Journal of Medicine in Scientific Research

Volume 4 | Issue 1 Article 1

Subject Area: Nephrology

Importance of PGC-1α gene expression in Egyptian hemodialysis patients

Ashraf Donia
National Institute of Urology and Nephrology

Azza A. I. Elmenyawi
National Institute of Urology and Nephrology, azzamenyawi@yahoo.com

Recommended Citation

Donia, Ashraf and I. Elmenyawi, Azza A. (2021) "Importance of PGC-1α gene expression in Egyptian hemodialysis patients," *Journal of Medicine in Scientific Research*: Vol. 4: Iss. 1, Article 1.
DOI: https://doi.org/10.4103/JMISR.JMISR_109_20

Importance of PGC-1α gene expression in Egyptian hemodialysis patients

Azza A.I. Elmenyawi[a], Ashraf Donia[b]

Departments of [a]Clinical Pathology, [b]Nephrology, National Institute of Urology and Nephrology, Cairo, Egypt

Abstract

Introduction

The peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α) is an upstream transcriptional regulator of mitochondrial biogenesis and function. Its function is immediate elimination of reactive oxygen species to get rid of their cellular destruction sequelae. Decreased PGC-1α count and less mitochondrial function take part in renal failure. COX6C gene encodes mitochondrial oxidative phosphorylation proteins. Reaction of excessive reactive oxygen species with linolenic acid results in malondialdehyde (MDA) as a breakdown product. It is an important marker for evaluating oxidative damage.

Patients and methods

A total of 58 hemodialysis (HD) patients in the National Institute of Urology and Nephrology and 20 apparently healthy participants as controls were enrolled in the study. PGC-1α and COX6C gene expressions were assessed by quantitative real-time PCR. Plasma MDA was assayed by ELISA. We followed up patients for 50 months for cardiovascular disease (CVD) and mortality.

Results

PGC-1α expression level was insignificantly downregulated ($P = 0.07$), COX6C gene expression level was significantly downregulated ($P < 0.001$), and plasma MDA level was significantly higher ($P < 0.001$) in HD patients than in controls. There was a significant negative association between PGC-1α expression and MDA in both HD patient and control groups (r=−0.73 and r=−0.76, respectively; $P \leq 0.001$ for both). We also found in patients who developed HD-related CVD, lower PGC-1α gene expression ($P < 0.001$), lower COX6C gene expression ($P < 0.001$), and higher plasma MDA level ($P < 0.001$) when compared with HD patients without CVD. By multivariate regression analysis, we found that PGC-1α gene expression was an independent predictor factor ($P < 0.001$) of CVD development.

Conclusion

PGC-1α could be a risk factor for occurrence of HD-linked CVD. Pharmacological modification of PGC-1α protein activity might be a hopeful therapeutic way to minimize oxidative stress-related clinical complications in HD patients.

Keywords: Cardiovascular disease, hemodialysis patients, PGC-1α gene

Introduction

Chronic kidney disease (CKD) is a great general health problem leading to significant economic and communal load [1]. CKD is an independent risk factor for cardiovascular disease (CVD) morbidity and mortality. Patients with CKD and end-stage renal disease (ESRD) have a 5- to 10-fold higher risk for developing CVD compared with controls [2]. CVD mortality is approximately twice as high in patients with third-stage CKD (estimated glomerular filtration rate 30–59 ml/min per 1.73 m^2) and three times higher at fourth stage (15–29 ml/min per 1.73 m^2) compared with normal kidney function participants. It was found that the incidence of cardiovascular mortality in patients with mild to moderate CKD is much higher than the incidence of ESRD [3].

Access this article online

Quick Response Code:

Website: www.jmsr.eg.net

DOI: 10.4103/JMISR.JMISR_109_20

Correspondence to: Azza A.I. Elmenyawi, MD
Department of Clinical Pathology, National Institute of Urology and Nephrology, Cairo, Egypt, Postal/Zip Code:11865
Fax: 0222549157 / 0222522771 Tel: +20 122 240 6654;
E-mail: azzamenyawi@yahoo.com

Submitted: 20-Oct-2020 **Revised:** 30-Nov-2020 **Accepted:** 17-Dec-2020 **Published:** 26-Feb-2021

How to cite this article: I. Elmenyawi AA, Donia A. Importance of PGC-1α gene expression in Egyptian hemodialysis patients. J Med Sci Res 2021;4:1-10.

In the patients with CKD, CVD ranges from arterial vascular diseases such as atherosclerosis and arteriosclerosis to heart failure, which is dominant at advanced stages. Not only traditional cardiovascular risk factors, such as hypertension and diabetes, can explain the severely increased cardiovascular burden in these patients with CKD but also oxidative stress (OS) and chronic inflammatory state as the nontraditional CKD-related risk factors are included in CVD pathogenesis [4].

In hemodialysis (HD) patients, bad clinical consequences are due to inflammation as a result of periodontal disease, bio-incompatible dialysis membranes, impure dialysate, vascular access, malnutrition, and CVD [5].

Enhanced prooxidant activity [6] and reduced antioxidant mechanisms [7] related both to ESRD and HD processes lead to increased OS in HD patients [8]. Cellular destruction occurs due to reaction of excessive reactive oxygen species (ROS) with proteins, lipids, and nucleic acids. Oxidation of polyunsaturated fatty acids such as linolenic acid results in malondialdehyde (MDA) as a breakdown product. It is an important marker for evaluating oxidative damage [9]. It causes mutation and has cytotoxic effects by reaction with DNA and proteins [8]. It may be included in the pathogenesis of many diseases such as atherosclerosis [9]. MDA levels increase with the severity of renal dysfunction and in HD patients with long dialysis time [10].

ROS are molecules derived from oxygen that can easily oxidize other molecules. ROS have a physiological role at low levels and take part in evoking reproduction and survival in stress conditions, but enhanced or chronic stress results in excess mitochondrial ROS production, which will lead to more mitochondrial abnormality and cellular destruction [11].

The kidney is the next organ only to the heart in oxygen utilization and mitochondrial profusion. Proximal tubules give rise to ATP mostly through mitochondrial oxidative phosphorylation [12].

There are mitochondrial dysfunctions in CKD owing to the presence of alteration in mitochondrial shape and structure, increased mitochondrial OS, and remarkable reduction in mitochondrial biogenesis and in ATP output [13].

The peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α) is considered as the chief inducible upstream transcriptional regulator of mitochondrial biogenesis and function [14]. PGC-1α is a coactivator because it interacts with other transcription factors, involving peroxisome proliferator-activated receptor α/β and nuclear respiratory factor 1 and 2 [15]. Enhanced mitochondrial biogenesis occurs by rising cellular PGC-1α amount, leading to increased mitochondrial count, oxidative phosphorylation, and ROS production. The function of PGC-1α in regulating oxidative procedure is known as a 'clean energy program', as ROS are originated and immediately eliminated to get rid of their cellular destruction sequelae [16]. Decreased PGC-1α count, mitochondrial loss, and less mitochondrial function take part in several metabolic diseases, including renal failure [14].

COX6C gene is also one of the genes encoding mitochondrial oxidative phosphorylation proteins. A big number of the genes distinguishing CKD and HD patients from healthy individuals were included in the synthesis of important nuclear-encoded structural subunits of the oxidative phosphorylation complexes. *COX6C* encodes for a subunit of the cytochrome c oxidase (COX or complex IV), which is the terminal enzyme of the mitochondrial respiratory chain inducing the transport of electron from reduced cytochrome C to oxygen [17].

