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SYSTEMS-LEVEL QUALITY IMPROVEMENT

Evaluation of eSie VVI Technology on Left Ventricular Systolic Function Changes in Uremic Patients Undergoing Dialysis

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Abstract
To analyze the longitudinal peak systolic strain of left ventricular myocardium in three layers before and after hemodialysis in uremic patients, and to explore the value of eSie VVI (Velocity Vector Imaging) technology in evaluating the changes of left ventricular myocardial systolic function in uremic patients in a short time after hemodialysis, the longitudinal peak systolic laminar strain and global full-thickness strain of 17 segments of the left ventricle are obtained by eSie VVI software analysis, and the results are deduced in Excel form. Statistical analysis is made on the results of longitudinal peak systolic stratified strain of left ventricular inner, middle and outer layers and whole myocardium in uremic patients before and after hemodialysis. The results show that eSie VVI technology can more sensitively and accurately evaluate the changes of left ventricular myocardial systolic function after hemodialysis in uremic patients, and has certain clinical value.

Keywords Echocardiography · VVI · Uremia · Hemodialysis

Introduction

Chronic kidney disease has become an important disease endangering human health because of its high cost of treatment, poor prognosis and high morbidity. Uremia is the late stage of chronic renal failure. Due to the accumulation of many harmful metabolites and non-degradable endocrine hormones in the body for a long time, the shape, structure and function of the heart are damaged.

Chronic renal failure (CRF) is a clinical syndrome in the later stage of chronic kidney disease. Cardiovascular disease is the main cause of death in patients with end-stage renal failure, accounting for 45%-60% of the causes of death in uremic patients [1]. Recent studies have found that the incidence of cardiovascular complications in CRF patients is 10-30 times higher than that in the general population. The incidence of atherosclerotic cardiovascular disease and cardiovascular adverse events in uremic patients is about 15-20 times higher than that in the general population [2]. 40-50% of patients with chronic kidney disease die from cardiovascular complications. The annual mortality rate of cardiovascular disease in the general population of the United States is 0.27%, and that of hemodialysis uremia patients is 9.5%, which is 35 times higher than that of the former. US Renal Disease Data System (USRDS) data show that 60% of dialysis patients die from unknown cardiac arrest and arrhythmia [3]. A recent multicenter epidemiological study in China showed that the prevalence of chronic kidney disease was about 8%-10%, and that of cardiovascular disease was 9.8% in patients with CKD1-4 (the four stages of patients with chronic kidney diseases). Among these patients with cardiovascular diseases, the proportions of myocardial infarction, chronic heart failure, cerebrovascular disease and peripheral artery disease were 20.6%, 9.0%, 69.1% and 16.1%, respectively. Studies have shown that these adverse outcomes can be prevented or delayed by early detection and treatment. Therefore, early assessment of cardiac damage in uremic patients is particularly important [4].

The survival rate of uremic patients is significantly improved with the improvement of dialysis technology, but the incidence of cardiovascular complications is not reduced.

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Studies have shown that single hemodialysis has a negative impact on left ventricular function in uremic patients in a short time. During the process of hemodialysis, the incidence of cardiovascular events is quite high. Therefore, during and after dialysis, the cardiac function of patients should be closely monitored to reduce the occurrence of sudden cardiovascular events while completing hemodialysis smoothly [5]. Conventional echocardiography can display the anatomical structure, wall thickness and activity of the heart in real time, and quantify the hemodynamic information. It has been widely used in the diagnosis of many clinical diseases, such as coronary heart disease, hypertension, heart valvular disease, etc. It has become the most common non-invasive technique for measuring the structure and function of the heart []. However, up to now, there are few studies on the effect of single dialysis on left ventricular myocardial function in uremic maintenance hemodialysis patients at home and abroad, and there are still controversies. According to the principle of two-dimensional speckle tracking, eSie VVI (Velocity Vector Imaging) technology is independent of tissue Doppler frequency shift and is not affected by the angle between the direction of sound beam and wall motion [6]. It can more accurately reflect myocardial motion, thus significantly improving the accuracy of measurement, and having easy operation and good repeatability. It has important value in quantitative evaluation of local and global myocardial function. eSie VVI technology is used to analyze the systolic longitudinal peak strain of left ventricular inner, middle and outer layers and whole myocardium in uremic patients before and after hemodialysis to evaluate the effect of hemodialysis on left ventricular myocardial function.

