Evaluation of Subclinical Uremic Cardiomyopathy using Speckle Tracking Echocardiography Serum Soluble Klotho and Fibroblast Growth Factor-23

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Abstract

Background: Uremic cardiomyopathy (UC) is being described to contribute to left ventricular (LV) dysfunction. Fibroblast growth factor-23 (FGF-23) and soluble alpha-Klotho (a-klotho) are thought to be involved in the pathogenesis of UC. Despite heart failure being the most common cardiovascular disease in chronic kidney disease (CKD), ejection fraction evaluation is still challenging.

Objectives: We aimed to evaluate the myocardial performance of both ventricles in uremic patients using speckle tracking echocardiography (STE) and its possible relation to serum soluble a-Klotho and FGF-23.

Patients and methods: This cross-sectional study included 45 hemodialysis (HD) patients and 45 patients with moderate CKD stages 3 and 4. Global longitudinal strain (GLS) and right ventricular free-wall strain (RVFWS) obtained by STE were used to evaluate both ventricular performances.

Results: Impaired GLS ≤ 16% was found in 46.7% of HD patients and 28.9% of CKD patients (p = 0.082). Impaired RVFWS ≤ 20% was found in 44.4% of HD patients and 24.4% of CKD patients (p = 0.046). In HD patients, impaired GLS is associated with a history of hypertension (p = 0.011), left ventricular mass index (LVMI) (p=0.041), and RVFWS (p = 0.030). The history of hypertension in HD patients (p = 0.013) and LVMI in CKD patients (p = 0.051), independently predicted compromised GLS.

Conclusion: Reduced subclinical LV and right ventricular systolic function existed in patients with moderate CKD and became worse in dialysis patients. The history of hypertension in HD patients and LVMI in CKD were key determinants of impaired GLS.

Keywords: Fibroblast growth factor-23; Global longitudinal strain, soluble aKlotho; Speckle tracking echocardiography; Uremic cardiomyopathy.

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Introduction

Cardiovascular (CV) complications represent the foremost cause of increased morbidity and mortality among patients with chronic kidney disease (CKD). Cardiovascular disease (CVD) manifests earlier in the progression of CKD, affecting approximately 30 to 44% of individuals initiating hemodialysis (HD) **(Go et al., 2004; Saran et al., 2018).**

Uremic cardiomyopathy (UC) is marked by structural remodeling at both macroscopic and cellular levels, leading to alterations in ventricular function **(Gross and Ritz, 2008).** Patients with CKD and those undergoing HD are subjected to factors such as hemodynamic overload including pressure and volume overload, neurohormonal activation, a high output state due to the creation of an arteriovenous fistula (AVF) and significant microvascular dysfunction. These factors are believed to be implicated in the development of left ventricular (LV) diastolic and systolic dysfunction **(Ori et al., 1996; Maisel et al., 2011).**

The biochemical alterations linked to chronic kidney disease-mineral and bone disorder (CKD-MBD) are believed to play a direct or indirect role in the development of CKD-associated cardiomyopathy **(de Albuquerque Suassuna et al., 2018).** Fibroblast growth factor-23 (FGF23) is a phosphaturic hormone produced by osteocytes and osteoblasts **(Shimada et al., 2005).** Alpha-Klotho (a-klotho), primarily secreted by the kidneys, functions as a co-receptor for FGF23 **(Hu et al., 2016).**

FGF-23 levels begin to rise early in the progression of CKD and reach very high levels in dialysis patients **(Hu et al., 2013).** Conversely, circulating levels of soluble a-klotho decrease as CKD advances. A relationship between serum levels of FGF23 and soluble a-klotho and CV disease, especially myocardial hypertrophy has been suggested **(Grabner and Faul, 2016).**

It is important to acknowledge that despite the high prevalence of CV events and the worsening symptoms of heart failure (HF), the ejection fraction (EF) stays intact in most patients with CKD and regular HD. This discrepancy arises from the intricate pathophysiology of CV disease and the technical difficulties associated with EF evaluation in this population **(Park et al., 2012; Krishnasamy et al., 2015).**

Two-dimensional speckle tracking echocardiography (2D STE) with myocardial strain analysis is an automated, operator-independent method for evaluating LV systolic function through the measurement of global longitudinal strain (GLS). This technique uses natural acoustic markers (speckles) distributed throughout the myocardium, which are tracked throughout the cardiac cycle to trace myocardial motion. The gathered data can be processed to accurately identify regional segments with impaired contractility, such as scar tissue (**Liu et al., 2009).**

Global longitudinal strain measurements can identify subtle disruptions in viability and subendocardial contractility, commonly occurring before noticeable LV dysfunction, as evident by a reduction in EF (**Leitman et al., 2004).** Similarly, right ventricular (RV) strain can be assessed using the RV free wall in an RV-focused apical 4-chamber view. The strain values from the three segments of the RV free wall (basal, mid and apical) are averaged to determine the RV free wall strain (RVFWS) **(Fine et al., 2015).**

The aim of the present study was to compare the myocardial performance of patients with moderate CKD stages 3 and 4 and patients on regular HD. The diastolic function in both groups was evaluated by conventional transthoracic echocardiography (TTE). 2D STE was used to compare LV and RV systolic functions. Furthermore, the possible relationship between serum levels of

soluble a-Klotho and FGF-23 and echocardiographic parameters was tested.

Patients and methods

Patients

This cross sectional study included 90 subjects, divided into 45 maintenance HD patients and 45 patients with CKD stages 3 and 4. They were recruited from the HD unit and the nephrology outpatient clinic at Alexandria Main University Hospital. Dialysis patients were maintained on renal replacement therapy for more than 3 months. They perform three weekly, four hour HD sessions.

CKD patients had moderate to advanced stages of CKD including stage 3 with an estimated glomerular filtration rate (eGFR) of 30-59 ml/min/1.73 m^2 and stage 4 with an eGFR of 15-29 ml/min/1.73 m^2. The evidence of kidney damage was present for more than 3 months. The eGFR was assessed by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation **(Levey and Stevens, 2010).**

Patients with LV EF less than 50%, a history of acute coronary syndromes, moderate to severe valvular heart disease, chronic atrial fibrillation, and chronic inflammatory systemic conditions were excluded from our study.

All patients gave informed consent before undergoing the research. The research complies with the declaration of Helsinki and ethical approval has been obtained from the university ethical committee.