The aim of the present study was to evaluate PGC-1α expression in patients with ESRD on HD as an index of mitochondrial oxidative dysfunction and its relation to HD-related cardiovascular complications.

Patients and methods

Our study included 58 patients with ESRD on maintenance HD three times a week for 4 h per session (through arteriovenous fistulas) in the National Institute of Urology and Nephrology and apparently healthy participants as controls. Institutional Ethics Committee approval and written informed consent were obtained from all the participants before the study. Patients with diabetes mellitus, CVDs, systemic autoimmune diseases, malignancies, inflammatory diseases, patients with infectious diseases receiving corticosteroids or anti-inflammatory drugs, pregnant women, and unwilling patients were excluded.

The patients were followed up for 50 months to assess the development of HD-related CVD morbidity and mortality.

All the study participants were subjected to the following investigations: renal function tests, high-sensitivity C-reactive protein, lipid profile, and complete blood counts using commercial kits. Low-density lipoprotein (LDL)-cholesterol was calculated with Friedewald formula: LDL-cholesterol = total cholesterol− (high-density lipoprotein-cholesterol + triglycerides/5) [18].

Plasma MDA was assayed using ELISA commercial kits (Wuhan EIAab Science, Wuhan, China), according to the manufacturer's protocol.

PGC-1α and COX6C gene expressions were determined by quantitative real-time PCR. Total RNA was isolated by QIAamp RNA Blood Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. By Nano drop 2000/2000c spectrophotometer (Thermo Scientific, Cambridge, Massachusetts, USA), we measured the concentration and purity of RNA. Total RNA was kept at −80°C until further analysis.

Reverse transcription of purified RNA was performed to synthesize cDNA using the QuantiTect cDNA Reverse Transcription Kit (Qiagen, GmbH, Hilden, Germany), following the manufacturer's instructions. Overall, 1 μg of purified RNA was added to the reverse transcription reaction

mix in a total volume of 20 µl. The reaction mix was incubated in Biometra thermocycler at 42°C for 15 min, then 95°C for 3 min to inactivate Quant script Reverse Transcriptase. cDNA was stored at −20°C for real-time PCR.

For real-time PCR amplification reaction, we used 2 × Universal PCR Master Mix, PGC-1α and COX6C Gene Expression Assay (Assay ID: QT00001190 and QT00221137, respectively) and cDNA in a total volume of 25 µl according to manufacturer's instructions (QuantiTect real-time PCR using SYBR Green I, Qiagen). Thermal cycling was performed on Rotor-gene Q System and included activation of Taq polymerase at 95°C for 5 min followed by 40 cycles at 95°C for 5 s for denaturation and 60°C for 10 s for annealing and extension. Amplification specificity was controlled by a melting curve analysis. A negative control (no template control) was included in each run. We used glyceraldehyde-3-phosphate dehydrogenase as a normalization gene (Gene Expression Assay ID: QT0079247).

For data analysis, we used Rotor-gene Q Software (Qiagen, GmbH, Hilden, Germany) for data processing. We relatively quantified PGC-1α and COX6C gene expression by calculation using the comparative C_t method ($2^{-\Delta\Delta Ct}$) and presented as a fold change relative to the control group.

Statistical analysis

The analysis was conducted with the use of SPSS statistical software package, version 23, for MAC. (Armonk, New York, United States). Categorical data were compared between HD and control groups using the χ^2 test, or Fisher's exact test. Quantitative data were expressed as median and interquartile range, and we used Spearman's correlation test and Mann and Whitney test. Variables that were statistically significant on univariate analysis were introduced into a logistic regression model to detect independent predictors of CVD and mortality in HD group. All tests were bilateral, and a *P* value less than 0.05 was considered statistically significant.

Results

The study patients were from the National Institute of Urology and Nephrology. A total of 58 patients with ESRD on maintenance HD and 20 controls were enrolled in the study. Patients' ages ranged from 22 to 58 years. They consisted of 26 (53.1%) males and 32 (46.9%) females. The mean duration of HD was 97 ± 50 months. The main causes of CKD were hypertension in 22 cases, tubulointerstitial diseases in 14 cases, glomerulonephritis in nine cases, adult polycystic kidney disease in four cases, and unknown in nine cases. After follow-up for 50 months, 15 (32.7%) patients developed evidence of HD-related CVDs, where nine patients had ischemic heart disease and six patients had congestive heart failure. During follow-up, nine patients died: five patients died owing to CVDs, and four patients died owing to other causes. Table 1 shows demographic, laboratory, and clinical data for the study groups.

Fig. 1 shows PGC-1α expression level. It was insignificantly downregulated in HD patients than in controls (*P* = 0.07).

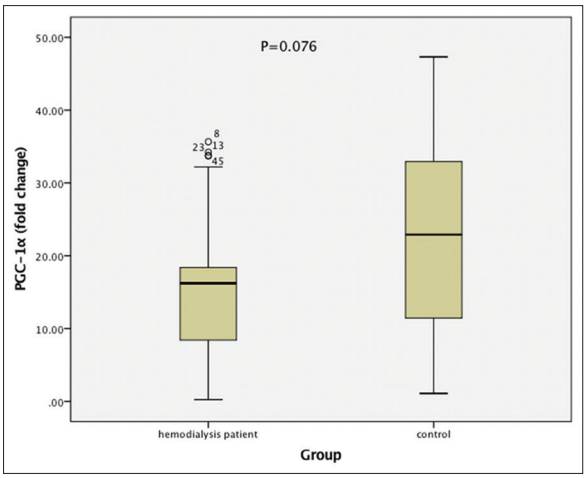

Figure 1: PGC-1α expression in hemodialysis and control groups. PGC-1α, peroxisome proliferator-activated receptor γ coactivator-1α.

It ranged from 0.25 to 35.66, with a median value of 16.22. We also asssesed COX6C gene expression level as one of the genes encoding mitochondrial OXPHOS proteins. It was significantly downregulated in HD patients than in controls; it ranged from 0.52 to 29.7, with a median value of 2.02 ($P < 0.001$) (Fig. 2).

There were significant associations between PGC-1α gene expression and cholesterol (r=−0.355, P = 0.006) and triglyceride (r=−0.336, P = 0.01) (Table 2).

Plasma MDA was significantly higher in HD patients when compared with control group ($P \leq 0.001$) (Fig. 3). It ranged from 3.8 to 26.9, with a median value of 13.52. We assesed MDA to asses the relationship between PGC-1α expression level and OS. MDA is one of thiobarbituric acid-reactive substances, which are products of OS-induced lipid damage. Figs. 4 and 5 show a significant negative association between PGC-1α expression and MDA in both HD patient and control groups (r=−0.73 and r=−0.76, respectively, $P < 0.001$).

We found in patients who developed HD-related CVD, lower PGC-1α gene expression ($P \leq 0.001$) (Fig. 6), lower COX6C gene expression ($P \leq 0.001$) (Fig. 7) and higher plasma MDA level ($P \leq 0.001$) (Fig. 8) when compared with HD patients without CVD. Moreover, patients with CVD showed significantly higher high-sensitivity C-reactive protein (P = 0.039), total cholesterol (P = 0.004), triglyceride (P = 0.001), and LDL levels (P = 0.048) (Table 3).