Materials and methods

Research object

Diagnostic criteria and classification method of uremia: In recent years, different concepts and stages of chronic kidney disease have been proposed at home and abroad. In 2012, the Expert Group on Improving the Prognosis of Global Kidney Diseases (KDIGO) defined chronic kidney disease as abnormal kidney structure or function for more than 3 months and GFR (Glomerular Filtration Rate) < 60 mL / (min 1.73 m^2) [7]. CRF is usually divided into four stages in clinical practice in China, including uremic stage with GFR < 10 mL/min and serum creatinine >707 μmol/L [8].

Inclusion criteria: This study was a prospective study. Thirty-five patients with uremia maintained hemodialysis in The Affiliated Hospital of Jining Medical University from October 2014 to December 2017 were selected, including 21 males and 14 females, aged 32-75 years, with an average age of 56.35 ± 9.62 years old. The diagnosis met the diagnostic criteria of 2012 K/DOQI: GFR < 10 mL/min, serum creatinine >707 μmol/L, which is equivalent to DOQI5 stage. The conditions for admission are: sinus heart rate; normal left ventricular ejection fraction; no obvious segmental motion abnormality of left ventricular wall by echocardiography; no pericardial disease; no mitral valve, aortic stenosis and mild or more incomplete closure; dialysis age < 5 years; and no congenital heart disease. The primary diseases are 16 cases of chronic glomerulonephritis, 10 cases of benign arteriolar nephrosclerosis, 6 cases of diabetic nephropathy and 3 cases of other unknown causes. Informed consent was signed by all patients or their families and this study was approved by the Ethics Committee of The Affiliated Hospital of Jining Medical University, and the informed consent was signed by all participants.

Thirty-five patients with arteriovenous fistula were treated with German F60 s high-throughput dialyzer (polysulfone membrane, surface area 1.3m^2) with ultrafiltration coefficient of 40 mL/(h·mmHg), Fresenius4008B volume ultrafiltration dialyzer, double reverse osmosis water dialysate, and sodium bicarbonate dialysate, three dialysis times a week, 4 h a dialysis, low molecular weight heparin (homogeneity) anticoagulation. The dialysate flow rate was 500-600 mL/min and the blood flow rate was 250-300 mL/min.

Exclusion criteria: Patients with left ventricular ejection fraction <50%, left ventricular wall segmental motion abnormality, ischemic heart disease, mitral and aortic stenosis and mild or more incomplete closure, congenital heart disease, complete left bundle branch block, artificial valve implantation, pacemaker implantation, pericardial lesion and unclear image were excluded.

Instrument and method

Instrument: Siemens color Doppler ultrasound diagnostic instrument ACUSON SC2000 was used, the heart probe 4V1c was selected, probe frequency was 1-4 MHz, and eSie VVI imaging technology was built in. Echocardiography was performed in uremic patients before and 1 h after dialysis [9]. The patient's name, age, dialysis age, serum creatinine and blood pressure before and after dialysis were recorded. Echocardiography was performed within 1 h before and after the first dialysis three times a week.

Method: Two-dimensional gray-scale dynamic images with frame rates of 60-90 frames/s were collected from the long axis of apical left ventricle, two-chamber and four-chamber cardiac sections for three consecutive cardiac cycles, and then online eSieVVI analysis was performed. Open eSieVVI function, freeze dynamic images, and adjust to the end of left ventricular systolic phase. Manual recording of subendocardial myocardium was performed. Subepicardial myocardium was automatically recognized by the instrument (Fig. 1). Subepicardial myocardium thickness was adjusted