Serum human FGF-23 and soluble a-Klotho concentrations

Venous blood samples were taken after an overnight fast at 8 a.m. In all HD patients, blood was withdrawn before the onset of the midweek HD session (and heparin administration). A serum separator tube was used, and samples were left to clot for 30 minutes before being centrifuged for 15 minutes. The separated serum was immediately frozen at -20C.

Serum human soluble α-Klotho concentrations were determined by the enzyme-linked immunosorbent assay (ELISA) method (Human soluble α-Klotho (S-KLα) ELISA kit; Glory Science, catalog #:90282, Del Rio, Texas, USA) [sensitivity: (minimum detectable concentration) is 18 pg/ml, Intra-assay CV: < 8%, Inter-assay CV: <10%]. Serum human FGF-23 concentrations were also determined by ELISA method (Human fibroblast growth factor-23 (FGF-23) ELISA kit; Glory Science, catalog #:15759, Del Rio, Texas, USA). [Sensitivity: (minimum detectable concentration) is 1 pg/ml, Intra-assay CV: < 8%, Inter-assay CV: <10%].

Venous blood samples were also used to determine serum hemoglobin, creatinine, calcium, phosphorus, parathyroid hormone (PTH), vitamin D, C-reactive protein (CRP) and lipid profile.

Conventional echocardiography

Standard TTE examinations comprised two-dimensional, pulsed-wave (PW) Doppler, PW tissue Doppler imaging (TDI) and M-mode. They were assessed and reported by experienced senior imaging cardiologists according to the European association of Cardiovascular Imaging guidelines utilizing Philips (EPIQ CVxi version, Philips Healthcare, Andover, MA, USA) equipped with an S5-1 phased array transducer. In HD patients, the echocardiographic examination was conducted on a mid-weekday between dialysis sessions to avoid interdialytic weight gain from volume accumulation if carried in the first HD session of the week with 3 day (long) interdialytic interval.

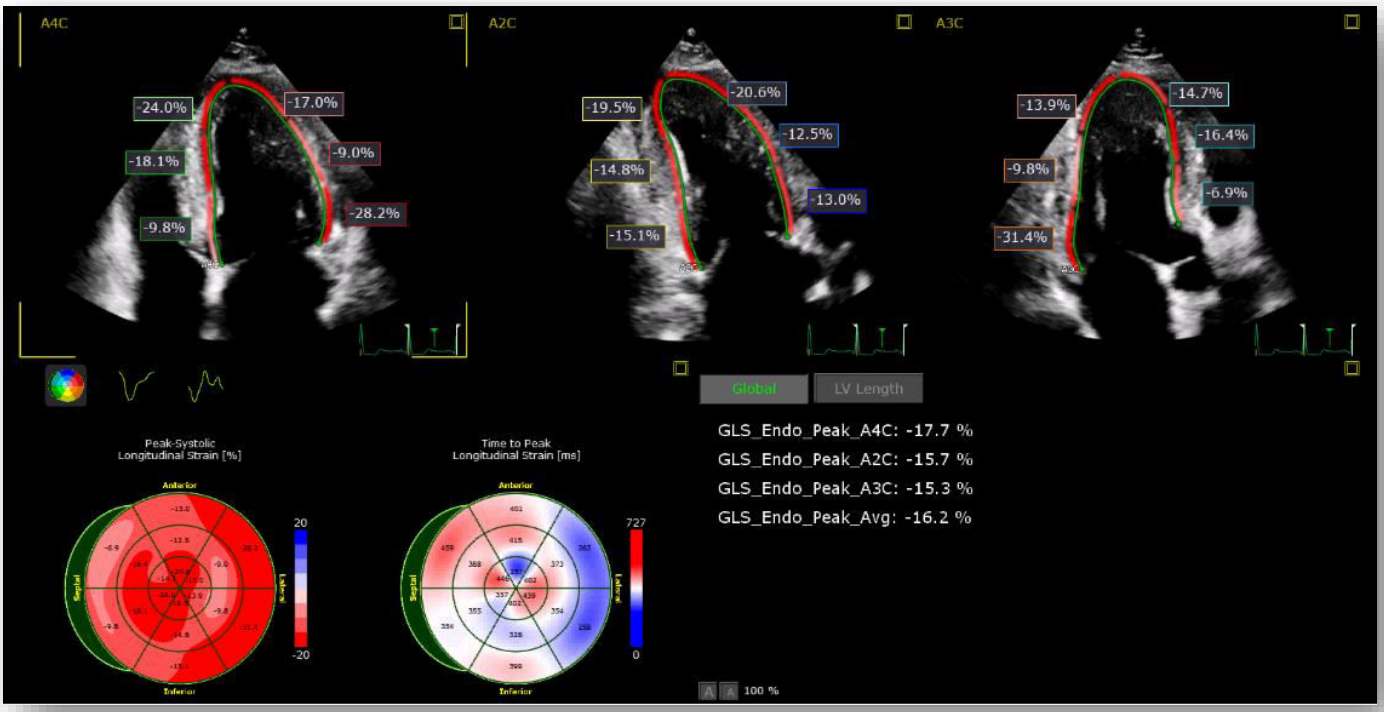
M-mode measurements of LV wall thickness and cavity dimensions, guided by two-dimensional imaging, were obtained in the parasternal long-axis view perpendicular to the LV long axis and assessed at the level of the mitral valve leaflet tips. 2D LV volumes were measured from the apical four- and two-chamber views using the biplane method of disk–summation (modified Simpson's rule) and further indexed by body surface

area (BSA) to calculate LV systolic function.

LV diastolic function was assessed by 1) PW Doppler which was utilized in the apical four-chamber view to acquire mitral inflow velocities for the assessment of LV filling. The measurements of mitral inflow included the peak early diastolic filling velocity (E-wave) and the late diastolic filling velocity (A-wave). 2) PW tissue Doppler imaging was performed in the apical views to acquire mitral annular velocities along with mitral peak E velocity (E/e′ ratio). 3) Measurement of left atrial (LA) volume was obtained using the apical 4-chamber and 2-chamber views.

