% in the CKD and dialysis groups correspondingly. In the CKD group, the most common primary disease was atrophied kidney in 20%, PUV in 16.7% then neurogenic bladder and polycystic kidney in 10% each. In the dialysis group, the most common primary disease was atrophied kidney and HUS in 13.3% for each.

**Table (2):** Primary disease among the studied groups

| **Primary disease** | **CKD group (no=30)** | **Dialysis group (no=30)** |
|---|---|---|
| **PUV** | 5 (16.7%) | 1 (3.3%) |
| **Polycystic kidney** | 3 (10.0%) | 2 (6.7%) |
| **Congenital nephrotic syndrome** | 1 (3.3%) | 0 (0%) |
| **Accidently discovered CKD** | 6 (20.0%) | 5 (16.7%) |
| **Atrophied kidney** | 6 (20.0%) | 4 (13.3%) |
| **Vertebral anomaly** | 1 (3.3%) | 0 (0%) |
| **Neurogenic bladder** | 3 (10.0%) | 1 (3.3%) |
| **Solitary kidney** | 2 (6.7%) | 0 (0%) |
| **VUR** | 2 (6.7%) | 0 (0%) |
| **Renal artery stenosis** | 1 (3.3%) | 0 (0%) |
| **HUS** | 0 (0%) | 4 (13.3%) |
| **RPGN** | 0 (0%) | 2 (6.7%) |
| **Cystinosis** | 0 (0%) | 1 (3.3%) |
| **congenital hepatic fibrosis and CKD** | 0 (0%) | 1 (3.3%) |
| **Iatrogenic renal artery cutoff during surgery** | 0 (0%) | 1 (3.3%) |
| **Glomerulonephritis** | 0 (0%) | 1 (3.3%) |
| **Idiopathic CKD** | 0 (0%) | 1 (3.3%) |
| **Alport syndrome** | 0 (0%) | 1 (3.3%) |
| **SRNS** | 0 (0%) | 1 (3.3%) |
| **Hydronephrosis** | 0 (0%) | 1 (3.3%) |
| **Nephronophthiasis** | 0 (0%) | 1 (3.3%) |
| **Hyperammonemia** | 0 (0%) | 1 (3.3%) |
| **Unknown cause** | 0 (0%) | 1 (3.3%) |

Table **(3)** displayed that the disease duration was significantly longer in the CKD group compared to the dialysis group (p<0.001). Family history was reported in 13.3% of the CKD group. There was a significant difference between both groups regarding the stage of kidney disease. The Ser Cr (p<0.001), serum phosphorous (p = 0.006) were statistically significantly increased in the dialysis group compared to the CKD group. In contrast, the serum calcium (p= 0.05) and estimated GFR (p<0.001) were statistically significantly greater in the CKD group compared to the dialysis group.

**Table (3):** Comparison of duration of illness, family history, stage of kidney disease, serum creatinine, calcium, phosphorus and estimated GFR among cases with CKD and cases on dialysis

| | **CKD group (no=30)** | **Dialysis group (no=30)** | **Test of significance** |
|---|---|---|---|
| **Duration of illness (years)** Mean ± SD | 6.07±3.54 | 3.25±1.86 | t=3.56 P≤0.001* |
| **Family history** | | | |
| Positive | 4 (13.3%) | 0 (0%) | FET |
| Negative | 26 (86.7%) | 30 (100%) | P=0.11 |
| **Stage of kidney disease** | | | |
| I | 2 (6.7%) | 1 (3.3%) | MC |
| II | 5 (16.7%) | 0 (0%) | P≤0.001* |
| III | 7 (23.3%) | 0 (0%) | |
| IV | 9 (30.0%) | 0 (0%) | |
| ESRD | 7 (23.3%) | 29 (96.7%) | |
| **Serum creatinine (mg/dl)** Median (Min-Max) | 1.8 (0.5-.5) | 9.23 (0.5-14.3) | Z=5.84 P≤0.001* |
| **Serum calcium (mg/dl)** Mean ± SD | 9.27±0.95 | 8.74±1.08 | t=2.00 P=0.05* |
| **Serum phosphorus (mg/dl)** Mean ± SD | 4.60±1.05 | 5.74±1.91 | t=2.85 P=0.006* |
| **Estimated GFR** Median (Min-Max) | 24 (6.7-112.3) | 5.5 (3.7-123) | Z=6.08 P≤0.001* |

t: Independent t- test, FET: Fisher exact test, MC: Monte carlo test

Table **(4)** displayed that there was insignificant difference between the three study groups regarding the CIMT of the right CC, the left CC, minimum diameter and maximum dimeter at baseline and at follow up. The right CC and left CC showed a significant increase during follow up compared to baseline in the dialysis group only. The maximum diameter at follow up showed a significant decrease compared to baseline in the dialysis group only, but not in the CKD group, where the reduction didn't achieve a significant value. The minimum diameter at follow up showed a significant decrease compared to baseline in the CKD group only, but not in the dialysis group, where the reduction didn't achieve a significant value.