Fig. 1 Automatically generated velocity vector motion trajectory

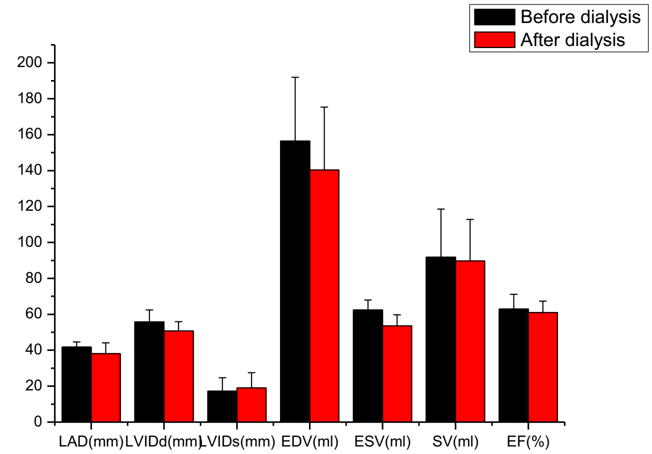

Fig. 3 Comparison of routine echocardiographic parameters before and after dialysis

according to the actual thickness of the myocardium. By clicking the Analysis key, the instrument can automatically track the subintimal myocardium (endo), middle myocardium (myo), and EPI (Fig. 2). The system defaults that the basal and middle segments of the left ventricle are divided into anterior septum, posterior septum, anterior wall, lateral wall, posterior wall and inferior wall. The apical segment is divided into septum, anterior wall, lateral wall and inferior wall, and the apical cap has 17 segments. Click the Summary key, and then it automatically displays the longitudinal peak systolic strain of each myocardial segment corresponding to the cardiac cycle, and the analysis results are derived in Excel form (Figs. 3 and 4).

Results and discussion

Comparison of routine dialysis parameters between pre-dialysis and 1 h post-dialysis in uremic patients

The left atrial anteroposterior diameter (LAD), left ventricular end-diastolic and end-systolic internal diameter (LVIDd and LVIDs), left ventricular end-diastolic and end-systolic volume (EDV, ESV), stroke volume (SV) decreased with statistical significance ($P < 0.05$); left ventricular ejection fraction (LVEF) had no statistical significance ($P > 0.05$) (Table 1 and Fig. 1).