Speckle-tracking echocardiography

Speckle tracking echocardiography was used to assess LV GLS. With ECG gaited, three standard 2D transthoracic apical views including apical two chamber (A2C), apical three chamber (A3C), and apical four chamber (A4C) views were acquired. Offline analysis using the LV Autostrain software (TOMTEC imaging systems, GmbH Company) was done. The software will automatically generate a topographic representation of all 18 LV segments from which the mean GLS is calculated and displayed automatically by the software. A GLS of less than –16% (GLS ≤16%) indicates reduced LV systolic function **(Fig.1).**



Fi.g.1. Speckle tracking echocardiography. Global longitudinal strain (GLS) measurement from the three standard two-dimensional transthoracic apical views (apical two chamber (A2C), apical three chamber (A3C), apical four chamber (A4C) views). A topographic representation of all 18 LV segments is generated automatically; from which the average GLS is calculated and displayed automatically by the software.

The assessment of the RV free wall was conducted using longitudinal strain measurements obtained from an apically focused RV view. The peak RV longitudinal strain was determined by averaging the peak segmental strain values displayed by the software. An RVFWS of

less than ≤ 20% signifies reduced RV function.

Statistical analysis

Data were entered into a computer and analyzed using IBM SPSS software version 20.0 (Armonk, NY: IBM Corp). Continuous data were tested for normality using the Shapiro-Wilk test. For normally distributed quantitative variables, data were expressed as mean and standard deviation, and comparisons between two groups were made using the Student's t-test. For non-normally distributed quantitative variables, data were expressed as median and interquartile range (IQR), and the Mann-Whitney test was used for comparisons. Categorical data were presented as numbers and percentages. The Chi-square test was employed to examine the relationship between two qualitative variables. Correlation analysis using Pearson's correlation coefficient was used to correlate between two normally distributed quantitative variables. Logistic regression analysis was used to detect the most independent factor predicting the impaired GLS. The significance level was set at 5%.

Results

Baseline characteristics of the study subjects

The baseline characteristics of the study groups are shown in **(Table.1).** A total of 90 subjects, divided into 45 maintenance HD patients and 45 patients with moderate CKD stages 3 and 4 were included. The mean age of the whole studied sample was 53.72 ± 12.96 years, and 50 patients (55.5%) were male. The mean BMI was 29.33 ± 4.67 kg/m^2 and there were more hypertensive patients in the HD group compared to CKD (51.1% vs. 40%; p = 0.290). No significant difference was found between the mean values of systolic and diastolic blood pressures. Approximately 15% of HD patients had an unknown cause of ESRD while 20% of the CKD group had chronic glomerulonephritis as a cause of CKD.

There was a statistically significant increase in serum phosphorous, PTH, vitamin-D, FGF-23 and soluble a-klotho in the HD group compared to the CKD group. Other clinical and laboratory data of the patients are shown in **(Table.1).**

Table 1. Baseline characteristics of HD and CKD groups

Parameters	HD (n = 45)	CKD (n = 45)	Test of Sig.	p
Demographics				
Age (years)	51.33 ± 13.78	56.11 ± 11.76	t=1.769	0.080
Gender (Male)	21 (46.7%)	29 (64.4%)	χ^2=2.880	0.090
BMI (kg/m^2)	26.88 ± 4.14	31.79 ± 6.99	t=4.057*	**<0.001***
BSA (m^2)	1.80 ± 0.23	1.96 ± 0.20	t=3.519*	**0.001***
Clinical characteristics				
Dialysis vintage (years)	5.0 (1.20 – 13.0)	–	–	–
Etiology of CKD /ESRD				
Hypertension	23 (51.1%)	18 (40%)	χ^2=1.120	0.290
Diabetes	1 (2.2%)	5 (11.1%)	χ^2=2.857	^{FE}p=0.203
Chronic glomerulonephritis	3 (6.7%)	9 (20%)	χ^2=3.462	0.063
Chronic pyelonephritis	3 (6.7%)	2 (4.4%)	χ^2=0.212	^{FE}p=1.000
Obstructive uropathy	1 (2.2%)	3 (6.7%)	χ^2=1.047	^{FE}p=0.616

Others	7 (15.6)	5 (11.1)	χ^2=0.385	0.535
Unknown	7 (15.6%)	3 (6.7%)	χ^2=1.800	0.180
CKD stage				
3A	–	3 (6.7%)	–	–
3B	–	16 (35.6%)		
4	–	26 (57.8%)		
Systolic BP (mmHg)	129 ± 24.65	137.6 ± 19.04	t=1.867	0.065
Diastolic BP (mmHg)	79.24 ± 13.44	82.93 ± 11.68	t=1.389	0.168
Laboratory parameters				
Hemoglobin (g/dl)	9.12 ± 1.89	9.74 ± 2.05	t= 1.475	0 .144
Creatinine (mg/dl)	9.46 ± 3.07	2.65 ± 0.94	t=14.245*	**<0.001***
CRP (mg/l)	10.50 (4.80 – 19.50)	15.80 (5.80 – 27.0)	U=912.00	0.417
Calcium (mg/dl)	8.52 ± 1.20	8.28 ± 1	t=1.031	0.306
Phosphorus (mg/dl)	5.52 ± 1.52	4.52 ± 1.01	t=3.643*	**<0.001***
PTH (pg/ml)	400 (228 – 678)	86.40(42.10 – 157)	U=283.00*	**<0.001***
Vitamin-D (ng/ml)	14.60 (9.46 – 26.20)	8.51 (4.19 – 11.30)	U=514.50*	**<0.001***
FGF-23 (pg/ml)	41.09 ± 15.09	29.61 ± 7.05	t=4.624*	**<0.001***
Soluble a-klotho (pg/ml)	505.5 (212 – 603.2)	573.5 (519.4 – 623)	U=631.00*	**0.002***

t: Student t-test; U: Mann Whitney test; χ^2: Chi square test; ;***bold:** significant; BMI: body mass index; BP: blood pressure; BSA: body surface area; CKD: chronic kidney disease; CRP: C- reactive protein; ESRD: end-stage renal disease; FGF-23: Fibroblast growth factor-23; PTH: parathyroid hormone.

Echocardiographic parameters of the study subjects

The main findings on conventional and speckle tracking echocardiography are shown in (**Table. 2).** There was a statistically significant higher left ventricular mass index (LVMI) in HD patients (142.6 ± 61.79 g/m^2) compared to CKD (116.1 ± 37.01 g/m^2).