By multivariate regression analysis, we found that PGC-1α gene expression was an independent predictor factor (P = 0.017) of CVD development, with an odds ratio of 1.583 (1.084–2.311), that is, every 1 U decrease in PGC-1 increases the probability of development of CVD by 1.6 folds. However, other factors became no longer significant (Table 4).

We found that HD patients who died showed lower PGC-1α expression ($P \leq 0.001$) (Fig. 9), lower COX6C expression (P = 0.004) (Fig. 10), and higher plasma MDA (P = 0.001) (Fig. 11) when compared with survivors (Table 5). There was no

Table 1: Comparison between the two studied groups regarding demographic, clinical, and laboratory data

	Hemodialysis patients ($n=58$)	Controls ($n=20$)	*P*
Male	26 (44.83)	8 (40)	0.7
Female	32 (55.17)	12 (60)	
Age (years)	50 (22-58)	40.5 (28-55)	0.001
Duration of hemodialysis (months)	97±50		
Systolic blood pressure (mmHg)	142.7±7.1	117±4	0.001
Diastolic blood pressure (mmHg)	86.4±7.3	79±4.5	0.19
High-sensitivity C-reactive protein (mg/l)	6 (1-23)	1.5 (0.6-2.4)	<0.001
Creatinine (mg/dl)	9.25 (4.6-13.8)	0.7 (0.4-1.1)	0.001
Urea (mg/dl)	123.65 (59-225.1)	26.4 (17.2-34.9)	<0.001
Cholesterol (mg/dl)	190 (109-325)	173.5 (158-191)	0.083
Triglyceride (mg/dl)	173.5 (55-501)	151 (96-176)	0.048
High-density lipoprotein (mg/dl)	33.35 (14-58)	38.85 (35.1-42.9)	0.019
Low-density lipoprotein (mg/dl)	116.6 (58.8-241.8)	105.55 (95.8-132.8)	0.31
Hemoglobin (g/dl)	8.9±0.8	13.5±1.23	<0.001
WBCs ($\times10^3$/cmm)	8.1 (3.7-17.8)	5.9 (4.1-8.8)	0.006
Platelets ($\times10^3$/cmm)	186 (129-449)	236 (159-428)	<0.001
PGC-1α (fold change)	16.22 (0.25-35.66)	22.9 (1.09-47.32)	0.07
COX6C (fold change)	2.02 (0.52-29.7)	21.5 (1.01-35.65)	<0.001
Malondialdehyde (nmol/ml)	13.52 (3.8-26.9)	4.24 (1.66-6.4)	<0.001

PGC-1α, peroxisome proliferator-activated receptor γ coactivator-1α; WBC, white blood cell.

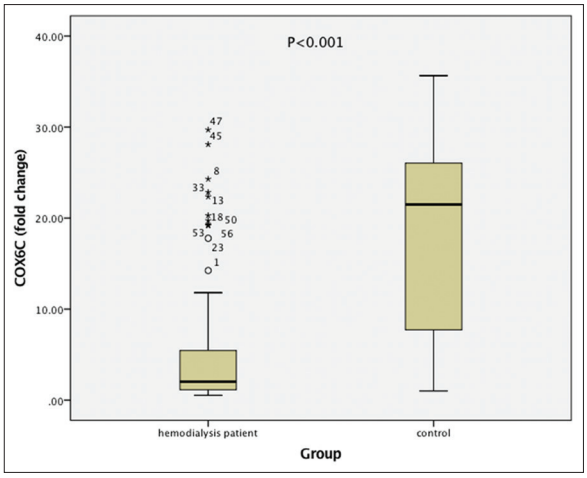

Figure 2: COX6C expression in hemodialysis and control groups.

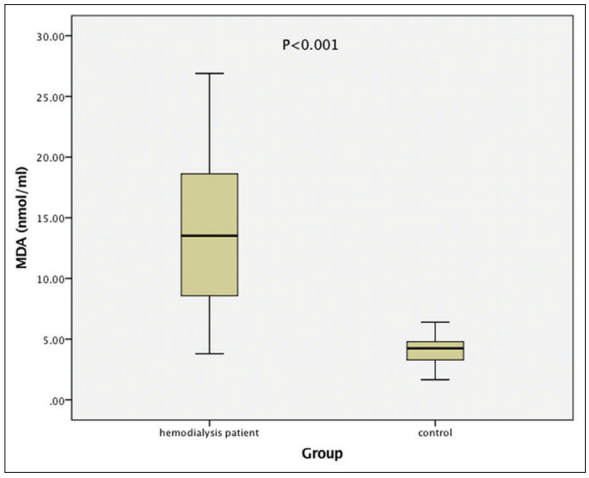

Figure 3: Malondialdehyde (MDA) in hemodialysis and control groups.

association between these parameters and mortality in these HD patients by using multivariate regression analysis (Table 6).

Discussion

The hazard of mortality and CVD in HD patients is high. Increased mortality and atherosclerosis are owing to not only conventional risk factors but also enhanced OS and chronic inflammation. OS occurs when oxidant substances overcome antioxidants. OS increase appears in late stages of CKD and is enhanced by the HD procedure per se [7].

The mechanisms of OS related to the pathogenesis of CVD in CKD included uremic toxins bound to protein and chronic activation of the renin–angiotensin–aldosterone and sympathetic nervous system, leading to OS-inflammation-fibrosis processes [19,20]; advanced glycation end products, resulting in receptor-mediated and receptor-independent increase of OS, inflammation, and vascular damage [21]; innate immune system activation, resulting in micro-inflammation and vascular dysfunction; and lastly, mitochondrial dysfunction, leading to ATP depletion [22].

On the contrary, increased OS in HD may be owing to situations present in HD patients like diabetes mellitus, dyslipidemia, hypertension, old age, and atherosclerosis [23]; reduction of antioxidant weapons; the associating chronic inflammation and prooxidant mechanisms activation by HD procedure [24], and reducing fruits and vegetables with malnutrition, leading to low vitamins intake (C, D, and E) [25]. Moreover, dialyzer membranes, anticoagulation, type of vascular access, and duration of HD session may participate in OS pathogenesis [23].

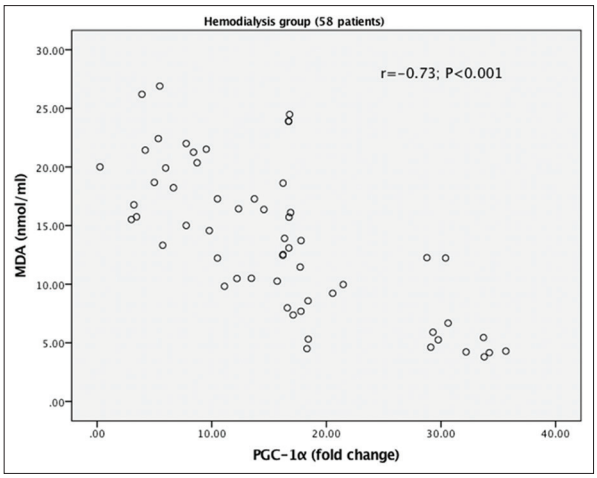

Figure 4: Correlation between PGC-1α expression and malondialdehyde (MDA) in hemodialysis group. PGC-1α, peroxisome proliferator-activated receptor γ coactivator-1α.