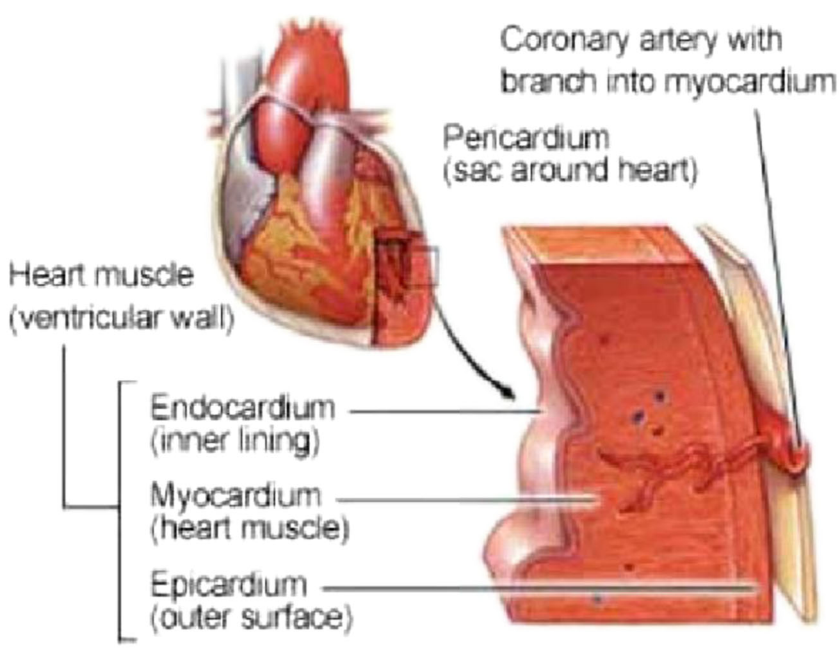

Fig. 2 Left ventricular inner, middle and outer myocardium

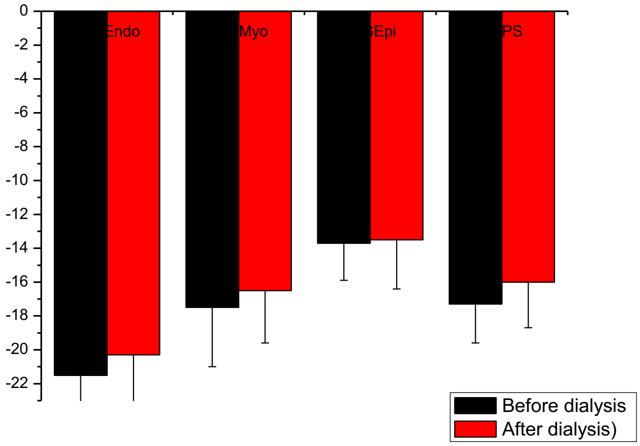

Fig. 4 Comparison of left ventricular myocardial stratification and longitudinal peak systolic strain before and after dialysis

Comparison of stratified strain parameters between before dialysis and 1 h after dialysis in uremic patients

Compared with before dialysis, the longitudinal peak systolic strain of left ventricular whole, inner, basal, middle and outer layers, middle and inner layers decreased significantly after dialysis, and the difference had statistical significance ($P < 0.05$); there was no significant difference in the longitudinal peak systolic strain of the whole middle, outer layers, middle and outer layers, and apical full-thickness, internal and external three-layer myocardium ($P > 0.05$) (Tables 2, 3, 4, and 5; Figs. 5, 6, and 7).

Significance of evaluation of left ventricular function before and after dialysis

Hemodialysis uses osmotic pressure and ultrafiltration pressure to remove excessive water, correct electrolyte disturbance and acid-base imbalance, remove most of uremic toxins, and reduce water and sodium retention, thus regulating the quality and quantity of blood, achieving the purpose of purifying blood, and correcting water and electrolyte and acid-base balance, so that it is close to physiological status, and the survival rate of uremic patients is improved. However, hemodialysis does not reduce cardiovascular mortality in uremic patients. Sudden cardiac death (SCD) is the most common cause of death in maintenance hemodialysis patients. The incidence of SCD is 30 times higher than that of normal people. Recent studies have shown that 25% of hemodialysis patients die from SCD, especially secondary to severe arrhythmia or cardiac arrest [10, 11]. During hemodialysis, ST segment of electrocardiogram (ECG) can be significantly reduced, which has a negative impact on left ventricular function in a short time, leading to a significant increase in cardiovascular events. The influence of single hemodialysis on left ventricular systolic and diastolic function has been different from previous studies. Foreign scholars such as Dincer and Agmon believe that the left ventricular diastolic function decreases significantly in the short term after single dialysis. Lee T Y et al. considered that the left ventricular systolic function did not change significantly and the diastolic function decreased after acute hemodialysis. Selby N M et al. considered that left ventricular long axis systolic and diastolic function decreased after acute hemodialysis. Hayshi et al. believed that single hemodialysis could significantly improve left ventricular diastolic function. Palecek T considered that left ventricular systolic function was improved and diastolic function was not changed after acute hemodialysis. Bauer et al. considered that left ventricular diastolic function was not affected by single hemodialysis. Chaihongli and Ni Ruizhi found that the mitral annular velocity (Ve/Va) of uremic patients before and after dialysis was significantly lower than that of healthy control group and significantly higher after dialysis than that before dialysis. When Gao Jun and Xia Taozi found no significant difference in E/A between before dialysis and after dialysis in uremia group, TEI (comprehensive ejection isovolumic diastolic index) decreased significantly compared with that before dialysis, which also indicated the existence of pseudonormalization in conventional echocardiography. Gao Yulan and Zhou Qichang found that the myocardial velocity, strain and strain rate of left ventricular long axis systolic segments decreased after dialysis compared with those before dialysis. The different conclusions may be related to the admission criteria, the status of patients, the difference of cardiac load before and after dialysis, the different dialysis volume, and the different research methods. At present, there are few studies