Two-dimensional parameters including LV EF, left ventricular end-diastolic volume indexed (LV EDVI) and left ventricular end-systolic volume indexed (LV ESVI) showed no significant difference between the two groups. On the contrary, LA ESVI was significantly higher in the HD group (44.81 ± 24.36 ml/m^2) compared to the CKD group (33.42 ± 9.83 ml/m^2).

There was a statistically significant higher peak early diastolic transmitral velocity (E) in HD patients (87.22 ± 30.17 cm/sec) compared to CKD patients (73.36 ± 19.06 cm/sec). There was no difference in peak late diastolic transmitral velocity (A) or E/A ratios between the two groups. Additionally, early diastolic mitral annular velocities at the interventricular septum (medial e' velocity) and the lateral wall (lateral e' velocity), as well as the ratio of peak early diastolic transmitral velocity to early diastolic mitral annular velocity (E/e') measured by tissue Doppler imaging, showed no significant differences between the HD and CKD groups.

Regarding the data obtained from speckle tracking echocardiography, the mean GLS in both groups showed no significant difference being -16.38 ± 4.47% in HD group compared to -17.39 ± 3.68% in CKD group. Patients with impaired GLS (GLS ≤ 16 %) represented approximately 47% in the HD group and 29% in the CKD group. The difference between the two studied groups was not significant **(Fig.2)**. The mean RVFWS in both groups showed no significant

difference being -21.33 ± 6.97% in HD group compared to -23.33 ± 6.11% in CKD group. Patients with impaired RVFWS (GLS ≤ 20 %) represented approximately 44.4% in the HD group and 24.4% in the CKD group. The difference between the two groups was statistically significant **(Fig.2)**.

Table 2. Echocardiographic parameters of HD and CKD groups

Parameters	HD (n = 45)	CKD (n = 45)	Test of sig.	P
M-mode dimensions				
LVIDd (mm)	51.98 ± 7.48	53.34 ± 7.40	t=0.866	0.389
LVIDs (mm)	33.14 ± 8.47	32.72 ± 6.43	t=0.268	0.789
LA dimension (mm)	39.57 ± 9.24	40.56 ± 5.70	t=0.608	0.545
AoR diameter (mm)	27.0 (23.0 – 31.0)	28.0 (25.0 – 30.0)	U=928.00	0.494
IVS thickness (mm)	12.63 ± 3.54	12.14 ± 2.46	t=0.764	0.447
LVPW thickness (mm)	11.13 ± 2.87	11.43 ± 2.28	t=0.544	0.588
LVMI (g/m^2)	142.6 ± 61.79	116.1 ± 37.01	t=2.459*	**0.016***
2D volumes				
LVEF (%)	60.89 ± 6.51	61.53 ± 6.23	t=0.480	0.633
LV EDVI (ml/m^2)	72.0 (55.73 – 93.82)	73.09(64.40 – 93.82)	U=892.50	0.333
LV ESVI (ml/m^2)	27.80 (19.0 – 39.80)	29.70(21.20 – 38.40)	U=963.00	0.690
LA ESVI (ml/m^2)	40.04 (26.80 – 54s)	31.70(26.60 – 39.70)	U=753.50*	**0.037***
Mitral flow Doppler				
E of mital inflow (cm/sec)	87.22 ± 30.17	73.36 ± 19.06	t=2.605*	**0.011***
A of mitral inflow (cm/sec)	89.50 ± 23.08	81.10 ± 23.67	t=1.703	0.092
Mitral valve E/A	0.90 (0.70 – 1.10)	0.86 (0.70 – 1.10)	U=921.50	0.461
Mitral annular velocities				
Medial e´ velocity	7.35 ± 1.92	7.63 ± 2.70	t=0.571	0.570
Lateral e´ velocity	10.65 ± 3.15	9.68 ± 3.20	t=1.448	0.151
E/ average e´	9.10 (7.20 – 13.08)	7.91 (6.96 – 11.10)	U=887.00	0.311
Speckle tracking (strain)				
Impaired GLS	21 (46.7%)	13 (28.9%)	χ^2=3.025	0.082
GLS (-ve %)	16.38 ± 4.47	17.39 ± 3.68	t=1.173	0.244
Impaired RVFWS	20 (44.4%)	11 (24.4%)	χ^2=3.986*	**0.046***
RVFWS (-ve %)	21.33 ± 6.97	23.33 ± 6.11	t=1.444	0.152

t: Student t-test; U: Mann Whitney test; χ^2: Chi square test; ;***bold:** significant; A: peak late diastolic transmitral velocity; AoR: Aortic root ; E: peak early diastolic transmitral velocity; e': early diastolic mitral annular velocity; GLS: global longitudinal strain of left ventricle; IVS: interventricular septum; LA: left atrial; LA ESVI: left atrial end-systolic volume indexed; LV EDVI: left ventricular end-diastolic volume indexed; LVEF: left ventricular ejection fraction; LVIDd: left ventricular end diastolic dimension ; LVIDs: left ventricular end systolic dimension; LVMI: left ventricular mass index; LVPW: left ventricular posterior wall; LV ESVI: left ventricular end-systolic volume indexed; RVFWS: Right-ventricular free-wall longitudinal strain.

