A pilot study on gene expression of endoplasmic reticulum unfolded protein response in chronic kidney disease

Rasha G. Mostafa a,⁎, Abd El-Aleem Hassan Abd Al-Aleem b, Eman Abdella Mahmoud Fouda c, Fardous Rabea Ahmed Taha c, Khaled Mohamed Amin Elzorkany d

a Department of Medical Microbiology and Immunology, Faculty of Medicine – Menoufia University, Egypt
b Department of Organic Chemistry, Faculty of Science – Menoufia University, Egypt
c Department of Biochemistry, Faculty of Science – Menoufia University, Egypt
d Department of Internal Medicine, Faculty of Medicine- Menoufia University, Egypt

ARTICLE INFO

Keywords:
CDK
ER stress
PERK
IRE1 and ATF6 gene expressions

ABSTRACT

Background: Chronic kidney disease (CKD) is a worldwide public health problem due to its increasing prevalence worldwide. Endoplasmic reticulum (ER) stress has been shown to play a role in the pathogenesis of various renal diseases in humans. It leads to the activation of the unfolded protein response (UPR) which triggers three known trans membrane sensors in the ER: activating transcription factor 6 (ATF6), inositol-requiring enzyme 1 (IRE1), and PKR (double-stranded RNA-dependent protein kinase)-like ER protein kinase (PERK). The activation of these signal transduction pathways can result in cell death, inflammation, and fibrosis in the context of CKD.

Objectives: The aim of this study was to detect the level of gene expression of activating transcription factor 6 (ATF6), inositol-requiring enzyme 1 (IRE1), and PKR (double-stranded RNA-dependent protein kinase)-like ER protein kinase (PERK) in chronic kidney disease patients.

Subjects and methods: This study was carried out on eighty subjects, 50 patients with CKD (25 with hypertension and 25 without hypertension) and 30 healthy subjects served as controls. All studied subjects underwent laboratory investigations, including CBC, Serum Lipid profile: Total cholesterol, Triglycerides, HDL-cholesterol and LDL-cholesterol, liver and kidney functions, fasting and 2 h postprandial blood glucose and HbA1C, serum level of IL6 and gene expression of ATF6, IRE1 and PERK using real time PCR technique.

Results: There was a significant increase in relative quantitation (RQ) of gene expression of IRE1, ATF6 and PERK in chronic kidney patient groups with hypertension and without hypertension compared to control group. Also, there was a significant positive correlation of PERK and ATF6 gene expressions and a significant negative correlation of PERK gene expressions and GFR in groups I&II.

Conclusion: Endoplasmic reticulum (ER) stress occurs in CKD with activation of gene expression of three transmembrane sensors in the ER: activating transcription factor 6 (ATF6), inositol-requiring enzyme 1 (IRE1), and PKR (double-stranded RNA-dependent protein kinase)-like ER protein kinase (PERK).

1. Introduction

Chronic kidney disease (CKD) is defined as kidney damage. The severity of CKD is classified into five stages according to the level of glomerular filtration rate (GFR) [1]. The major outcomes of chronic kidney disease, regardless of cause, include progression to kidney failure, and cardiovascular disease (CVD). Some of these adverse outcomes can be prevented or delayed by early detection and treatment [2].

Endoplasmic reticulum (ER) stress has been shown to play a role in the pathogenesis of various renal diseases in humans [3]. Endoplasmic reticulum (ER) stress occurs when ER show accumulation of unfolded/misfolded protein or calcium depletion. The unfolded protein response (UPR), comprising of inositol-requiring enzyme 1a (IRE1α), double-stranded RNA-dependent protein kinase (PKR)-like ER kinase (PERK) and activating transcription factor 6 (ATF6) signaling pathways, is a protective cellular response activated by ER stress. However, UPR activation can also induce cell death upon persistent ER stress [4].

The IRE1α and PERK pathways of the UPR result in inflammation...
through the activation of NF-κB and AP-1, transcription factors responsible for the up regulation of cytokines, chemokines, complement, and acute phase proteins [5]. Released, NF-κ B activates the transcription of a large number of target genes: RANTES (regulated upon activation normal T cell expressed and secreted), interleukin (IL)-1, IL-2, IL-6, MCP, tumour necrosis factor-alpha (TNF-alpha), adhesion molecules, and several other pro-inflammatory mediators. The inhibition of TNF-alpha and NF-B-inducible gene, has been shown to delay the progression of hypertension and renal damage by reducing proteinuria, urinary MCP-1 excretion, and renal macrophage infiltration in animal models [6,7].