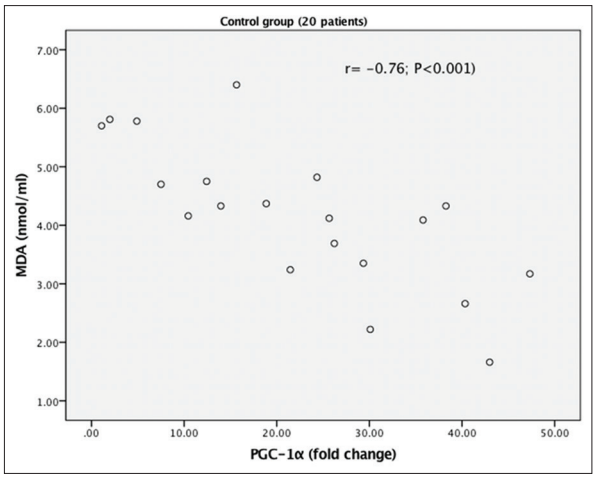

Figure 5: Correlation between PGC-1α expression and malondialdehyde (MDA) in control group. PGC-1α, peroxisome proliferator-activated receptor γ coactivator-1α.

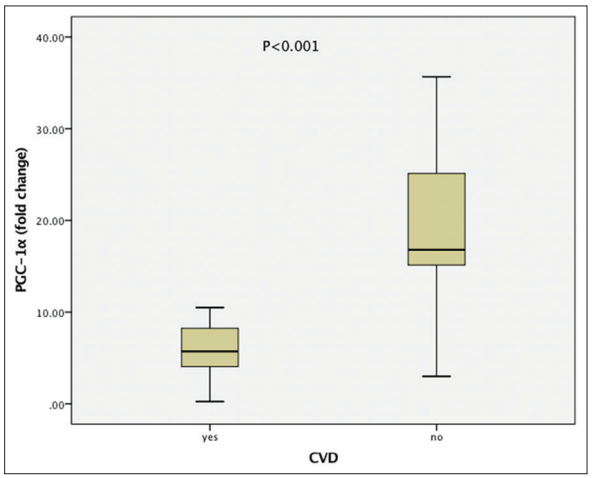

Figure 6: PGC-1α expression in hemodialysis patients with and without cardiovascular diseases. PGC-1α, peroxisome proliferator-activated receptor γ coactivator-1α.

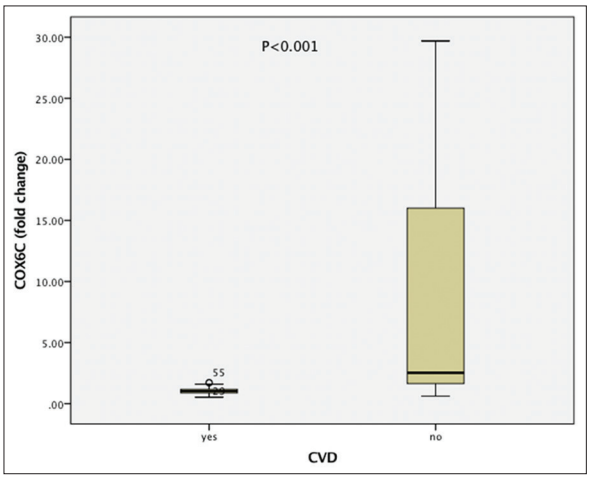

Figure 7: COX6C expression in hemodialysis patients with and without cardiovascular diseases.

Table 2: Comparison between the two studied groups regarding PGC-1 gene expression and laboratory data

	r	*P*
Creatinine (mg/dl)	−0.213	0.108
Urea (mg/dl)	−0.019	0.88
Cholesterol (mg/dl)	−0.355	0.006
Triglyceride (mg/dl)	−0.336	0.01
High-density lipoprotein (mg/dl)	−0.036	0.787
Low-density lipoprotein (mg/dl)	−0.252	0.056
Malondialdhyde (nmol/ml)	−0.741	<0.001

OS and mitochondrial deregulation are essential in several diseases, such as diabetes, CVD, and cancer [26]. ROS production mostly takes place in mitochondrial oxidative phosphorylation system via oxygen metabolism. Oxygen molecules are transformed to superoxide anions when some electrons are discharge from the electron transport chain and directly convert oxygen molecules to the superoxide anion. In stress conditions, more electrons are discharged from the respiratory chain, leading to enhanced superoxide production [27].

CKD and HD patients had an increased intracellular ROS generation, which affected cell functions, destructing proteins, lipids, and nucleic acids [28], and reduced the action of many components of the cellular respiratory chains [29]. An enhanced formation of ROS owing to the effect of proinflammatory mediators leads to severe reduction of the oxidative phosphorylation system causing a compensatory 'hypertrophy' of its elements leading to a continuous discharge of ROS. Granata *et al.* [30] declared the inability to differentiate between CKD and HD patients regarding oxidative phosphorylation system and considered this observation as a genomic hallmark of CKD itself which is not changed by HD treatment. It was revealed that there is a strong relation between mitochondrial dysfunction and OS

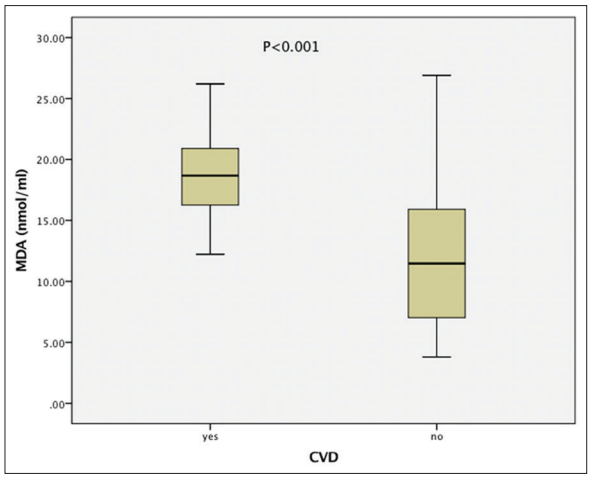

Figure 8: Malondialdehyde (MDA) in hemodialysis patients with and without cardiovascular diseases.

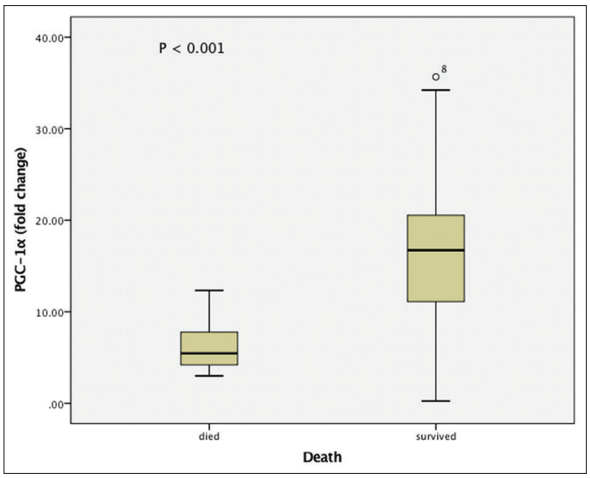

Figure 9: PGC-1α gene expression in survived and died hemodialysis patients. PGC-1α, peroxisome proliferator-activated receptor γ coactivator-1α.