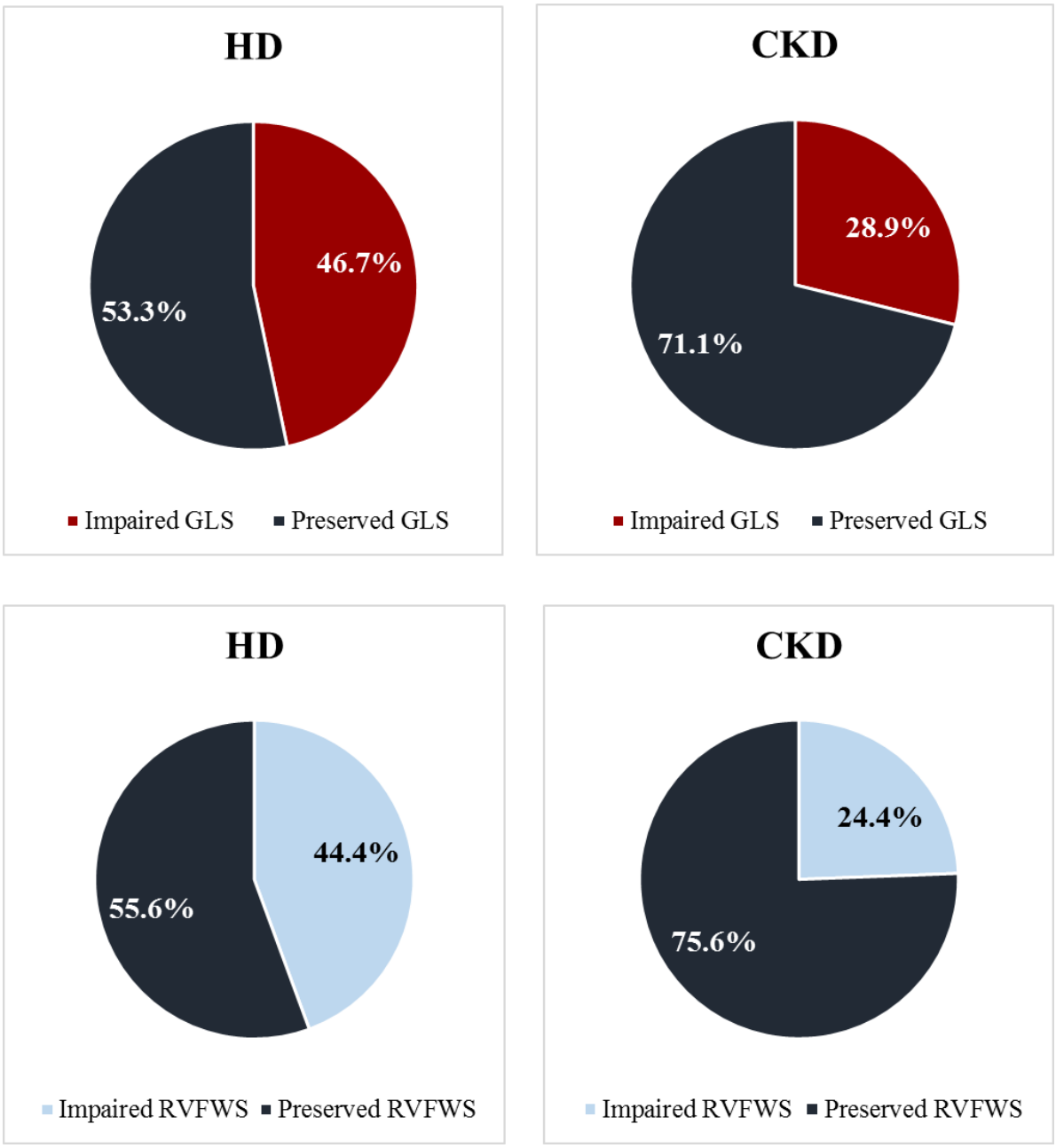


Fig.2. Distribution of hemodialysis (HD) and chronic kidney disease (CKD) patients according to whether they have impaired or preserved global longitudinal strain (GLS) or right ventricular free wall strain (RVFWS).

Clinical characteristics of HD and CKD patients according to preserved and impaired GLS

Participants were classified into preserved and impaired GLS: impaired GLS ≤ 16 % and preserved GLS ≥ 16%. The association between GLS and laboratory, clinical and echocardiographic parameters are shown in **(Table.3)**. In HD patients, impaired GLS showed an association with a history of hypertension (p=0.011), LVMI (p=0.041), and RVFWS (p=0.030). There was an association between CRP, LVMI and impaired GLS in CKD patients (p=0.010, p=0.014; respectively). However, impaired GLS was not associated with FGF-23 or soluble a-klotho in both groups.

Table 3. Clinical, laboratory and echocardiographic characteristics according to preserved and impaired GLS

Variables	HD (n = 45)			CKD (n = 45)		
	Preserved GLS (n = 24)	Impaired GLS (n = 21)	p	Preserved GLS (n = 32)	Impaired GLS (n =13)	p
Age (years)	51.67 ± 12.93	50.95 ± 15.01	0.865#	54.38 ± 11.86	60.38 ± 10.78	0.121#
Gender (Male)	9 (37.5%)	12 (57.1%)	0.188@	19 (59.4%)	10 (76.9%)	FEp=0.322
BMI (kg/m²)	27.34 ± 3.68	26.35 ± 4.64	0.431#	31.77 ± 6.94	31.73 ± 7.17	0.987#
Hypertension	8 (33.3%)	15 (71.4%)	**0.011***@	10 (31.3%)	8 (61.5%)	0.060@
Laboratory parameters						
Hemoglobin (g/dl)	9.23 ± 1.49	9.0 ± 2.30	0.696#	10.0 ± 2.27	9.09 ± 1.22	0.091#
CRP (mg/l)	9.75 (5.15 – 15.50)	13 (4.50 – 35)	0.488$	9.40 (3.50 – 21.05)	27.0 (15.80 – 72)	**0.010***$
FGF-23 (pg/ml)	39.73 ± 14.72	42.64 ± 15.71	0.526#	29.71 ± 7.82	29.38 ± 4.91	0.889#
Soluble a-klotho (pg/ml)	489.7 (273.1–580.4)	505.5 (107 – 611)	0.865$	563.5 (521.5–628.7)	584.3 (519.4–608.4)	0.940$
PTH (pg/ml)	481.6 (203.8 –983)	362 (228 – 600)	0.394$	71.50 (42.95–143.5)	89.60 (40 – 181)	0.661$
Vitamin D (ng/ml)	11.75 (7.95 – 21)	16.20 (10.30–33.20)	0.165$	8.67 (4.01 – 13.40)	6.23 (4.19 – 9.64)	0.523$
Phosphorus (mg/dl)	5.67 ± 1.53	5.34 ± 1.53	0.471#	4.49 ± 0.78	4.61 ± 1.48	0.785#
Calcium (mg/dl)	8.45 ± 1.31	8.60 ± 1.09	0.691#	8.45 ± 0.79	7.85 ± 1.33	0.065#
Echo parameters						
M-mode dimensions						
IVS thickness (mm)	11.73 ± 3.07	13.65 ± 3.83	0.070#	11.47 ± 2.29	13.78 ± 2.15	**0.003***#
LVPW thickness (mm)	10.51 ± 2.66	11.85 ± 3.0	0.119#	10.78 ± 1.85	13.02 ± 2.53	**0.002***#
LVMI (g/m²)	125.0 ± 55.58	162.6 ± 63.71	**0.041***#	107.7 ± 34.60	137.0 ± 35.57	**0.014***#
2D volumes						
LVEF (%)	63.15 ± 7.11	58.30 ± 4.69	**0.011***#	61.61 ± 6.51	61.33 ± 5.71	0.892#
LV EDVI (ml/m²)	63.85 (51.06–91.34)	74 (60 – 94.60)	0.446$	69.15 (60.20–95.85)	83.12 (71.50–86.41)	0.438$
LV ESVI (ml/m²)	24.62 (17.90–34.04)	29.60 (24.80–48.11)	0.080$	26.03 (20.95–40.29)	31.60 (24.48–33.30)	0.548$
LA ESVI (ml/m²)	34.51 (26.48–51.01)	44 (28 – 55.65)	0.228$	28.47 (25.48–38.65)	36 (33 – 39.75)	0.086$
RVFWS (-ve %)	23.42 ± 6.68	18.94 ± 6.67	**0.030***#	23.30 ± 6.61	23.39 ± 4.89	0.964#