Table 1 Comparison of routine echocardiographic parameters before and after dialysis

Time	LAD (mm)	LVIDd (mm)	LVIDs (mm)	EDV (mL)	ESV (mL)	SV (mL)	EF %
Before dialysis	41.84 ± 2.82	55.73 ± 6.72	17.2 ± 7.44	156.4 ± 35.57	62.4 ± 5.61	91.84 ± 26.77	62.92 ± 8.25
After dialysis	38.07 ± 6.06	50.75 ± 5.16	19 ± 8.51	140.34 ± 35.03	53.6 ± 6.15	89.70 ± 23.05	60.96 ± 6.32
P	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	>0.05

Compared with before dialysis, $P < 0.05$ has statistical significance after dialysis, and $P > 0.05$ has no statistical significance

LAD left atrial anteroposterior diameter, *LVIDd* left ventricular end-diastolic diameter, *LVIDs* left ventricular end-systolic diameter, *EDV* left ventricular end-diastolic volume, *ESV* left ventricular end-systolic volume, *SV* stroke volume, *EF* left ventricular ejection fraction

Table 2 Comparison of routine echocardiographic parameters before and after dialysis

Time	LPSEndo	LPSMyo	LPSEpi	GLPS
Before dialysis	−21.5 ± 3.1	−17.5 ± 3.5	−13.7 ± 2.2	−17.3 ± 2.3
After dialysis	−20.3 ± 3.3*	−16.5 ± 3.1**	−13.5 ± 2.9**	−16.0 ± 2.7*

Compared with before dialysis, * indicates that $P < 0.05$, there is significant difference; ** suggests that $P > 0.05$, there is no statistical difference after dialysis

LPSEndo Longitudinal peak strain of inner myocardium, *LPSMyo* longitudinal peak strain of middle myocardium, *LPSEpi* longitudinal peak strain of outer myocardium, *GLPS* longitudinal peak strain of whole myocardium

on the effect of acute hemodialysis on left ventricular systolic function in uremic maintenance hemodialysis patients at home and abroad. In order to further understand the effect of hemodialysis on left ventricular inner, middle and outer myocardium and whole myocardial systolic function, it is necessary to reduce sudden cardiovascular events while completing hemodialysis. eSieVVI technology was used to evaluate the occurrence of vascular events.

Advantages and limitations of 2DE, TDI, RT-3DE, 2D-STI and 3D-STI in evaluating left ventricular function

TDI technology can measure the relationship between peak systolic velocity at different parts of the myocardium and ECG activity at corresponding time. It can more accurately and quantitatively analyze mechanical asynchrony and local function, and make differential diagnosis and curative effect detection of various cardiovascular diseases. However, the evaluation of overall myocardial function is limited. 2DE and RT-3DE can accurately evaluate left ventricular ejection function in uremic patients, but the sensitivity of evaluating regional wall function changes is low. 2D-STI objectively reflects the longitudinal, radial and circumferential deformation of myocardium, but there is “cross-plane tracking”. 3D-STI can track myocardial motion from three-dimensional direction at the same time, but the image quality is worse than 2DE, the accuracy and repeatability are poor, and the time resolution and spatial resolution need to be improved.

Feasibility and superiority of eSie VVI technology in detecting left ventricular myocardial function in uremic patients before and after dialysis

Based on the principle of two-dimensional speckle tracking, eSie VVI technology displays the velocity and method of myocardial motion by vectors. It is not affected by the whole translation, rotation and contraction of adjacent segments. It can perform myocardial motion imaging directionally and freely, and quantitatively measure the parameters such as velocity, displacement, strain and strain rate on the long axis, short axis and circumferential directions of myocardium, so as to evaluate the systolic and diastolic function of the local myocardium more accurately. It obviously improves the accuracy of measurement, and it is easy to operate and has good repeatability. It has important value in quantitative evaluation of the function of the local and the whole myocardium. Piart et al. confirmed that VVI technology could accurately measure the longitudinal and circumferential motion of myocardium. The latest eSie VVI technology can track the left ventricular subendocardial myocardium, middle myocardium and subepicardial myocardium, reflect the systolic longitudinal strain of three-layer myocardium and whole myocardium more accurately, and evaluate the changes of left ventricular myocardial systolic function by hemodialysis.