**Table (4):** CIMT at baseline and after follow up among the studied groups

| CIMT | CKD group (no=30) | Dialysis group (no=30) | Control group (no=30) | Test of significance | | |
|---|---|---|---|---|---|---|
| | | | | P1 | P2 | P3 |
| **Right CC baseline** | 0.41±0.08 | 0.39±0.06 | 0.37±0.06 | t=1.24 P=0.223 | t=0.845 P=0.406 | t=0.629 P=0.533 |
| **Right CC Follow up** | 0.38±0.08 | 0.43±0.06 | - | - | - | t=1.25 P=0.232 |
| Paired t test P value | t=2.45 P=0.07 | t=2.78 P=0.021* | - | - | - | - |
| **Left CC baseline** | 0.42±0.09 | 0.39±0.07 | 0.36±0.05 | t=1.91 P=0.067 | t=1.01 P=0.322 | t=1.197 P=0.240 |
| **Left CC Follow up** | 0.44±0.09 | 0.42±0.06 | - | - | - | t=0.370 P=0.717 |
| Paired t test P value | t=2.14 P=0.09 | t=2.41 P=0.04* | - | - | - | - |
| **Maximum diameter baseline (mm)** | 5.93±0.78 | 5.97±1.09 | 5.44±0.82 | t=1.59 P=0.123 | t=1.376 P=0.181 | t=0.160 P=0.874 |
| **Maximum diameter Follow up (mm)** | 5.44±0.23 | 5.76±1.25 | - | - | - | t=0.554 P=0.589 |
| Paired t test P value | t=1.75 P=0.155 | t=2.52 P=0.033* | - | - | - | - |
| **Minimum diameter baseline (mm)** | 4.67±0.68 | 5.01±0.88 | 4.60±0.65 | t=0.267 P=0.792 | t=1.32 P=0.196 | t=1.31 P=0.199 |
| **Minimum diameter Follow up (mm)** | 4.10±0.16 | 4.88±1.15 | - | - | - | t=1.48 P=0.163 |
| Paired t test P value | t=11.0 P≤0.001* | t=1.54 P=0.16 | - | - | - | - |

Table **(5)** displayed that there was a significant positive correlation between serum klotho level and estimated GFR in the dialysis group (r= 0.518, p = 0.003), while there was a significant negative correlation between serum klotho level and estimated serum creatinine in the dialysis group (r=-0.467, p=0.009). Other correlations didn't show a significant correlation. There was insignificant correlation between serum klotho level with CIMT either at baseline or during follow up.

**Table (5):** Correlation between Serum creatinine, estimated GFR, stage of kidney disease, CIMT and serum klotho level

| | Serum klotho level | | | |
|---|---|---|---|---|
| | CKD group (no=30) | | Dialysis group (no=30) | |
| | r | p | r | p |
| **Serum Creatinine** | 0.217 | 0.249 | -0.467 | 0.009* |
| **Estimated GFR** | -0.220 | 0.244 | 0.518 | 0.003* |
| **Stage of kidney disease** | 0.214 | 0.255 | -0.268 | 0.152 |
| **CIMT** | | | | |
| → Right CC baseline | 0.117 | 0.633 | -0.199 | 0.443 |
| → Right CC Follow up | 0.685 | 0.202 | -0.147 | 0.685 |
| → Left CC baseline | 0.192 | 0.432 | -0.121 | 0.644 |
| → Left CC Follow up | 0.406 | 0.497 | -0.056 | 0.879 |
| → Maximum diameter baseline | -0.181 | 0.458 | -0.052 | 0.844 |
| → Maximum diameter Follow up | -0.143 | 0.819 | 0.138 | 0.705 |
| → Minimum diameter baseline | 0.040 | 0.872 | -0.119 | 0.649 |
| → Minimum diameter Follow up | 0.458 | 0.438 | 0.061 | 0.868 |

Table **(6)** displayed that there was insignificant difference in the serum klotho level as compared based on the stage of CKD (p= 0.553).