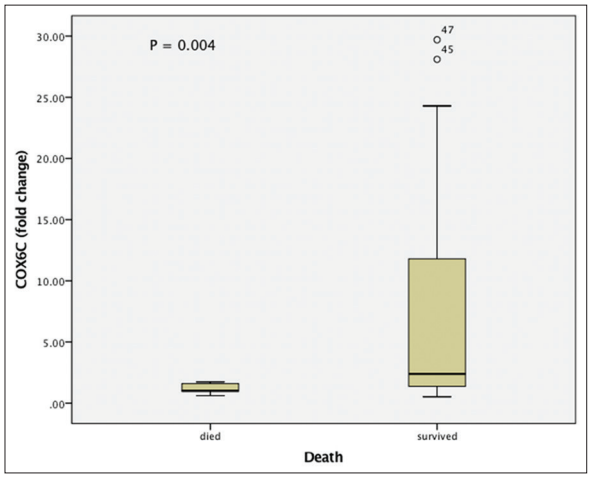

Figure 10: COX6C expression in survived and died hemodialysis patients.

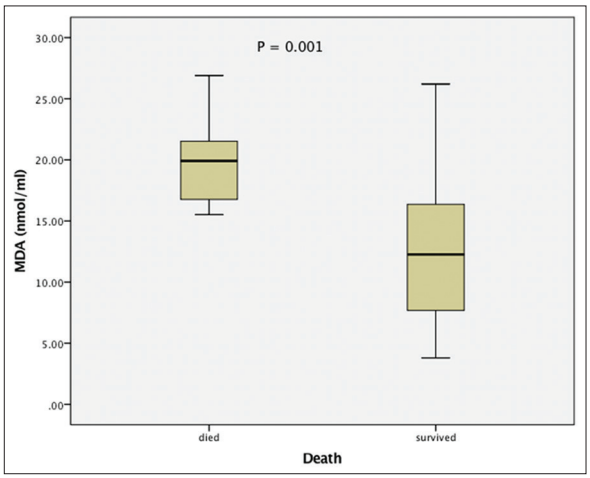

Figure 11: Malondialdehyde (MDA) in survived and died hemodialysis patients.

in (CKD) patients on conservative treatment and HD patients. The study by Gamboa *et al.* [31] concluded that mitochondrial OS and deregulation, evaluated by mitochondrial DNA copy number in Peripheral blood mononuclear cells (PBMCs), is elevated in HD patients more than stages 3–4 CKD patients.

Presence of chronic inflammation and malnutrition in dialysis patients is a common stimulator of ROS generation [32]. White blood cells and platelets are activated because of contact to bioincompatible dialysate, dialyzers, heparin utilization and intravenous iron injection, resulting in acute generation of ROS, after HD session start [33].

It is well known that anemia enhances OS in HD patients, whereas its correction improves the oxidation procedure [34]. It was observed that erythropoietin-stimulating agents decrease OS in HD patients [35], whereas Mircescu *et al.* [36] stated that erythropoietin-stimulating agent treatment in HD patients did not diminish OS in spite of correction of anemia. Utilization of central venous catheters for vascular access for long time and arteriovenous grafts and fistulae failure promote OS, inflammation, and vascular calcification [37].

Our real-time PCR assay showed that the expression extent of PGC-1α included in mitochondrial oxidative phosphorylation system is significantly decreased in HD patients compared with controls. PGC-1α expression manifests cellular energy requirements, in case of conditions of high energy necessities, giving rise to its expression [38]. We found also a decrease of COX6C gene, one of the PGC-1α downstream genes encoding for oxidation phosphorylation system (OXPHOS) subunits, showing a decrease reduction in the OXPHOS activity. COX6C and COX7C, the two subunits of the cytochrome C oxidase (COX or complex IV), are the terminating enzyme of the mitochondrial respiratory chain. Moreover, HD patients in the present study had significantly elevated higher plasma concentration of MDA, which is an OS index [39], compared with normal participants. Decreased PGC-1α expression was negatively correlated with MDA concentration. This is supported by the results of Elsayed *et al*. [40].

The origin of energy for cells are mitochondria, mitochondrial activity, and expression of PGC-1α are increased in the

Table 3: Comparison between laboratory data of hemodialysis patients with and without cardiovascular diseases

	Cardiovascular diseases		*P*
	No [*n*=43 (74.1%)]	Yes [*n*=15 (25.9%)]	
Male	19 (44.2)	7 (46.7)	0.868
Female	24 (55.8)	8 (53.3)	
Age (years)	50 (22-58)	54 (47-58)	0.048
High-sensitivity C-reactive protein (mg/l)	4 (1-16)	9 (3-21)	0.039
Creatinine (mg/dl)	9 (4.6-13.4)	10.3 (5.8-13.8)	0.051
Urea (mg/dl)	122.5 (59-225.1)	137 (81.6-187)	0.29
Cholesterol (mg/dl)	184 (108-299)	216 (156-325)	0.004
Triglyceride (mg/dl)	151 (55-452)	238 (87-501)	0.001
High-density lipoprotein (mg/dl)	34 (14-58)	31 (22-47)	0.58
Low-density lipoprotein (mg/dl)	105 (58.8-223)	136 (95.6-241.8)	0.048
Hemoglobin (g/dl)	9.8±0.8	8.9±0.9	0.86
PGC-1α (fold change)	16.79 (2.99-35.66)	5.72 (2.59-10.5)	<0.001
COX6C (fold change)	2.52 (0.61-29.7)	1.02 (0.52-1.73)	<0.001
Malondialdhyde (nmol/ml)	11.46 (3.8-26.9)	18.68 (12.22-26.2)	<0.001

PGC-1α, peroxisome proliferator-activated receptor γ coactivator-1α.

Table 4: Multivariate logistic regression analysis for factors affecting cardiovascular diseases development in hemodialysis patients

	P	Odds ratio	95% CI of odds ratio	
			Lower	Upper
PGC-1α (fold change)	0.017	1.583	1.084	2.311
COX6C (fold change)	0.742	1.331	0.2430	7. 307
MDA (nmol/ml)	0.783	1.036	0.808	1.328

CI, confidence interval; MDA, malondialdehyde; PGC-1α, peroxisome proliferator-activated receptor γ coactivator-1α.

kidney in the proximal tubule and medullary thick ascending limb of Henle, where the kidney requires a constant level of ATP to transport solutes along nephrons [41]. The expression of PGC-1α was significantly decreased in kidney biopsy specimens taken from patients with CKD and in unilateral ureteral obstruction, and folic acid caused fibrosis models [42]. Recently, the study by Choi *et al.* [43] revealed that PGC-1α may treat renal fibrosis. So, it is a good target for CKDs.

In agreement with our data, Li and Susztak [44] showed that reduced PGC-1α expression and decrease fatty acid use are the main pathological mechanisms of CKD. In both acute and chronic kidney injury, the PGC-1α mitochondrial, fatty acid oxidation axis is impaired, leading to energy diminution resulting in cell death.

Kidney biopsy specimens from patients with CKD showed reduced PGC1α expression than normal participants [45,46]. These data agree with ours.

Consistent with our study, Zaza *et al.* [47] reported downregulation of PGC-1α and COX6C and COX7C genes in peritoneal dialysis patients' PBMCs. Moreover, there were high MDA concentrations.

Experimentally stimulation of PGC-1α upregulation in diabetic kidney disease rat resulted in mitochondrial biogenesis stimulation and renal function enhancement [48]. The increased formation of mitochondrial ROS was accompanied with reduced expression of PGC-1α [49]. Induction of OS via inhibiting superoxide dismutase, which is a ROS detoxifying enzyme, indicates the contribution of OS in the pathogenesis of CVD in CKD [50].