#: indicate student t-test, **$**: indicate Mann Whitney test and @: indicate Chi square test;***bold:** significant; Impaired GLS = GLS ≤16 %; preserved GLS = GLS ≥ 16 %; BMI: body mass index; CRP: C- reactive protein; FGF-23: Fibroblast growth factor-23; GLS: global longitudinal strain of left ventricle ; IVS: interventricular septum; LA ESVI: left atrial end-systolic volume indexed; LV EDVI: left ventricular end-diastolic volume indexed; LVEF: left ventricular ejection fraction; LVMI: left ventricular mass index; LVPW: left ventricular posterior wall; LV ESVI: left ventricular end-systolic volume indexed; PTH: parathyroid hormone; RVFWS: Right-ventricular free-wall longitudinal strain.

Correlation between GLS and echo Doppler parameters

The correlations between GLS and echocardiographic Doppler parameters are represented in **(Table.4).** A statistically significant inverse correlation was found between E/e' and GLS in HD group and CKD patients (r = -0.380, p = 0.010 and -0.367, p = 0.013, respectively) **(Fig.3).**

Table 4.Correlation between GLS and echo Doppler parameters

GLS (-ve %) vs.	HD (n = 45)		CKD (n = 45)	
	r	p	r	p
Mitral flow Doppler				
E of mitral inflow (cm/sec)	-0.246	0.103	0.058	0.704
A of mitral inflow (cm/sec)	-0.159	0.296	0.127	0.406
MV E/A	-0.143	0.348	-0.161	0.289
Mitral annular velocities				
Med e´vel (cm/s)	0.360*	**0.015***	0.142	0.354
Lat e´ vel (cm/s)	0.216	0.154	0.344*	**0.021***
E/ average e´	-0.380*	**0.010***	-0.367	**0.013***

r: Pearson coefficient; ***bold**: significant; A: peak late diastolic transmitral velocity; E: peak early diastolic transmitral velocity; e': early diastolic mitral annular velocity; GLS: global longitudinal strain of left ventricle

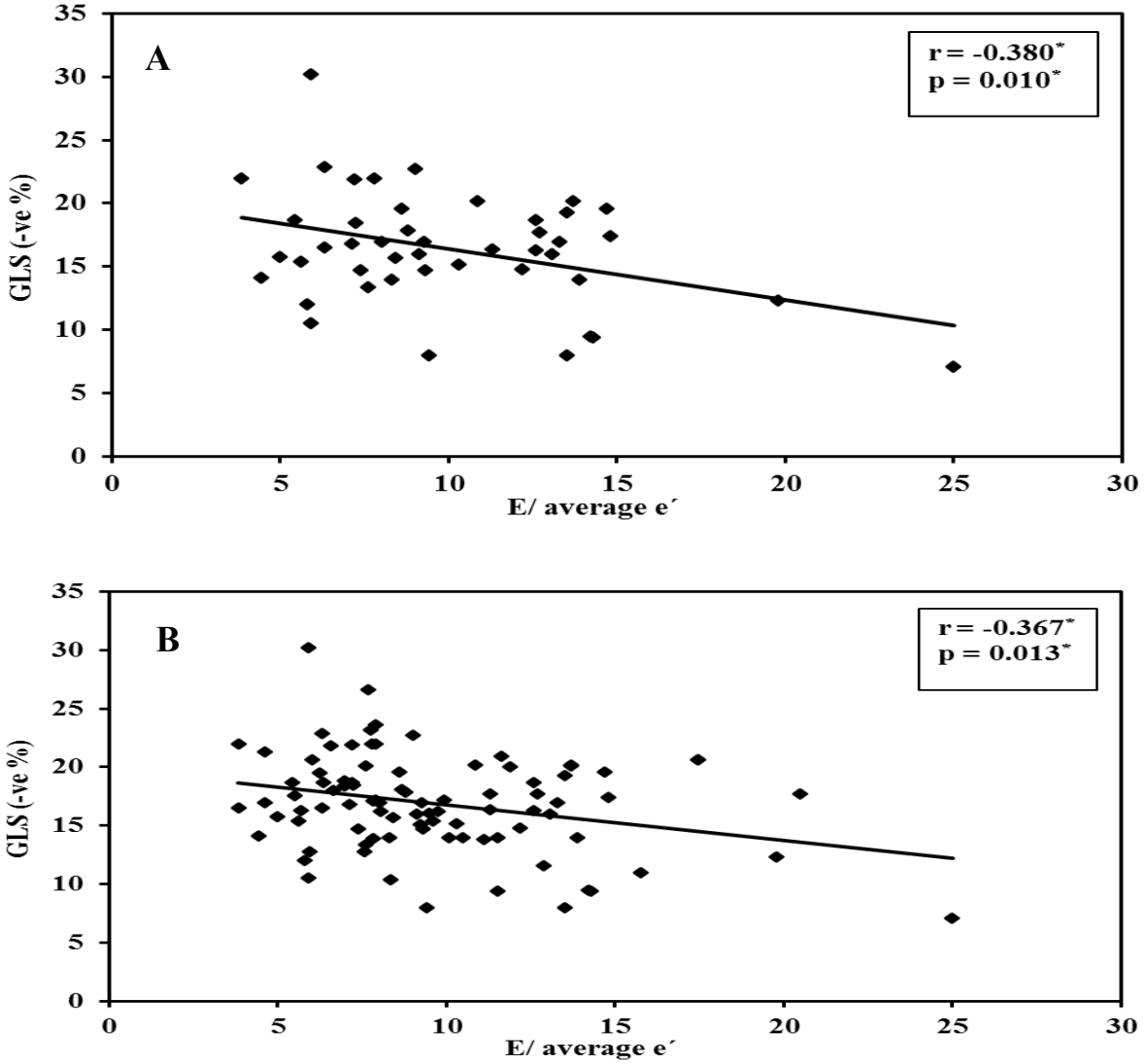


Fig.3. Correlation between the ratio of peak early diastolic transmitral velocity to early diastolic mitral annular velocity (E/e') and global longitudinal strain (GLS) in pic **(A)** HD patients and pic **(B)** CKD patients.