The UPR itself is mainly cyto-protective, aiming to alleviate the damage and restore cellular homeostasis via transcriptional induction of specific molecular chaperones. However, the UPR signaling pathway may switch to death-triggering pathways and lead to apoptosis through the tree signal trans-membrane molecules may occur in the case of prolonged activation of PERK-dependent UPR signaling pathway under severe and extensive ER stress conditions. Thus, the life-versus-death determination or coordination of adaptive and apoptotic responses are tightly regulated [8].

Changes in circulatory hemodynamics may result in electrolyte and fluid retention in chronic kidney disease and hormones released into the circulation in these states may act directly on certain cell types to induce ER stress [9]. UPR tunes immune responses through the modulation of cytokine production. It supports the development of specific immune cells and may determine shapes the immune responses in many cell types. Also, ER stress can directly promote inflammatory gene expression, including pro-inflammatory cytokines [9,10].

The aim of this study was to detect the level of gene expression of activating transcription factor 6 (ATF6), inositol-requiring enzyme I (IRE1), PKR (double-stranded RNA-dependent protein kinase)-like ER protein kinase (PERK) and serum interleukin 6 in chronic kidney disease patients.

1.1. Subject and methods

This study was carried out by cooperation between, Biochemistry department, faculty of science, Menoufia University and Internal Medicine, Microbiology & Immunology Departments, Faculty of Medicine, Menoufia University in the period from February to August 2019. It was conducted on 80 subjects classified into group I: included 25 patients with kidney disease with hypertension and group II included 25 patients with kidney disease without hypertension. The diagnosis of chronic kidney diseases can be ascertained by the presence of albuminuria, defined as albumin-to-creatinine ratio >30 mg/g in two of three spot urine specimens. GFR can be estimated from calibrated serum creatinine and estimating equations, such as the Modification of Diet in Renal Disease (MDRD), patients with diabetes mellitus, cardiovascular disease and end-stage renal disease were excluded from the study. Group III: 30 healthy subjects served as controls, age, and gender matched apparently healthy control subjects were volunteers from the hospitals staff, medical and nursing students and members of the local community.

Prior to collection of blood samples, written informed consent (approved from Committee of Ethics and Human Rights in Research at Faculty of Medicine, Menoufia University) was obtained from all subjects enrolled in this study. They were subjected to the following: Thorough medical history taking, Complete physical examination, Ultrasound, and Routine investigations including the following: CBC, Serum Lipid profile: Total cholesterol, Triglycerides, HDL-cholesterol and LDL-cholesterol, liver and kidney functions, fasting and 2 h post-prandial blood glucose and HbA1C.

1.2. Sample collection and assay

Seven ml of venous blood were collected from all subjects included in this study after overnight fasting by venipuncture from the cubital vein, and were divided as follow: 2 ml of blood were put into EDTA containing tubes, for RNA extraction. The remaining 5 ml were divided in plain vacutainer tube, left 15 min for coagulation, then centrifuged at 3000 rpm for 10 min then the serum was separated into aliquots for measurement of serum fasting glucose level by glucose oxidase method using (Spinreact diagnostics kit, Spain). Measurement of lipid profile [10]: high density lipoproteins cholesterol (HDLC), total cholesterol (TC) and triglycerides (TG) using standard enzymatic colorimetric kits (Spinreact diagnostics kit, Spain), calculation of low-density lipoprotein cholesterol (LDL-c) according to the Friedewald equation [11]. Determination of serum IL6 by ELISA technique using Quantikine Kit, USA.

1.3. ATF6, IRE1 and PERK mRNA gene expression by real time PCR

Total RNA was extracted from fresh serum samples using miR Neasy kit, QIAGEN, USA. The yield and purity of RNA were measured by Nano-Drop instrument (Thermo Scientific, USA). RNA extract was stored at -80 C. Sensifast cDNA synthesis Kit, Bioline, Germany was used for reverse transcription step and production of complementary DNA (cDNA). Each reaction was carried out on ice with a total volume of 20 μl, containing 1 μl of reverse transcriptase enzyme, 4 μl of reverse transcriptase Buffer, 10 μl of template RNA and 5 μl of nuclease free water. Incubation was done using 2720 thermal cycler, Applied Bio systems (Singapore) for one cycle as follows: 10 min at 42 C then, 5 min at 95 C to inactivate reverse transcriptase enzyme and finally for 5 min at 4C. cDNA produced was stored at -20C till the real-time PCR step. Real-time PCR was performed using Sensifast TM SYBR low ROX Kit, USA. Total volume of 20 μl was applied, in the form of 10 μl of SYBR green Master Mix; 1 μl of Nuclease-free water, 6 μl of template cDNA and 1.5 μl of each primer (forward & reverse). The following primers (Midland, Texas) were used; forATF6 gene: forward primer sequence was: CAGGGAGAAGGACTTTGTA and reverse primer primer was::ACTGACCGAGGAGACGAGA, for IRE1 gene: forward primer sequence was: GCGGAAGTTGAGTGAATC and reverse primer primer was: AATCCTGGAAAGGCGGATGA, for PERK forward primer sequence was: ATGTGCATTGCTGTTAC and reverse primer primer sequence was: GACTCCTCCTCTTCGCTGTT and for (GAPDH) as an endogenous control Forward primer 5-CCACTCCCTCACCCCATGAC and Reverse primer 5-ACCCGTTGCTGCTGAC-3.