Analysis of research results

There is a clear correlation between renal function and cardiovascular risk factors. Traditional risk factors include age, lipid metabolism disorders, diabetes, hypertension, smoking, sedentary and so on. New cardiovascular risk factors include oxidative stress, endothelial dysfunction, anemia, cardiovascular calcification and sleep disorders. The overlapping effects of these risk factors have a common impact on the heart, leading to further myocardial damage. Myocardial interstitial fibrosis, decreased compliance and diastolic dysfunction in uremic patients affect systolic function, resulting in delayed myocardial contraction, reduced degree of contraction and asynchrony of contraction.

Myocardial fibers are divided into three layers: longitudinal myocardium in the inner layer, circular myocardium in the

Table 3 Comparison of longitudinal peak systolic laminar strain of left ventricular basal segment before and after dialysis

Time	bLPSndo	bLPSMyo	bLPSEpi	bGLPS
Before dialysis	−16.0 ± 3.3	−14.5 ± 3.1	−12.0 ± 2.5	−14.6 ± 3.2
After dialysis	−14.5 ± 3.1*	−13.7 ± 3.4*	−12.1 ± 2.6*	−13.6 ± 3.3*

Table 4 Comparison of longitudinal peak delamination strain of left ventricular systole before and after dialysis

Time	mLPSndo	mLPSMyo	mLPSEpi	mGLPS
Before dialysis	−22.6 ± 3.3	−18.8 ± 2.9	−15.0 ± 2.1	−19.1 ± 2.2
After dialysis	−21.3 ± 2.2*	−18.7 ± 2.4**	−15.1 ± 2.3**	−18.8 ± 2.4*

middle layer, and oblique myocardium in the outer layer. The contraction of the inner and outer myocardium shortens the ventricle, while the contraction of the middle myocardium shortens the ventricular cavity. Because of the unique structure of myocardial fibers and the different effects of myocardial layers, the effects of different pathological factors on the function of myocardial layers may be different. Therefore, understanding the effect of each layer of myocardium on cardiac deformation is helpful to correctly distinguish and evaluate different myocardial lesions and understand their pathological mechanism.

LAD, LVIDd, LVIDs, EDV, ESV and SV decreased after dialysis, and the difference was statistically significant. However, there was no significant difference in LVEF, indicating that single hemodialysis could not significantly improve left ventricular systolic function in uremic patients with normal systolic function. LVEF could only objectively reflect the global systolic function of left ventricular myocardium, but could not directly reflect the local and global function of left ventricular myocardium in pathological state. The results of eSieVVI stratified strain analysis showed that the systolic longitudinal peak strain of whole and inner layer of left ventricle, and whole, inner, middle and outer layers of basal segments, middle and inner layers of left ventricle in 1 h after dialysis was significantly lower than that before dialysis, and there was statistical significance ($P < 0.05$); the longitudinal peak strain of whole middle and outer layers, middle and outer layers of middle parts, apical full-thickness, internal and external three-layer myocardium was significantly lower than that before dialysis, and there was no significant difference ($P > 0.05$). The reason why the left ventricular longitudinal peak strain changes significantly in a short time after hemodialysis may be related to the following mechanisms. Mechanism 1 is that the available and ingested calcium concentration in cardiac myocytes decreases during hemodialysis, resulting in impaired contractile and diastolic functions of cardiac myocytes. Mechanism 2 is that, during hemodialysis, arteriovenous fistula shunt increases cardiac load, causes unstable blood pressure, increases sympathetic excitability, activates adrenaline, causes abnormal cardiovascular system, and changes cardiac hemodynamics. Mechanisms 3 is that resistance vascular lesions in the coronary circulation of dialysis patients lead to decreased coronary reserve and increased myocardial oxygen demand. In a short time, the effective blood volume decreases sharply, electrolytes are replaced rapidly, the oxygen supply of coronary artery decreases and myocardial oxygen demand increases, which leads to insufficient blood supply of coronary artery, acute myocardial ischemia, disturbance of myocardial cell self-discipline, changes of wall motion and increase of cardiovascular events. Mechanisms 4 is that hemodialysis can increase peroxide and decrease antiperoxide, resulting in damage to myocardial cells.