Multivariate regression analysis of factors affecting GLS in HD and CKD patients.

In a multiple regression model, hypertension was the only significant independent determinant of impaired GLS in HD patients while in CKD patients; LVMI was the only significant independent determinant **(Table.5 and 6).**

Table 5. Predictors of impaired GLS in HD patients by logistic regression analysis

Variables	Univariate		#Multivariate	
	p	OR (LL – UL 95%C. I)	p	OR (LL – UL 95%C. I)
Hypertension	0.013*	5.000 (1.402 – 17.830)	0.013*	7.967 (1.546 – 41.042)
LVMI (g/m²)	0.053	1.011 (0.999 – 1.023)		
LVEF (%)	0.018*	0.873 (0.779 – 0.977)	0.026*	0.863 (0.757 – 0.983)
E/ average e´	0.436	1.059 (0.916 – 1.225)		
RVFWS (-ve %)	0.040*	0.899 (0.813 – 0.995)	0.022*	0.852 (0.743 – 0.977)

Hosmer and Lemeshow Test (χ^2=9.607; p=0.212); OR: Odd`s ratio; C.I: Confidence interval; LL: Lower limit; UL: Upper Limit; #: All variables with p<0.05 was included in the multivariate; *: significant; E: peak early diastolic transmitral velocity; e': early diastolic mitral annular velocity; LVEF: left ventricular ejection fraction; LVMI: left ventricular mass index; RVFWS: right-ventricular free-wall longitudinal strain.

Table 6.Predictors of impaired GLS in CKD patients by logistic regression analysis

Variables	Univariate		#Multivariate	
	p	OR (LL – UL 95%C. I)	p	OR (LL – UL 95%C. I)
Hypertension	0.067	3.520 (0.918 – 13.501)		
CRP (mg/l)	0.050*	1.017 (1.000 – 1.033)	0.134	1.013 (0.996 – 1.031)
LVMI (g/m²)	0.022*	1.023 (1.003 – 1.044)	0.051	1.020 (0.9999 – 1.041)

Hosmer and Lemeshow Test (χ^2=5.129; p=0.644) ; OR: Odd`s ratio; C.I: Confidence interval; LL: Lower limit; UL: Upper Limit; #: All variables with p<0.05 was included in the multivariate; *: significant CRP: C- reactive protein; LVMI: left ventricular mass index

Discussion

The CVD represents a significant complication in patients with CKD and end-stage renal disease (ESRD). The risk of mortality following cardiac events, such as myocardial infarction and HF, increases progressively with advancing stages of CKD **(Menon et al., 2005).**

In our study, we used GLS as measured by 2D STE as a more sensitive index of subclinical myocardial systolic function**.** A low GLS greater than −16%, indicative of LV systolic dysfunction, was observed in 38% (34/90) of our patients, despite the fact that all participants had a normal LVEF. This finding supports previous research indicating that strain imaging-derived GLS can detect functional changes before alterations in EF are apparent using conventional echocardiography **(Panoulas et al., 2015).** Additionally, GLS has been shown to be a prognostic marker for all-cause and CV mortality in CKD patients (**Krishnasamy et al., 2015).**

In our subgroup analysis, 46.7 % of dialysis patients had impaired GLS compared to 28.9% of patients with a moderate CKD stage. Although the difference between the two groups is not statistically significant, it seems that subclinical LV myocardial systolic dysfunction is more prevalent in HD patients compared to CKD. In line with our results, **Liu et al. (2011)** demonstrated that impaired GLS correlated significantly with the deterioration in eGFR in 153 patients 37 with stages 3-5 CKD, 60 with ESRD on regular HD and 56 without CKD.

The most plausible explanation could be that left ventricular longitudinal

dysfunction, as assessed by GLS, reflects early myocardial changes related to CKD, such as myocardial ischemia, hypertrophy, and fibrosis. Subendocardial longitudinal myocardial fibers are particularly vulnerable to decreased coronary perfusion and increased wall stress. (**Panoulas et al., 2015).**

Adding to the aforementioned explanation, the higher incidence of impaired GLS in dialysis patients compared to CKD can be attributed to the circulatory stress imposed by HD. This will eventually lead to acute reversible segmental myocardial hypoperfusion that result in regional systolic contractile dysfunction, known as myocardial stunning. When this process occurs repeatedly, the cumulative injury leads to permanent systolic dysfunction (**McIntyre, 2010)**.

Furthermore, we thought to highlight in our study the possible association between impaired GLS and traditional CV risk factors in both groups. HD patients with a known history of hypertension had a significantly impaired GLS compared to normotensives. It is well known that hypertension, combined with metabolic alterations related to the uremic environment, can lead to vascular wall injury including exaggerated atherosclerosis and arteriosclerosis leading to arterial stiffness (**Fliser et al., 2011) .**

These observations align with prior research that has shown a link between aortic stiffness and impaired GLS. They concluded that LV systolic function may be impaired due to ventricular vascular stiffening in advanced CKD patients **(Krishnasamy et al., 2015).** Furthermore, in a meta-analysis carried out by **Yingchonacharoen et al. (2013)**, systolic blood pressure was a major determinant in the variation of GLS values.