The PCR condition for gene amplification consisted of three phases: initial activation phase at 95 C for 5 min followed by 45 cycles at 95 C for 20 s; 60 C for 30 s; 72 C for 1 min; and a final extension phase at 72 C for 10 min. Finally, fluorescence detection and data analysis were done using 7500 real time PCR instrument (Applied Biosystems, USA) v.2.0.1. The relative quantification (RQ) of gene expression was calculated using comparative DDCt method [12]. Fig. 1 shows the amplification plot of gene expression.

1.4. Statistical analysis

Results were collected, tabulated and statistically analyzed by IBM personal computer and statistical package SPSS version 20(IBM Corporation, Armond, NY, USA). Chi-square test (χ2was used to study the association between two qualitative variables. Odds ratio (OR) describes the probability that people who are exposed to a certain factor will have a disease compared to people who are not exposed to the factor. Student’s t-test was used for comparison between two groups having quantitative variables. Analysis of variance (ANOVA) (F) test was used
for comparison between three or more groups having quantitative variables. Multiple regression analysis was performed to calculate the effects of risk factors as independent ORs with the effects of other confounders removed. P-value < 0.05 was statistically significant.

2. Results

The present study included 80 individuals (29 male and 51 female), there was a non-significant statistical difference between two patient groups compared to control group regarding age (p = 0.621) and gender (p = 0.177) indicating group matching. While there was a significant statistical difference in SBP, DBP in chronic kidney disease patients with hypertension compared to chronic kidney disease patients without hypertension and control groups (p < 0.001). As regards kidney and liver function tests there was a significant statistical difference of increase in urea and creatinine in chronic kidney disease patients with hypertension and without hypertension compared to controls (p < 0.001). As regards kidney and liver function tests there was a significant statistical difference of increase in urea and creatinine in chronic kidney disease patients with hypertension and without hypertension compared to controls (p < 0.001). As regards kidney and liver function tests there was a significant statistical difference of increase in urea and creatinine in chronic kidney disease patients with hypertension and without hypertension compared to controls (p < 0.001). On the other hand, there was non-significant statistical difference of liver function tests between the three studied groups (p > 0.05).

A significant increase in triglyceride (TG) and total cholesterol (TC) levels in chronic kidney patients with hypertension compared to chronic kidney disease without hypertension and control groups, while there was a significant increase in LDLc, pro-inflammatory Cytokines (IL6) and decrease in HDLc chronic kidney patients with and without hypertension compared to controls. (Table 1).

A significant increase in relative quantitation (RQ) of gene expression of PERK, ATF6 and IRE1 in chronic kidney patient groups with hypertension and without hypertension compared to control group (Table 2, Fig. 2 & 3).

Receiver operating characteristic (ROC curve) of PERK, ATF6 and IRE1 gene expression at cut off point 52, 12.7 and 4.99 respectively for differentiation between chronic kidney disease patients with and without hypertension have 100% sensitivity for PERK, ATF6 and IRE1 gene expressions, has 76% Specificity for PERK gene expression, 100% specificity for ATF6 gene expression and 80% specificity for IRE1 gene expression (Table 3 and Fig. 4).

Table 1 showed a significant positive correlation of PERK with each of ATF6 gene expression, serum IL6 level and a significant negative correlation of PERK gene expressions with GFR in chronic kidney patients with hypertension, while a significant positive correlation of PERK gene expression and urea level and significant negative correlation of PERK gene expression and IRE1 are present in control group. On the other hand, a significant positive correlation of ATF6 gene expressions with HDLc serum IL6 levels in chronic kidney patients with hypertension (Table 5).