The results of this study suggest that hemodialysis has different effects on the changes of function of the three layers of myocardium. The change of the inner myocardium is the most obvious. The reason is that the inner myocardium is mainly longitudinal myocardium, which plays a leading role in the contractile movement along the longitudinal axis. The inner myocardium has stronger contractile force than the middle and outer myocardium, and the inner myocardium is located in the end of blood supply, and it is sensitive to early ischemic changes. Left ventricular longitudinal peak strain basal segment > middle segment > apical segment before and after dialysis, suggesting that hemodialysis mainly affects the basal

Table 5 Comparison of longitudinal peak systolic laminar strain of left ventricular apical segment before and after dialysis

Time	aLPSndo	aLPSMyo	aLPSEpi	aGLPS
Before dialysis	−31.0 ± 3.8	−24.2 ± 2.7	−17.0 ± 2.4	−23.1 ± 3.1
After dialysis	−31.3 ± 3.3**	−23.6 ± 2.1**	−16.1 ± 2.6**	−23.3 ± 3.2**

Compared with before dialysis, * indicates that $P < 0.05$, there is significant difference; ** suggests that $P > 0.05$, there is no statistical difference after dialysis

bLPSEndo longitudinal peak systolic strain of basal inner myocardium, *bLPSMyo* longitudinal peak systolic strain of basal middle myocardium, *bLPSEpi* Longitudinal peak systolic strain of basal outer myocardium, *mLPSEndo* longitudinal peak systolic strain of middle inner myocardium, *mLPSMyo* longitudinal peak systolic strain of middle myocardium, *mLPSEpi* longitudinal peak systolic strain of the middle and outer myocardium, *aLPSEndo* longitudinal peak systolic strain of the inner apical myocardium, *aLPSMyo* longitudinal peak systolic strain of the middle apical myocardium, *aLPSEpi* longitudinal peak systolic strain of the outer apical myocardium

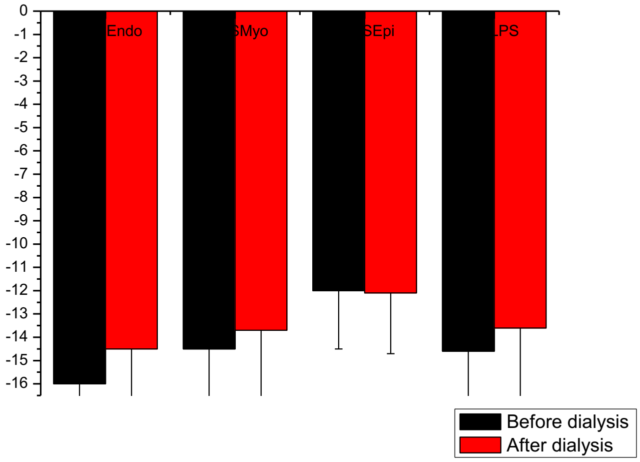

Fig. 5 Comparison of longitudinal peak systolic laminar strain of left ventricular basal segment before and after dialysis