**Table (6):** Association between stage of kidney disease and Serum klotho level

| Stage of kidney disease | Serum klotho level | Test of significance | P value |
|---|---|---|---|
| I | 4148.0±1120.60 | F=0.764 | 0.553 |
| II | 3726.4±502.50 | | |
| III | 4521.6±240.61 | | |
| IV | 4231.3±553.31 | | |
| ESRD | 4205.6±881.41 | | |

F: ANOVA test.

## DISCUSSION

CKD has been considered as a major health problem recently. Alterations in metabolic processes could happen due to impaired kidney functions [17]. The uremic type of CKD presents by an increased rate of accompanying age-related adverse events, which include vascular stiffening, osteoporosis, loss of muscle power, depressive manifestations, impaired cognition, and weakness [18]. Concerning aging, this may comprise numerous mediators, comprising oestrogen, androgen, L-arginine, and klotho [19].

Low klotho protein values were linked to adverse CV outcomes, as assessed by **Ságі *et al.*** [20] in adult cases with CKD on HD, comprising twenty four percent of HD cases secondary to diabetic nephropathy. Decreased s-klotho values were demonstrated in those cases; on the other hand, the investigators did not identify their correlation with vascular calcification.

However, there is shortage in studies about the association with s-klotho in pediatrics with CKD. So, our research was conducted to correlate s-klotho as a new biochemical marker to premature vascular aging to achieve better outcomes in children with CKD. The current study included 90 children who were distributed in 3 groups (each of 30 children). Group 1 included children with CKD (before dialysis) and group 2 included children on dialysis. Both groups were recruited from The Nephrology Unit of MUCH. Group 3 included healthy age- and sex-matched children as a control group who were recruited from General Outpatient Clinic of MUCH.

In this study, there was insignificant difference between the three studied groups regarding the age and sex distribution. The mean age was 9.48±4.36 years and 11.38±3.61 years in the CKD and dialysis groups respectively. Regarding sex distribution, males represented 56.7% and females represented 43.3% in both CKD and dialysis groups. This comes in agreement with **Sallam *et al.*** [21] who conducted a study that was conducted on 87 individuals, who were divided into three groups equally; CKD stage V on regular HD, CKD stage II-IV, & age- & sex-matched controls. The results displayed that there was insignificant difference between the studied groups concerning the age and sex distribution. In contrast, the current results disagree with **Afifi *et al.*** [22] who included 30 cases diagnosed with CKD on hemodialysis, 10 cases diagnosed with CKD and on conservative treatment based on Schwartz formula and 10 healthy children as controls. Their results revealed that age was significantly different between both groups.

There was insignificant difference between the three studied groups regarding the anthropometric measurements including weight, height and BMI. The current results are in disagreement with **Ghobrial *et al.*** [23] who displayed that there was a significant difference between the three groups in terms of weight percentiles (**P<0.001**). The study found that 70% of end-stage renal disease (ESRD) cases and 45.2% of CKD cases had weight below the 3rd percentile compared to healthy controls. Furthermore, this disagrees with **Afifi *et al.*** [22] who displayed that there were significant differences between patient' groups and control regarding weight percentiles. Dialysis group was more affected. Also, in contrast with current study, **Rodig** [24] found that the degree of weight affection increases as GFR drops, although a significant reduction in weight was detected at all renal function levels.

The mean SBP and DBP in our study were statistically significantly lower in the CKD group compared with the HD group (**p<0.001**) and controls (**p<0.001**). This is in accordance with **Mahmoud *et al.*** [25] who displayed that there was a significant increase of the numbers of hypertensive cases in HD group cases compared to CKD groups (p<0.01). However, this disagrees with **Ghobrial *et al.*** [23] who displayed that blood pressure were significantly increased in the CKD group compared to ESRD group (P <0.05).

In the current study, the disease duration was statistically significantly longer in the CKD group (p<0.001). There was a significant difference between both groups regarding the stage of kidney disease. Longer disease was reported in the CKD group stage I, II, III, IV and ESRD as 6.7%, 16%, 23.3%, 30% and 23.3% respectively. In the dialysis group, 96.7% of the cases were at ESRD. This agrees with **Ezzat *et al.*** [26] who included 50 children in their study, 20 were on maintenance dialysis for ESRD, ten were on conservative treatment for CKD, and ten were healthy control. The study revealed that the disease duration was accompanied by a significant increase in the CKD group. However, the current results are opposite to **Ghobrial *et al.*** [23] who showed that the age of onset of the main disease differed significantly between the CKD and ESRD (P=0.027).