Regarding IRE1 gene expressions, it showed non-significant correlation with other parameters in chronic kidney patients with hypertension and without hypertension and control group (Table 6).

for comparison between three or more groups having quantitative variables. Multiple regression analysis was performed to calculate the effects of risk factors as independent ORs with the effects of other confounders removed. P-value < 0.05 was statistically significant.

2. Results

The present study included 80 individuals (29 male and 51 female), there was a non-significant statistical difference between two patient groups compared to control group regarding age (p = 0.621) and gender (p = 0.177) indicating group matching. While there was a significant statistical difference in SBP, DBP in chronic kidney disease patients with hypertension compared to chronic kidney disease patients without hypertension and control groups (p < 0.001). As regards kidney and liver function tests there was a significant statistical difference of increase in urea and creatinine in chronic kidney disease patients with hypertension and without hypertension compared to controls (p < 0.001). On the other hand, there was non-significant statistical difference of liver function tests between the three studied groups (p > 0.05).

A significant increase in triglyceride (TG) and total cholesterol (TC) levels in chronic kidney patients with hypertension compared to chronic kidney disease without hypertension and control groups, while there was a significant increase in LDLc, pro-inflammatory Cytokines (IL6) and decrease in HDLc chronic kidney patients with and without hypertension compared to controls. (Table 1).

A significant increase in relative quantitation (RQ) of gene expression of PERK, ATF6 and IRE1 in chronic kidney patient groups with hypertension and without hypertension compared to control group (Table 2, Fig. 2 & 3).

Receiver operating characteristic (ROC curve) of PERK, ATF6 and IRE1 gene expression at cut off point 52, 12.7 and 4.99 respectively for differentiation between chronic kidney disease patients with and without hypertension have 100% sensitivity for PERK, ATF6 and IRE1 gene expressions, has 76% Specificity for PERK gene expression, 100% specificity for ATF6 gene expression and 80% specificity for IRE1 gene expression (Table 3 and Fig. 4).

Table 1 showed a significant positive correlation of PERK with each of ATF6 gene expression, serum IL6 level and a significant negative correlation of PERK gene expressions with GFR in chronic kidney patients with hypertension, while a significant positive correlation of PERK gene expression and urea level and significant negative correlation of PERK gene expression and IRE1 are present in control group. On the other hand, a significant positive correlation of ATF6 gene expressions with HDLc serum IL6 levels in chronic kidney patients with hypertension (Table 5).

Regarding IRE1 gene expressions, it showed non-significant correlation with other parameters in chronic kidney patients with hypertension and without hypertension and control group (Table 6).

for comparison between three or more groups having quantitative variables. Multiple regression analysis was performed to calculate the effects of risk factors as independent ORs with the effects of other confounders removed. P-value < 0.05 was statistically significant.

2. Results

The present study included 80 individuals (29 male and 51 female), there was a non-significant statistical difference between two patient groups compared to control group regarding age (p = 0.621) and gender (p = 0.177) indicating group matching. While there was a significant statistical difference in SBP, DBP in chronic kidney disease patients with hypertension compared to chronic kidney disease patients without hypertension and control groups (p < 0.001). As regards kidney and liver function tests there was a significant statistical difference of increase in urea and creatinine in chronic kidney disease patients with hypertension and without hypertension compared to controls (p < 0.001). On the other hand, there was non-significant statistical difference of liver function tests between the three studied groups (p > 0.05).

A significant increase in triglyceride (TG) and total cholesterol (TC) levels in chronic kidney patients with hypertension compared to chronic kidney disease without hypertension and control groups, while there was a significant increase in LDLc, pro-inflammatory Cytokines (IL6) and decrease in HDLc chronic kidney patients with and without hypertension compared to controls. (Table 1).

A significant increase in relative quantitation (RQ) of gene expression of PERK, ATF6 and IRE1 in chronic kidney patient groups with hypertension and without hypertension compared to control group (Table 2, Fig. 2 & 3).

Receiver operating characteristic (ROC curve) of PERK, ATF6 and IRE1 gene expression at cut off point 52, 12.7 and 4.99 respectively for differentiation between chronic kidney disease patients with and without hypertension have 100% sensitivity for PERK, ATF6 and IRE1 gene expressions, has 76% Specificity for PERK gene expression, 100% specificity for ATF6 gene expression and 80% specificity for IRE1 gene expression (Table 3 and Fig. 4).

Table 1 showed a significant positive correlation of PERK with each of ATF6 gene expression, serum IL6 level and a significant negative correlation of PERK gene expressions with GFR in chronic kidney patients with hypertension, while a significant positive correlation of PERK gene expression and urea level and significant negative correlation of PERK gene expression and IRE1 are present in control group. On the other hand, a significant positive correlation of ATF6 gene expressions with HDLc serum IL6 levels in chronic kidney patients with hypertension (Table 5).