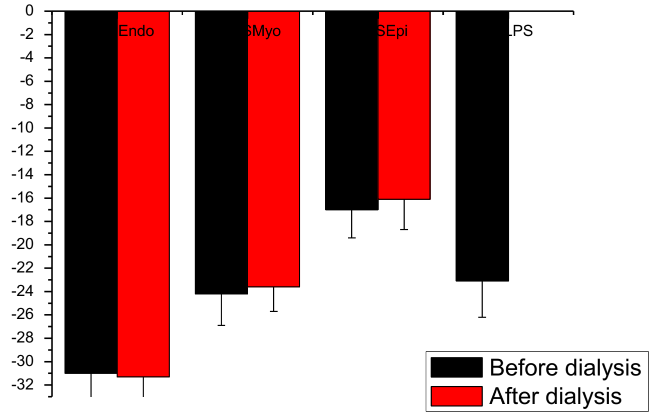

Fig. 7 Comparison of longitudinal peak systolic laminar strain of left ventricular apical segment before and after dialysis

segment and middle segment of myocardium, but has little effect on the apical segments of myocardium. The basal segment has the strongest contractile force, and the myocardium is under greater pressure. Therefore, it has a higher demand for energy and oxygen, and is more sensitive to pathological changes caused by various ischemia. The results of this study further confirm the conclusions of previous studies.

It is found that the cardiac function indexes of routine echocardiography in uremic hemodialysis patients do not change significantly before and after dialysis, but the indexes of stratified strain detection e change, which has statistical significance. It shows that stratified strain technique can detect the slight changes of myocardial function sensitively in the early stage before and after dialysis. It has certain application value to early warn the changes of cardiac function in such patients.

The limitations of this study are as follows: first, the sample size is small, and the results of the study need to be further validated by large samples; second, the technique requires high image quality. The recognition and description of endocardium and epicardium are influenced by many factors, such as respiration, heart rate, skeleton, subcutaneous adipose layer and muscular layer thickness, which will directly affect the accuracy, reproducibility and analysis results of data; third, myocardium is a three-dimensional motion, but eSie VVI technology is only a two-dimensional plane analysis, and it still has spatial dependence, so it cannot evaluate the characteristics of myocardial motion in all segments comprehensively at the same time.

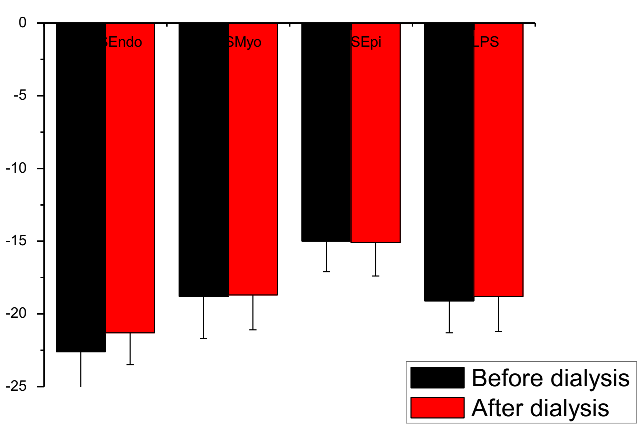

Fig. 6 Comparison of longitudinal peak delamination strain of left ventricular systole before and after dialysis

Conclusion

Hemodialysis has a certain negative impact on left ventricular systolic function in a short time. Therefore, it is necessary to closely observe the cardiac function of uremic patients after hemodialysis, and monitor it when necessary, so as to reduce the occurrence of sudden cardiovascular events while completing hemodialysis.

eSie VVI technology can more sensitively, accurately and quantitatively evaluate the local and global systolic function of left ventricular inner, middle and outer myocardium layer by layer, and timely evaluate the changes of longitudinal peak strain of left ventricular myocardium in uremic patients before and after hemodialysis. It is hopeful that eSie VVI technology can provide a new method for quantitatively evaluating the systolic dysfunction of left ventricular local and global in clinical hemodialysis patients.

Compliance with ethical standards

Conflict of interest Author Yafen Wang declares that he has no conflict of interest. Author Yiming Zhang declares that he has no conflict of interest. Author Weidong Liang declares that he has no conflict of interest. Author Liangdong Yuan declares that he has no conflict of interest. Author Shiqi Zhang declares that he has no conflict of interest. Author Yang Li declares that he has no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

This article does not contain any studies with animals performed by any of the authors.

Informed consent Informed consent was obtained from all individual participants included in the study.

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