Enhanced OS in HD patients is influenced by several factors such as age and uremic state. Membrane polyunsaturated fatty acid peroxidation by free radicals gives rise to some molecules such as MDA, which is helpful as an index for evaluating oxidative destruction. Presence of high serum MDA levels is observed in HD patients with CVD, than those without CVD, indicating the association of OS with the occurrence of atherosclerosis [51].

MDA epitopes present on dying cells in OS serve as waste markers for the immune cells, to be engulfed by phagocytes as a complement-mediated fast clearance. Meanwhile, increased formation of MDA epitopes with reduced clearance leads to proinflammatory responses. This is present in several chronic inflammatory diseases which are accompanied with diminished resolution such as atherosclerosis [52].

In consistence with our results, the study by Lakshmi *et al.* [53] revealed that MDA was significantly increased in HD patients when compared with controls. MDA, a small water-soluble molecule, can diffuse through dialysis membranes. Taking into consideration the clearance of MDA during dialysis, the ratio of MDA and creatinine was likely to give a better picture about MDA during dialysis. After correcting MDA for creatinine, they found a significant rise in MDA levels from predialysis to the postdialysis, suggesting that the elevation in MDA during HD was due to its increased generation owing to OS. This increase in post-HD MDA was a suggestion of the presence of OS during the dialysis session [54]. On the contrary, Ogunleye *et al.* [55] found similar MDA values before and after HD, which may be due

Table 5: Comparison between laboratory data of survived and died hemodialysis patients

	Mortality		*P*
	No [*n*=49 (48.5%)]	Yes [*n*=9 (15.5%)]	
Male	21 (42.9)	5 (55.6)	0.481
Female	28 (57.1)	4 (44.4)	
Age (years)	46.69±9.91	53.77±3.86	0.05
Cardiovascular disease: no	39 (79.6)	4 (44.4)	0.02
Yes	10 (20.4)	5 (55.6)	
High-sensitivity C-reactive protein (mg/l)	6 (2-21)	12.9 (9-23)	0.02
Creatinine (mg/dl)	9 (4.6-13.8)	10.9 (6.5-13.3)	0.16
Urea (mg/dl)	118.2 (69.9-225.1)	141.7 (59-187)	0.3
Cholesterol (mg/dl)	187 (109-325)	202 (155-310)	0.26
Triglyceride (mg/dl)	165 (55-452)	222 (87-501)	0.46
High-density lipoprotein (mg/dl)	33 (14-58)	35 (16-42)	0.75
Low-density lipoprotein (mg/dl)	115.4 (58.8-241.8)	136.1 (88.7-223)	0.34
Hemoglobin (g/dl)	9.6±0.8	9.4±0.7	0.32
PGC-1α (fold change)	16.72 (2.59-35.66)	5.46 (2.99-12.34)	<0.001
COX6C (fold change)	2.39 (0.52-29.7)	1.02 (0.61-1.73)	0.004
Malondialdhyde (nmol/ml)	12.26 (3.8-26.2)	19.91 (15.52-26.9)	0.001

PGC-1α, peroxisome proliferator-activated receptor γ coactivator-1α.

Table 6: Multivariate logistic regression analysis for factors affecting mortality risk in hemodialysis patients

	P	Odds ratio	95% CI of odds ratio	
			Lower	Upper
PGC-1α (fold change)	0.076	1.254	0.977	1.610
COX6C (fold change)	0.8860	1.104	0.284	4.288
MDA (nmol/ml)	0.213	0.872	0.703	1.082

MDA, malondialdehyde; PGC-1α, peroxisome proliferator-activated receptor γ coactivator-1α.

to taking samples 30 min after beginning dialysis leading to attaining equilibrium after HD.

Moreover, in agreement with our results, the studies of Rusu *et al.* [56], Wang *et al.* [57], and Liakopoulos *et al*. [58] found higher plasma concentration of MDA, well known as an OS marker, compared with normal participants.

Our patients were followed up for 50 months. We observed decreased PGC-1α and COX6C gene expression and increased MDA plasma level in HD patients with CVD when compared with HD patients without. Our results confirmed the study by Elsayed *et al.* [40], which reported PGC-1α and COX6C gene expression downregulation with enhanced MDA plasma level.

The current study showed that PGC-1 α gene expression was a predictor factor for CVD development but not increase in the risk of mortality by using multivariate logistic regression analysis. This is may be owing to relatively small sample size and consequently the small number of died patients .This is consistent with Elsayed *et al* [40], who found decreased PGC-1α gene expression and increased MDA in HD patients with CVD and HD patients who died. They also found that PGC-1 α gene is a predictor factor for CVD development without association between PGC-1α gene expression and MDA level with mortality.

The importance of OS in CVD pathogenesis in patients with CKD is established. Himmelfarb [59] reported that enhanced OS and its sequelae is the main sponsor to enhanced atherosclerosis and CV morbidity and mortality found in uremia. OS and inflammation cause decrease endothelial function and weaken vascular construction and function [60].

The study by La Russa *et al.* [61] reported that HD patients with previous cardiovascular episodes had elevated assays of OS and antioxidant barrier compared with patients without. In consistence with our results that showed in HD patients, the clinical and prognostic relevance of OS differed than normal people.

In Chronic renal failure (CRF), atherosclerosis occurs owing to endothelial dysfunction which leads to accumulation of atherosclerotic plaques. The severity of endothelial dysfunction is proportional to OS extent [62].

It is well known that mitochondrial changes occur in hearts as renal function is reduced. It was found that there was mitochondrial fragmentation and apoptosis, and cardiomyocyte dysfunction in hearts of mice subjected to bilateral renal ischemia reperfusion in an experimental design of cardio renal syndrome [63].

A significant cardiac dysfunction in PGC-1α-deficient mice was found, showing significant cardiac dysfunction owing to severe decrease of oxidative phosphorylation genes expression and elevated ROS [64].

There was a positive association correlation between PGC-1α expression in PMNCS and myocardial cells in patients sustaining a cardiac surgery. So, PMNCS PGC-1α expression could be used to manifest the myocardial expression of PGC-1α [65].

PGC-1α gene could be a precious target for pharmacological interference in HD patients to decrease OS, and consequently decrease complications. Some studies in cell and animal models of diseases with mitochondrial dysfunction revealed better mitochondrial functions by PGC-1α activation [66].

Acute kidney injury occurring in suprarenal vascular surgery, as well as cardiothoracic surgery requiring cardiopulmonary bypass, can be improved by enhancing PGC1α expression or action [67]. Resveratrol, a PGC-1α activator drug, acts by reducing PGC-1α acetylation, leading to enhanced PGC-1α activity and its downstream genes [68]. Meanwhile, there is a powerful restriction to utilize PGC-1α in therapy, as its functions are extremely cell-specific. Therefore, it is important to asses new objectives targeting mitochondrial biogenesis [11]. The main limitations of our study were the small sample size, a single-center study, and the assessment of genes' expression only once at the start of the study. Regardless of these limitations, our study showed a delicate modulated intracellular biochemical network related to OS in HD patients.

Conclusion

We concluded that PGC-1α could be a risk factor for the occurrence of HD-linked CVD. So, pharmacological modification of PGC-1α protein activity might be a hopeful therapeutic way to minimize OS-related clinical complications in HD patients.