Another aim of the research was to investigate the possible contribution of CKD-related CV risk factors especially mineral bone disease on LV dysfunction. Recently, FGF-23 and Klotho appeared to be important regulator of calcium-phosphate homeostasis. They may represent the missing clue in the link between CKD and CVD **(Shimada et al., 2004).**

As CKD progresses, serum FGF-23 becomes higher due to phosphate retention, while soluble a-Klotho levels decline. In accordance with the literature, we found significantly higher FGF-23 levels and lower soluble a-klotho levels in HD patients compared to patients with a moderate CKD stage. However, no association was found between both serum levels and GLS values in both groups. According to our findings, the incorporation of a new technique, such as 2D STE in combination with FGF23 and soluble a-klotho, didn't increase the diagnostic sensitivity of detecting subclinical myocardial systolic dysfunction.

There are probable justifications for these results. CVD and disorders of mineral homeostasis start to arise in the earlier CKD stages. Thus, our study groups with moderate to advanced CKD stages have probably been subjected to an environment prompting myocardial systolic or even DD for a long duration of time. In this study, we only assessed soluble a-Klotho and FGF-23 levels at the time of enrollment. Consequently, both levels do not represent the burden of mineral bone disease in our population. Hence, the relationship between both biomarkers and subclinical myocardial systolic dysfunction as assessed by GLS might be weakened by the time patients have reached advanced CKD stages.

Adding strength to our observations, in a study conducted by **Tanaka et al. (2016)**, FGF23 correlated significantly with low EF in patients with early CKD stages G1, G2, and G3a. On the contrary, the relationship was not significant in patients with advanced CKD (CKD G3b-G5). On the other hand, the relationship between α-Klotho and left ventricular systolic dysfunction was not

significant among patients with no CKD (stage G1 and G2). In our study, only 6.7 % of our patients were at stage G3a while the remaining were at a more advanced CKD stage including those on maintenance HD. This could provide a possible explanation for the lack of association between both biomarkers and GLS. To conclude, mineral bone disease parameters could serve as an early CV risk biomarker in patients with earlier stages of CKD.

In our study, apart from being affected by hypertension (increased afterload), impaired GLS correlated significantly with impaired E/e' ratio (assessed by tissue Doppler imaging) in both studied groups. Elevated E/e' ratio serves as a dependable and early indicator of diastolic dysfunction with a proven association with poor CV outcome in patients with renal disease (**Untersteller et al., 2016).** It is plausible that early subclinical diastolic and systolic dysfunction coincide in renal disease, potentially contributing to the elevated rates of mortality and morbidity observed independent of disease stage. Similar to ours, in a study conducted by **Ravera et al. (2019)**, the E/e' ratio was the most powerful independent predictor of abnormal GLS in CKD, HD and renal transplant patients.

Interestingly, apart from being a sensitive indicator of subclinical myocardial dysfunction, researches have proposed that GLS may provide further information. **De Vore et al. (2016)** demonstrated that GLS could serve as an indicator of myocardial fibrosis and thus, diastolic dysfunction. This was evidenced by the connection between impaired GLS and elevated levels of N-terminal pro-brain natriuretic peptide (NT-proBNP) and biomarkers of collagen synthesis in the blood. In our study, the observation that individuals with impaired GLS exhibited elevated E/e' ratios, which is indicative of diastolic dysfunction, further supports the hypothesis that myocardial fibrosis may explain the observed diastolic impairment.

In our study, increased LVMI was significantly linked with impaired GLS in CKD and ESRD patients. Our findings extend the report of **Hensen et al. (2018)** who found a high prevalence of impaired LV GLS in patients diagnosed with CKD stage 3b–5 which was independently associated with higher LVMI.

From the pathophysiologic point of view, the link between low GLS and higher LVMI values can be attributed to the following: myocardial hypertrophy led to the activation of autophagic and apoptotic signals that raised collagen synthesis within the extracellular matrix subsequently leading to myocardial fibrosis (**Graham-Brown et al., 2017).** In addition, left ventricular hypertrophy increases the myocardial oxygen demand that leads to worse myocardial perfusion and further interstitial fibrosis (**Graham-Brown et al., 2017).** These structural changes contribute to abnormal LV contractility, which can be analyzed with LV GLS.

In the multiple regression model, which included known confounding variables, we tried to distinguish the independent determinants of impaired GLS in both groups. A statistically significant association existed between GLS and a history of hypertension in HD patients while LVMI seems to be the most important determinant of abnormal GLS in CKD patients.

At the end of our study, we sought to extend our observations by evaluating RV systolic function in the studied sample. A low RVFWS (≤ 20%) suggestive of RV systolic dysfunction was found in 44.4 % of dialysis patients compared to 24.4% in patients with moderate CKD stage. The difference between the two groups was statistically significant. The impaired RV function in dialysis patients correlated significantly with the impaired LV systolic function as assessed by GLS. Thus, STE

echocardiography demonstrated an interconnected biventricular systolic dysfunction in asymptomatic ESRD patients on maintenance hemodialysis.

Our results are consistent with the retrospective cohort study carried out by **Mavrakanas et al. (2018)**, which demonstrated that patients with worse eGFR had more structural and functional LV and RV impairments including RV hypertrophy, systolic impairment, or dilated RV than those with more preserved renal function. Furthermore, **Hickson et al. (2016)** provided evidence supporting the link between RV and LV in HD patients, showing that the death rates were two-fold higher in those with biventricular dysfunction compared to those with only impaired LV function.

RV dysfunction in ESRD patients could be attributed to fluid retention, anemia, uremia, hyperparathyroidism and a high output state from the AVF. **Paneni et al. (2010)** showed that AVF has a crucial role in RV dysfunction in HD patients. AVF induces chronic volume or pressure overload in the RV, particularly in brachial AVF compared to radial AVF. This chronic overload leads to a leftward shift of the interventricular septum, thereby impairing LV function (**Paneni et al., 2010**). This fact could in part explain the subclinical biventricular affection observed in our dialysis patients.

Conclusion

Subclinical LV and RV systolic dysfunction are present in patients with moderate CKD stages 3 and 4 and becomes worse in dialysis patients. The impaired RV function in dialysis patients was significantly associated with impaired GLS suggesting interconnected biventricular dysfunction in asymptomatic ESRD patients. The history of hypertension in HD patients and LVMI in CKD were the most powerful independent predictors of abnormal GLS. Finally, the incorporation of a new technique, such as 2D STE in combination with FGF23 and soluble a-klotho, didn't increase the diagnostic sensitivity of detecting subclinical myocardial systolic dysfunction.

Conflict of interest: None.

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