In the current study, serum Cr (p<0.001) and serum phosphorous (p=0.006) were statistically significantly greater in the dialysis group compared to the CKD

group. In contrast, serum Ca (p=0.05) and estimated GFR (p<0.001) were statistically significantly greater in the CKD group compared to the dialysis group. This agrees with **Ghobrial *et al.*** [23] who displayed that serum Cr was significantly diminished in the CKD group compared to ESRD group, whereas Ca, and phosphorus were associated with a significant elevation in the CKD group compared to ESRD group (P< 0.05).

Of note, the CIMT examination allows clinicians to assess subclinical changes in wall structure that can predict future cardiovascular clinical events [27]. In the current study, there was insignificant difference between the three study groups regarding the CIMT of the right CC, the left CC, minimum diameter and maximum dimeter at baseline and at follow up. Likewise, **El Khayat *et al.*** [28] showed that the CKD group and dialysis group had comparable right and left ICA and CCA IMT. On the contrary, **Kumar *et al.*** [29] stated that CIMT in ESRD children was greater than that in the controls even before initiating dialysis.

The mean level of serum klotho in the current study was 4231.13±955.5, 4175.87±552.42 and 4338.77±555.6 in the CKD group, dialysis group and healthy controls respectively. There was insignificant difference between the three groups concerning the serum klotho level. This was opposite to **Gamrot *et al.*** [30] who showed a significant elevation in serum klotho compared to healthy children in a control group. The current results also contradict those of **Keryakos *et al.*** [31] who included 40 hemodialysis patients and 20 healthy controls. Their results showed that serum klotho value was significantly diminished in HD cases compared to healthy controls.

Our study revealed that there was insignificant difference in serum klotho value as compared based on the stage of CKD (p=0.553). In one study, a major study of 312 cases with stages II–IV CKD were followed and it was displayed that the level of s-klotho wasn't accompanied by the GFR [12]. **Kim *et al.*** [32] recorded conflicting outcomes where they demonstrated that the basal s-klotho could be used as an indicator for CKD advancement in a study of 243 cases with stages I–V CKD after five years of follow-up. Also, opposite to the current study, **Qian *et al.*** [33] displayed a significant reduction in the basal plasma α-klotho level from stage I-II cases to stage V cases. A significant negative association was recorded between basal klotho value and CKD stages. **Seiler *et al.*** [12] analysed plasma values of Klotho among cases in CKD stages II–IV and revealed no decrease with disease progression. The investigators displayed no association between Klotho values and estimated GFR or parameters accompanied by Ca-P metabolism.

In the current study, there was a significant positive relationship between serum klotho level and estimated GFR in the dialysis group (r=0.518, p=0.003), while there was a significant negative relationship between serum klotho level and estimated serum creatinine in the HD group (r=-0.467, p=0.009). Other correlations didn't show a significant correlation. This agrees with **Qian *et al.*** [33] who included 112 adults with stages I–V CKD in their study. The results showed that the basal plasma klotho values were positively accompanied by basal estimated GFR (p=0.017), but not age, Ca, phosphorus, or PTH values. In the same line, a comparable positive relationship between plasma klotho levels and estimated GFR was demonstrated in children with CKD. This result suggests that the reduction in s-klotho could mirror an estimated GFR reduction in cases with CKD [34]. On the other hand, some authors display negative results. **Seiler *et al.*** [12] conducted their study on a total of 312 patients (stage II-IV CKD) and demonstrated that klotho value weren't significantly accompanied by estimated GFR or other Ca-P metabolism parameters in these cases.

There was insignificant correlation between klotho levels with CIMT either at baseline or during follow up in our study. However, this is in disagreement with **Wungu *et al.*** [35] who revealed that Klotho was negatively correlated with arterial calcification ($p = 0.001$) and CIMT ($p < 0.00001$). The difference could be due to differences in the degree of disease severity and affection in the included cases. Despite the obtained results, there are some limitations in our study mainly in the form of small sample size included and being a single center study.

## CONCLUSION

We included that there was no correlation between serum klotho level with CIMT either at baseline or during follow up and no correlation between it and the CKD stage. The lack of significant correlation may be due to the small included sample size as was illustrated in the study limitations, but this point could be handled in subsequent studies.

**Conflict of interest:** None.
**Fund:** None.

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