Regarding IRE1 gene expressions, it showed non-significant correlation with other parameters in chronic kidney patients with hypertension and without hypertension and control group (Table 6).
Discussion

ER stress plays an important role in the pathogenesis of chronic diseases associated with the accumulation of mis-folded proteins. These include neurodegenerative diseases such as Alzheimer’s and Parkinson’s diseases, atherosclerosis diabetes mellitus and chronic kidney disease. ER stress results in the activation of the unfolded protein response, an evolutionarily conserved cellular response [13].

Changes in circulatory hemodynamics may result in electrolyte and fluid retention in chronic kidney disease and may also result in cardiac hypertrophy. As well, hormones released into the circulation in these pathophysiologic states may act directly on certain cell types to induce ER stress [15].

In this study, there was a significant statistical difference in SBP, DBP in chronic kidney disease patients with hypertension compared to chronic kidney disease patients without hypertension and control groups (p < 0.001).

Table 2

Mean distribution of relative quantitation (RQ) for gene expression of PERK, ATF6 and IRE1 among the studied groups.

| Groups | Kruskal-Wallis (P value) | Post hoc value |
|--------|--------------------------|----------------|
| I (N = 25) | II (N = 25) | III (N = 30) |
| Mean ± SD | Mean ± SD | Mean ± SD |
| RQ of Perk | 111.48 ± 49.36 | 36.08 ± 17.61 | 1.21 ± 0.64 |
| RQ of ATF6 | 24.16 ± 7.86 | 8.69 ± 2.06 | 0.55 ± 0.63 |
| RQ of IRE1 | 9.10 ± 2.52 | 3.72 ± 1.07 | 0.88 ± 0.25 |

*significant.

The UPR involves the activation of processes such as apoptosis and inflammation that determine the fate of cell survival and tissue scarring. Therefore, ER stress is an important factor in the development of renal diseases and the study of the UPR pathway is likely to reveal molecular targets that influence disease progression [14].

Table 3

Validity of PERK, ATF6 and IRE1 gene expressions between the studied groups (I vs. II).

| Group | Gene | AUC | Cutoff point | Sensitivity % | Specificity % | Accuracy % | PPV % | NPV % |
|-------|------|-----|--------------|--------------|---------------|------------|-------|-------|
| I vs. II | Perk | 0.96 | ≥52 | 100.0 | 76.0 | 88.0 | 80.6 | 100.0 |
| ATF6 | 1.0 | ≥12.70 | 100.0 | 100.0 | 100.0 |
| IRE1 | 0.97 | ≥4.99 | 100.0 | 80.0 | 90.0 | 83.0 | 100.0 |

*significant.

3. Discussion

ER stress plays an important role in the pathogenesis of chronic diseases associated with the accumulation of mis-folded proteins. These include neurodegenerative diseases such as Alzheimer’s and Parkinson’s diseases, atherosclerosis diabetes mellitus and chronic kidney disease. ER stress results in the activation of the unfolded protein response, an evolutionarily conserved cellular response [13].
Correlation of ATF6 gene expressions and other variables among the studied groups.

| Variables Groups | I | II | III |
|------------------|---|----|-----|
| Rs P value rs P value rs P value |
| Age (years) | 0.135 | 0.520 | 0.016 | 0.941 | -0.275 | 0.142 |
| SBP (mmHg) | -0.022 | 0.916 | 0.213 | 0.307 | 0.132 | 0.488 |
| DBP (mmHg) | -0.001 | 0.998 | 0.242 | 0.244 | -0.031 | 0.872 |
| Urea (mg/dl) | 0.296 | 0.151 | 0.071 | 0.737 | 0.371 | 0.043* |
| Creatinine (mg/dl) | 0.383 | 0.059 | 0.024 | 0.909 | 0.104 | 0.584 |
| GFR (ml/min) | -0.499 | 0.011* | -0.007 | 0.973 | -0.095 | 0.616 |
| AST (IU/L) | -0.083 | 0.069 | -0.102 | 0.628 | 0.087 | 0.674 |
| ALT (IU/L) | -0.085 | 0.685 | -0.433 | 0.030 | -0.032 | 0.865 |
| TG (mg/dl) | -0.176 | 0.400 | -0.246 | 0.236 | 0.188 | 0.320 |
| TC (mg/dl) | 0.116 | 0.581 | -0.449 | 0.024 | -0.277 | 0.138 |
| HDLc (mg/dl) | 0.454 | 0.023 | -0.253 | 0.223 | 0.157 | 0.407 |
| LDLc (mg/dl) | 0.127 | 0.545 | -0.368 | 0.070 | -0.350 | 0.058 |
| Serum IL6 (pg/ml) | 0.658 | <0.001* | 0.1394 | 0.535 | 0.321 | 0.112 |
| ATP6 | 0.694 | <0.001* | 0.142 | 0.498 | 0.028 | 0.883 |
| IRE1 | 0.018 | 0.931* | 0.184 | 0.378 | -0.404 | 0.027* |

*a significant.