Recommendation

Further studies on a larger scale are required to evaluate the degree of risk of CVD accompanied with changes in these OS markers in HD patients.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Zoccali C, Vanholder R, Massy ZA, Ortiz A, Sarafidis P, Dekker FW, *et al*. The systemic nature of CKD. Nat Rev Nephrol 2017; 13:344–358.
2. Jager KJ, Lindholm B, Goldsmith D, Fliser D, Wiecek A, Suleymanlar G, *et al*. Cardiovascular and non-cardiovascular mortality in dialysis patients: where is the link?. Kidney Int 2011; 1:21–23.
3. van der Velde M, Matsushita K, Coresh J, Astor BC, Woodward M, Levey A, *et al*. Lower estimated glomerular filtration rate and higher albuminuria are associated with all-cause and cardiovascular mortality. A collaborative meta-analysis of high-risk population cohorts. Kidney Int 2011; 79:1341–1352.
4. Cachofeiro V, Goicochea M, de Vinuesa SG, Oubiña P, Lahera V, Luño J. Oxidative stress and inflammation, a link between chronic kidney disease and cardiovascular disease. Kidney Int 2008; 74 (Suppl 111):S4–S9.
5. Raj DS, Carrero JJ, Shah VO, Qureshi AR, Barany P, Heimburger O, *et al*. Soluble CD14 levels, interleukin 6, and mortality among prevalent hemodialysis patients. Am J Kidney Dis 2009; 54:1072–1080.
6. Inal M, Kanbak G, Sen S, Akyüz F, Sunal E. Antioxidant status and lipid peroxidation in hemodialysis patients undergoing erythropoietin and erythropoietin-vitamin E combined therapy. Free Radic Res 1999; 31:211–216.
7. Locatelli F, Canaud B, Eckardt KU, Stenvinkel P, Wanner C, Zoccali C. Oxidative stress in end-stage renal disease: an emerging threat to patient outcome. Nephrol Dial Transplant 2003; 18:1272–1280.
8. El Mesallamy FA, Elhefnawy KA, El Said HH. Plasma retinol and malondialdehyde levels among hemodialysis patients. Int J Sci Res 2015; 4:193–200.
9. Sung CC, Hsu YC, Chen CC, Lin YF, Wu CC. Oxidative stress and nucleic acid oxidation in patients with chronic kidney disease. Oxid Med Cell Longev 2013; 2013:301982.
10. Peti A, Csiky B, Guth E, Kenyeres P, Mezosi E, Kovacs GL, Bajnok L. Effect of oxidative stress in hemodialysed patients. EJIFCC 2011; 22:45–51.
11. Galvan DL, Green NH, Danesh FR. The hallmarks of mitochondrial dysfunction in chronic kidney disease. Kidney Int 2017; 92:1051–1057.
12. Wirthensohn G, Guder WG. Renal substrate metabolism. Physiol Rev 1986; 66:469–497.
13. Zschiedrich S, Bork T, Liang W, Wanner N, Eulenbruch K, Munder S, *et al*. Targeting mTOR signaling can prevent the progression of FSGS. J Am Soc Nephrol 2017; 28:2144–2157.
14. Arany Z, He H, Lin J, Hoyer K, Handschin C, Toka O, *et al*. Transcriptional coactivator PGC-1 alpha controls the energy state and contractile function of cardiac muscle. Cell Metab 2005; 1:259–271.
15. Handschin C, Spiegelman BM. Peroxisome proliferator-activated receptor gamma coactivator 1 coactivators, energy homeostasis, and metabolism. Endocr Rev 2006; 27:728–735.
16. Finkel T. Cell biology: a clean energy programme. Nature 2006; 444:151–152.
17. Lenka N, Vijayasarathy C, Mullick J, Avadhani NG. Structural organization and transcription regulation of nuclear genes encoding the mammalian cytochrome c oxidase complex. Prog Nucleic Acid Res Mol Biol 1998; 61:309–344.
18. Roberts WC. The Friedewald-Levy-Fredrickson formula for calculating low-density lipoprotein cholesterol, the basis for lipid-lowering therapy. Am J Cardiol 1988; 62:345–346.
19. Lekawanvijit S, Kompa AR, Krum H. Protein-bound uremic toxins: a long overlooked culprit in cardiorenal syndrome. Am J Physiol 2016; 311:F52–F62.
20. Giam B, Kaye DM, Rajapakse NW. Role of renal oxidative stress in the pathogenesis of the cardiorenal syndrome. Heart Lung Circ 2016; 25:874–880.
21. Stinghen AEM, Massy ZA, Vlassara H, Striker GE, Boullier A. Uremic toxicity of advanced glycation end products in CKD. J Am Soc Nephrol 2016; 27:354–370.
22. Zewinger S, Schumann T, Fliser D, Speer T. Innate immunity in CKD-associated vascular diseases. Nephrol Dial Transplant 2016; 31:1813–1821.
23. Liakopoulos V, Roumeliotis S, Gorny X, Dounousi E, Mertens PR. Oxidative stress in hemodialysis patients: a review of the lit.erature. Oxid Med Cell Longev 2017; 2017:3081856.
24. Duni A, Liakopoulos V, Rapsomanikis KP, Dounousi E. Chronic kidney disease and disproportionally increased cardiovascular damage: does oxidative stress explain the burden?. Oxid Med Cell Longev 2017; 2017:9036450.
25. Ross R. Atherosclerosis – an inflammatory disease. N Engl J Med 1999; 340:115–126.
26. Rains JL, Jain SK. Oxidative stress, insulin signaling, and diabetes. Free Radic Biol Med 2011; 50:567–575.
27. West AP, Shadel GS, Ghosh S. Mitochondria in innate immune responses. Nat Rev Immunol 2011; 11:389–402.
28. Stadtman ER, Levine RL. Free radical-mediated oxidation of free amino acids and amino acid residues in proteins. Amino Acids 2003; 25:207–218.
29. Han Z, Chen YR, Jones CI3rd, Meenakshisundaram G, Zweier JL, B Rita Alevriadou BR. Shear-induced reactive nitrogen species inhibit mitochondrial respiratory complex activities in cultured vascular endothelial cells. Am J Physiol Cell Physiol 2007; 292:C1103–C1112.
30. Granata S, Zaza G, Simone S, Villani G, Latorre D, Pontrelli P, *et al*. Mitochondrial dysregulation and oxidative stress in patients with chronic kidney disease. BMC Genomics 2009;10:388.
31. Gamboa JL, Billings FT, Bojanowski MT, Gilliam LA, Yu C, Roshanravan B, *et al*. Mitochondrial dysfunction and oxidative stress in