Correlation of PERK gene expressions and other variables among the studied groups.

| Variables Groups | I | II | III |
|------------------|---|----|-----|
| Rs P value rs P value rs P value |
| Age (years) | 0.240 | 0.248 | 0.025 | 0.905 | 0.070 | 0.713 |
| SBP (mmHg) | -0.042 | 0.841 | -0.273 | 0.186 | 0.210 | 0.265 |
| DBP (mmHg) | 0.023 | 0.912 | -0.210 | 0.314 | -0.112 | 0.557 |
| Urea (mg/dl) | 0.126 | 0.550 | -0.146 | 0.487 | -0.229 | 0.223 |
| Creatinine (mg/dl) | 0.271 | 0.190 | 0.044 | 0.836 | 0.251 | 0.180 |
| GFR (ml/min) | -0.351 | 0.085 | -0.185 | 0.350 | 0.059 | 0.757 |
| AST (IU/L) | 0.002 | 0.992 | 0.215 | 0.302 | -0.326 | 0.079 |
| ALT (IU/L) | -0.015 | 0.943 | 0.131 | 0.533 | -0.072 | 0.706 |
| TG (mg/dl) | 0.015 | 0.943 | -0.293 | 0.155 | -0.247 | 0.188 |
| TC (mg/dl) | 0.269 | 0.194 | 0.076 | 0.716 | 0.068 | 0.721 |
| HDLc (mg/dl) | 0.441 | 0.027* | 0.197 | 0.346 | 0.249 | 0.185 |
| LDLc (mg/dl) | 0.207 | 0.320 | 0.023 | 0.915 | -0.044 | 0.816 |
| Serum IL6 (pg/ml) | 0.6792 | <0.001* | 0.227 | 0.275 | 0.3279 | 0.093 |
| IRE1 | -0.066 | 0.753 | -0.098 | 0.642 | -0.107 | 0.572 |

Kumari and Srinivas who studied the lipid profile in CKD patients and state several mechanisms may underlie the reductions in HDLc levels include, diminished activity of LCAT (the enzyme responsible for the esterification of free cholesterol in HDL particles) as well as increased activity of cholesterol ester transfers protein that facilitates the transfer of cholesterol esters from HDL to TGL-rich lipoproteins that reduce serum concentrations of HDL cholesterol [17].

Also, the down-regulation of the expression of several genes along with the changes in the composition of lipoprotein particles and the direct inhibitory effect of various uremic toxins on the enzymes involved in lipid metabolism represents the most important pathophysiological mechanism underlying the development of hypertriglyceridemia in renal failure [17].

The present study demonstrates that IL-6 is significantly increased in the plasma of CKD patients as compared to normal subjects, these results are in accordance of Hua et al. study who concluded that IL-6 participates in renal instinct cell injury and repair process, as well as a variety of immune, metabolic, ischemic, and toxemic-mediated renal diseases [18].

Increased plasma levels of IL-6 and TNF alpha in not yet dialyzed patients are associated to an activation state of monocytes. The presence of activated T cells as reflected by increased expression of their membrane interleukin-2 receptors [19].

Although activated monocytes and T cells could both be responsible for increased circulating TNF alpha and IL-6 levels in these patients, one must also consider the possibility that chronic renal failure itself could participate in dysregulation of the production and elimination of these cytokines. Indeed, the reduced glomerular filtration rate could lead to their defective clearance from the plasma [20].

In this study, a significant increase in gene expression of PERK, ATF6 and IRE1 was detected in chronic kidney patients groups with hypertension and without hypertension compared to controls. This was in agree with Liu et al. [21] who detected activated expression of these three ER stress genes in cardiac tissue of diabetic animals in their study and they found dramatically increased p-PERK/t-PERK ratio, p-IRE1/-t-IRE1 ratio and cleaved ATF6 expression, as well as the up-regulated levels of the proapoptotic protein called C/EBP homologous protein (CHOP), indicating all three ER stress sensors were activated, leading to myocyte apoptosis. Rather than IRE1 or ATF6, PERK governed signaling pathway is probably the major pathway conducting apoptotic signaling in ROS-induced ER stress mediated myocyte apoptosis in Diabetic cardiomyopathy.

Also, the study of Robert et al. suggests that both ATF6a and ATF6β filtration rate in chronic kidney disease patients with hypertension and without hypertension compared to controls (p < 0.001). On the other hand, liver function tests showed non significant stastical difference between the three studied groups (p > 0.05).

Chronic renal disease results in profound lipid disorders due to the deregulation of high-density lipoproteins (HDL) and triglyceride-rich lipoprotein metabolism which increases the risk of arteriosclerotic cardiovascular disease, the leading cause of mortality among chronic kidney disease (CKD) patients. In this study, there was a significant increase in triglyceride (TG) and total cholesterol (TC) levels in chronic kidney patients with hypertension compared to chronic kidney disease without hypertension and control groups, while there was a significant increase in LDLc and decrease in HDLc chronic kidney patients with and without hypertension compared to controls. These results were consistent with
are important for the development of compensatory cardiac hypertrophy following pressure overload stress [22]. While, The study of Zahraa et al. found that phosphorylated IRE1α are upregulated in patients with hypertensive nephrosclerosis and ER stress inhibitors were shown to decrease renal fibrosis [14]. Comparing to this study, Shaqoun et a study I reported that PERK phosphorylation was detected in post-ischemic kidney disease and the ratio of p-PERK/P ERK was significantly increased [23].

4. Conclusion

• ER stress and deregulated UPR are closely associated with the pathogenesis of CKD. Targeting the components of the UPR machinery to optimize UPR activity and alleviating ER stress have been considered to be promising strategies for treating or delaying the progression of CKD.

• Patients with CKD have a higher prevalence of dyslipidemias compared to the general population and so, are at high risk of developing cardiovascular disease. Most CKD patients do not develop kidney failure but die as a result of CVD. Therefore, it is important not only to identify these patients early but also to treat their dyslipidemias intensively before they develop end-stage renal disease. Most patients will require lifestyle modification and lipid-lowering therapy. CKD patients should be considered as patients at very high risk for CVD and treated accordingly.

• Serum pro-inflammatory cytokines (IL-6) may be predictive of the development of chronic kidney disease and the disease activity, more researches needed to evaluate the role of IL-6 levels in the clinical assessment of CKD, while their therapeutic neutralization could be of assistance in its treatment.

Credit author statement

Rasha Galal Mostafa: Writing- Reviewing and Editing, Eman Abdella Mahmoud Fouda: Data collection, Writing- Original draft preparation, Fardous Rabea Ahmed Taha and Khaled Mohamed Amin Elzorkany: Sample collection- Investigation, Abd El-Aleem Hassan: Supervision- Methodology.

Funding

This article was not funded.

Ethical Approval

Research Involving Human Participants. The study was conducted in accordance with the Declaration of Helsinki. All participants provided written informed consent, and the Ethics Committee of Faculty of Medicine, Menoufia University approved the study protocol.

Declaration of competing interest

The authors declare that they have no conflict of interest.

Acknowledgements

Thanks to Central laboratory – Faculty of Medicine- Menoufia University for providing technical advice for this study. Also thanks to all laboratory members of the central lab for their support.