patients with chronic kidney disease. Physiol Rep 2016; 4:e12780.
32. Modaresi A, Nafar M, Sahraei Z. Oxidative stress in chronic kidney disease. Iran J Kidney Dis 2015; 9:165–179.
33. Yang CC, Hsu SP, Wu MS, Hsu SM, Chien CT. Effects of vitamin C infusion and vitamin E-coated membrane on hemodialysis-induced oxidative stress. Kidney Int 2006; 69:706–714.
34. Wiswedel I, Peter D, Gardemann A, Carluccio F, Hampl H, Siems W. Serum concentrations of F2□isoprostanes and 4□hydroxynonenal in hemodialysis patients in relation to inflammation and renal anemia. Biomark Insights 2008; 3:419–428.
35. Katavetin P, Tungsanga K, Eiam□Ong S, Nangaku M. Antioxidative effects of erythropoietin. Kidney Int Suppl 2007; 107:S10–S15.
36. Mircescu G, Capusa C, Stoian I, Maracine M, Muscurel C, Garneata L, Barbulescu C. Influence of epoietinum therapy on the oxidative stress in haemodialysis patients. Nephron Clin Pract 2005; 100:c126–c132.
37. Himmelfarb J, Stenvinkel P, Ikizler TA, Hakim RM. The elephant in uremia: oxidant stress as a unifying concept of cardiovascular disease in uremia. Kidney Int 2002; 62:1524–1538.
38. Liang H, Ward WF. PGC-1alpha: a key regulator of energy metabolism. Adv Physiol Educ 2006; 30:145–151.
39. Yagi K. Simple assay for the level of total lipid peroxides in serum or plasma. Methods Mol Biol 1998; 108:101–106.
40. Elsayed ET, Nassra RA, Naga YS. Peroxisome proliferator-activated receptor-γ–coactivator 1α (PGC-1α) gene expression in chronic kidney disease patients on hemodialysis: relation to hemodialysis-related cardiovascular morbidity and mortality. Int Urol Nephrol 2017; 49:1835–1844.
41. Tran M, Tam D, Bardia A, Bhasin M, Rowe GC, Kher A, *et al*. PGC-1alpha promotes recovery after acute kidney injury during systemic inflammation in mice. J Clin Invest 2011; 121:4003–4014.
42. Han SH, Wu MY, Nam BY, Park JT, Yoo TH, Kang SW, *et al*. PGC-1alpha protects from notch-induced kidney fibrosis development. J Am Soc Nephrol 2017; 28:3312–3322.
43. Choi H, Park JS, Kim D, Kim CS, Bae EH, Ma SK, Kim SW. PGC-1α suppresses the activation of TGF-β/Smad signaling via targeting TGFβRI downregulation by let-7b/c upregulation. Int J Mol Sci 2019; 20:5084.
44. Li SY, Susztak K. The role of peroxisome proliferator-activated receptor gamma coactivator 1 α (PGC-1α) in kidney disease. Semin Nephrol 2018; 38:121–126.
45. Kang HM, Ahn SH, Choi P, Ko YA, Han SH, Chinga F, *et al*. Defective fatty acid oxidation in renal tubular epithelial cells has a key role in kidney fibrosis development. Nat Med 2015; 21:37–46.
46. Tran MT, Zsengeller ZK, Berg AH, Khankin EV, Bhasin MK, Kim W, *et al*. PGC1α drives NAD biosynthesis linking oxidative metabolism to renal protection. Nature 2016; 531:528–532.
47. Zaza G, Granata S, Masola V, Rugiu C, Fantin F, Gesualdo L, *et al*. Downregulation of nuclear-encoded genes of oxidative metabolism in dialyzed chronic kidney disease patients. PLoS ONE 2013; 8:e77847.
48. Dugan LL, You YH, Ali SS, Diamond-Stanic M, Miyamoto S, DeCleves AE, *et al*. AMPK dysregulation promotes diabetes-related reduction of superoxide and mitochondrial function. J Clin Invest 2013; 123:4888–4899.
49. Guo K, Lu J, Huang Y, Wu M, Zhang L, Yu H, *et al*. Protective role of PGC-1α in diabetic nephropathy is associated with the inhibition of ROS through mitochondrial dynamic remodeling. PLoS ONE 2015; 10:e0125176.
50. Baldelli S, Aquilano K, Ciriolo MR. PGC-1alpha buffers ROS-mediated removal of mitochondria during myogenesis. Cell Death Dis 2014; 5:e1515.
51. Tucker PS, Dalbo VJ, Han T, Kingsley MI. Clinical and research markers of oxidative stress in chronic kidney disease. Biomarkers 2013; 18:103–115.
52. Milicevic NP, Busch CJ, Binder CJ. Malondialdehyde epitopes as targets of immunity and the implications for atherosclerosis. Adv Immunol 2016; 131:1–59.
53. Lakshmi BS, Devi NH, Suchitra MM, Rao PVLNS, Kumar VS. Changes in the inflammatory and oxidative stress markers during a single hemodialysis session in patients with chronic kidney disease. Ren Fail 2018; 40:534–540.
54. Ramakrishna P, Reddy EP, Suchitra MM, Bitla AR, Srinivasa Roa PV, Sivakumar V. Effect of reuse of polysulfone membrane on oxidative stress during haemodialysis. Indian J Nephrol 2012; 22:200–205.
55. Ogunleye A, Akinbodewa AA, Adejumo OA, Oluwafemi TT, Akinfaderin DA. Changes in antioxidant status associated with haemodialysis in chronic kidney disease. Ghana Med J 2018; 52:29–33.
56. Rusu CC, Rasacan S, Kasco IM, Moldovan D, Potra A, Patiu IM, *et al*. Malondialdehyde can predict survival in hemodialysis patients. Clujul Med 2016; 89:250–256.
57. Wang Z, Yu C, Li X, Deng B. The prognostic value of oxidative stress and inflammation in Chinese hemodialysis patients. Ren Fail 2017; 39:54–58.
58. Liakopoulos V, Roumeliotis S, Zarogiannis S, Eleftheriadis T, Mertens PR. Oxidative stress in hemodialysis: causative mechanisms, clinical implications, and possible therapeutic interventions. Semin Dial 2019; 23:58–71.
59. Himmelfarb J. Linking oxidative stress and inflammation in kidney disease: which is the chicken and which is the egg?. Semin Dial 2004; 17:449–454.
60. Himmelfarb J. Uremic toxicity, oxidative stress, and hemodialysis as renal replacement therapy. Semin Dial 2009; 22:636–643.
61. La Russa D, Pellegrino D, Montesanto A, Gigliotti P, Perri A, La Russa A, Bonofiglio R. Oxidative balance and inflammation in hemodialysis patients: biomarkers of cardiovascular risk?. Oxid Med Cell Longev 2019; 2019:8567275.
62. Wu-Wong JR. Endothelial dysfunction and chronic kidney disease: treatment options. Curr Opin Investig Drugs 2008; 9:970–982.
63. Sumida M, Doi K, Ogasawara E, Yamashita T, Hamasaki Y , Kariya T, *et al*. Regulation of mitochondrial dynamics by dynamin-related protein-1 in acute cardiorenal syndrome. J Am Soc Nephrol 2015; 26:2378–2387.
64. Rowe GC, Jiang A, Arany Z. PGC-1 coactivators in cardiac development and disease. Circ Res 2010; 107:825–838.
65. Fabregat-Andres O, Paredes F, Monsalve M, Milara J, Ridocci-Soriano F, Gonzalez-Hervas S, *et al*. mRNA PGC-1α levels in blood samples reliably correlates with its myocardial expression: study in patients undergoing cardiac surgery. Anatol J Cardiol 2016; 16:622–629.
66. Hofer A, Noe N, Tischner C, Kladt N, Lellek V, Schauss A, *et al*. Defining the action spectrum of potential PGC-1alpha activators on a mitochondrial and cellular level in vivo. Hum Mol Genet 2014; 23:2400–2415.
67. Lynch MR, Tran MT, Parikh SM. PGC1α in the kidney. Am J Physiol Renal Physiol 2018; 314:F1–F8.
68. Long J, Badal SS, Ye Z, Wang Y, Ayanga BA, Galvan DL, *et al*. Long noncoding RNA Tug1 regulates mitochondrial bioenergetics in diabetic nephropathy. J Clin Invest 2016; 126:4205–4218.