References

[1] V. Jha, et al., Chronic kidney disease: global dimension and perspectives, Lancet 382 (2013) 260–272.
[2] KDIGO: Kidney Disease Improving Global Outcomes, KDIGO clinical practice guideline for the diagnosis, evaluation, prevention, and treatment of chronic kidney disease-mineral and bone disorder (CKD-MBD), Kidney Int. July 7 (1) (2017) 1–59.
[3] J.G. Dickhout, R.E. Carlisle, R.C. Austin, Interrelationship between cardiac hypertrophy, heart failure, and chronic kidney disease endoplasmic reticulum stress as a mediator of pathogenesis, Circ. Res. 107 (2010) 1185–1197.
[4] X. Liu, R.M. Green, Endoplasmic reticulum stress and liver diseases liver, Research 3 (2019) 55e64.
[5] K. Zhang, R.J. Kaufman, From endoplasmic reticulum stress to the inflammatory response, Nature (Lond.) 454 (2008) 455–462.
[6] A.A. Elmarakby, J.E. Quigley, D.M. Pollock, J.D. Imig, Tumor necrosis factor blockade increases renal Cyp2c23 expression and slows the progression of renal damage in salt- sensitive hypertension., J. Hypertension 47 (3) (2006) 557–562.
[7] A.A. Elmarakby, J.E. Quigley, J.D. Imig, J.S. Pollock, D. Pollock, TNF-inhibition reduces renal injury in DOCA-salt hypertensive rats, in: A.A. Elmarakby, J. E. Quigley, J.D. Imig, J.S. Pollock, D.M. Pollock (Eds.), Am. J. Physiol. Regul. Integr. Comp. Physiol. 294 (1) (2008) R76–R83.
[8] Z. Liu, N. Liu, S.R. Cheng, The unfolded protein response: a novel insight into chronic kidney disease (CKD), Nephrol. Open J. 3 (1) (2017) e15-e17, https://doi.org/10.17101/NPOJ-3-e008.
[9] J. Grootjans, A. Kaser, R.J. Kaufman, R.S. Blumberg, The unfolded protein response in immunity and inflammation, Nat. Rev. Immunol. 16 (2016) 469–484.
[10] Z.M. Ali, G.L. Cruz, J.G. Dickhout, Croststalk between the unfolded protein response and NF-B-mediated inflammation in the progression of chronic kidney disease, Journal of Immunology Research (2015) 10. Article ID 428508.
[11] Metabolic and hereditary disorders, in: J.B. Wallach (Ed.), Interpretation of Diagnostic Tests, sixth ed., Little Brown and Company, Boston, MA, 1996.
[12] M. Dorak, Real-time PCR, Clin. Chem. 50 (2004) 1680–1682.
[13] V. Siwecka N, W.R. edek, Y. Darianz Pytel, Wawrzynkiewicz Adam, Dzikl Adam, J. LukaszDzikl, Alan diehl and IreneuszMajsterek: dual role of endoplasmic reticulum stress-mediated unfolded protein response signaling pathway in Carcinogenesindot, J. Mol. Sci. 20 (2019) 4354.
[14] Zahraa Mohammed-Abli, Gaile L. Cruz, Jeffrey G. Dickhout, Croststalk between the unfolded protein response and NF-B-mediated inflammation in the progression of chronic kidney disease, J Immunol Res (2015) 428508.
[15] R.W. Schrier, W.T. Abraham, Hormones and hemodynamics in heart failure, N. Engl. J. Med. 341 (1999) 97–105. Article ID 428508.
[16] T.W. Meyer, T.H. Hostetter, Uremic study of lipid profile in patients with chronic kidney disease on conservative management andHemodialysis, N. Engl. J. Med. 357 (2007) 1316–1325.
[17] K Rajani Kumari, Bindi Srinivas, Study of lipid profile in patients with chronic kidney disease on conservative management and hemodialysis, Int. J. Sci. Stud. 6 (7) (2018), 10.17354.
[18] Hua Su, Chun-Tao Lei, Chun Zhang, Interleukin-6 signaling pathway and its role in kidney disease: an update, Front. Immunol. 8 (2017) 405.
[19] O.M. Akchurin, F. Kaskel, Update on inflammation in chronic kidney disease, Blood Purif. 39 (2015) 84–92.
[20] A. Bruchfeld, J.J. Carrero, A.R. Qureshi, B. Lindholm, P. Barany, O. Heimburger, et al., Elevated serum macrophage migration inhibitory factor (MIF) concentrations in chronic kidney disease (CKD) are associated with markers of oxidative stress and endothelial activation, Mol. Med. 15 (2009) 70–75.
[21] Z.-W. Liu, H.-T. Zhu, Kun-Lun Chen, Xin Dong, Wei Jin, Chuan Qiu, Jia-Hong Xue, Protein kinase RNA- like endoplasmic reticulum kinase (PERK) signaling pathway plays a major role in reactive oxygen species (ROS)-mediated endoplasmic reticulum stress-induced apoptosis in diabetic cardiomyopathy Cardiovascular, Diabetology 12 (2013) 15.
[22] Robert N. Correll, Kelly M. Grimes, Vikram Prasad, Jeffrey M. Lynch, Hadi Khalil, D. Jeffery, Molkentin Overlapping and Differential Functions of ATF6 versus ATF6 in the Mouse Heart Scientific Reports, vol. 9, 2019, p. 2059, https://doi.org/10.1038/s41598-019-39515-5.
[23] Shaoqun Shu, Jiefu Zhu, Zhiven Liu, Chengyuan Tang, Jian Cai, Dong Zheng, Endoplasmic reticulum stress is activated in post-ischemic kidneys to promote chronic kidney disease, EBioMedicine 37 (2018) 269–280.