Research Square

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Expression of Serum GRP78 and CHOP in Endoplasmic Reticulum Stress Pathways of Chinese Type 2 Diabetic Kidney Disease Patients

Ning Ma
Lianyungang No 1 People's Hospital
Ning Xu
Lianyungang No 1 People's Hospital
Dong Yin
Lianyungang No 1 People's Hospital
Ping Zheng
Lianyungang No 1 People's Hospital
Weiwei Liu
Lianyungang No 1 People's Hospital
Guofeng Wang
Lianyungang No 1 People's Hospital
Yuan Hui
Lianyungang No 1 People's Hospital
Guanjun Han
Lianyungang No 1 People's Hospital
Chuanhui Yang
Lianyungang No 1 People's Hospital
Xingbo Cheng (20174132022@stu.suda.edu.cn)

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Abstract

Background: The kidney has a rich endoplasmic reticulum system. A close relationship exists between endoplasmic reticulum stress (ERS) and diabetic kidney disease (DKD). The current study aimed to investigate serum glucose-regulated protein 78 (GRP78) as well as CCAAT/enhancer binding protein homologous protein (CHOP) concentrations in type 2 diabetes mellitus (T2DM) Chinese patients, especially those with microalbuminuria.

Methods: We evaluated the relationships between serum GRP78 or CHOP levels and DKD. We recruited 67 patients with T2DM and 63 control subjects. We determined serum GRP78 and CHOP concentrations by ELISA, collected anthropometric data, and measured biochemical parameters in a clinical laboratory.

Results: Compared with control groups, Chinese T2DM patients showed decreased serum levels of GRP78 [0.21 (0.16–0.24) vs. 0.16 (0.16–0.19) ng/mL, $p < 0.01$] and CHOP [3.8 (3.0–5.5) vs. 5.5 (3.7–7.9) ng/mL, $p < 0.01$]. Reduction in GRP78 and CHOP serum levels was more pronounced in patients with more severe categories of microalbuminuria. Amounts of serum GRP78 correlated directly with serum fasting c-peptide, cystatin-c (cys-c), creatinine (Cr), blood urea nitrogen (BUN), and uric acid, and inversely with glomerular filtration rates. Serum CHOP level was positively correlated with age, Cr, BUN, cys-c, urinary microalbumin/creatinine (UmALB/Cr), and eGFR. Serum GRP78 was predicted independently by Cr, BUN, serum uric acid, eGFR, and cys-c, while CHOP depended on age, Cr, BUN, serum uric acid, eGFR, UmALB/Cr, and cys-c. After controlling for confounding factors, GRP78 and CHOP expression was significantly associated with DKD (binary logistic regression, $p < 0.01$).

Conclusions: T2DM patients showed increased serum GRP78 and CHOP concentrations. Receiver operating characteristic (ROC) areas under the curve for predicting DKD based on GRP78 and CHOP were 0.686 [95% CI: 0.558–0.813] and 0.670[0.524–0.816], respectively.

Background

Diabetic kidney disease (DKD) represents an important health problem worldwide with millions of people affected. As a microvascular complication of diabetes mellitus (DM), it is responsible for substantial proportion of end-stage kidney disease cases. It is estimated that there are approximately 120 million people with chronic kidney disease in China [[1]]. DKD accounts for 30–47% of cases of end-stage renal disorders (ESRD) and is a major cause of death in DM patients [[2]]. However, the underlying pathophysiological mechanisms of DKD remain incompletely understood, hampering the development of new therapeutic approaches. Over the years, numerous basic and clinical studies have confirmed that advanced glycation end products (AGEs), oxidative stress, inflammation, as well as activation of protein kinases C, renin-angiotensin aldosterone system, and others have made valuable contributions to the pathogenesis and development of DKD. Among these, it is believed that increased production of reactive oxygen species (ROS) and subsequent oxidative stress contributes significantly to DKD development [[3]].

In attempt to counteract numerous environmental stressors and preserve normal cell function, kidney cells in DM patients developed delicate signaling systems, such as a homeostatic pathway for regulating membrane structure and secretory activity of endoplasmic reticulum (ER) (unfolded protein response, UPR). It was reported that activation of UPR in kidneys of DM patients contributes to ER's functional restoration and preserved cell viability [[4]]. Growing evidence now suggests that endoplasmic reticulum stress (ERS) plays critical roles in the pathophysiological mechanisms of DKD. Studies have shown that changes in ER regulation of protein folding pathways cause ROS imbalance and increase their production, indirectly interfering with ER and redox balance [[5]].

ER's main function in normal conditions is related to folding, modification and degradation of secretory binding proteins of the plasma membrane [[6, 7]]. We know that disturbed homeostasis in DM due to various factors, such as ROS, high serum glucose, free fatty acids, etc. lead to ERS reflected in ER accumulation of unfolded proteins [[8]]. Earlier studies showed that ERS plays a key role in diabetes [[9–13]]. By identifying disease-causing mutations in epithelial-restricted genes, recent studies have indicated the importance of severe or prolonged ERS for multiple organs' degenerative diseases and fibrosis, including the pro-fibrosis role of UPR signaling in different cell types [[14]]. Many stressors encountered upon kidney injury may trigger ERS. In the kidney, despite ERS involvement in both acute and chronic histological damage, it may have nephroprotective effects and promote cellular adaptation [[15]]. Yet, pathological ERS activation may lead to inflammatory response, cell apoptosis, and alteration in protective processes, such as autophagy and mammalian target of rapamycin complex (mTORC) activation [[4]]. However, the way ERS participates in the generation and development of DKD is still not fully understood.

Glucose-regulated protein 78 (GRP78) belongs to the family of heat-shock proteins (HSP70 family). It is also known as the immunoglobulin heavy chain binding protein (BiP). It is an ER lumen protein whose expression is induced during ERS and plays a novel protective role in preventing ERS-induced cell death [[16]]. As an ER chaperone, GRP78 modulates the UPR signaling network. In conditions of ERS, it dissociates from protein kinase R-like ER kinase (PERK) and binds to unfolded or misfolded proteins [[17]].

CCAAT/enhancer binding protein (C/EBP) homologous protein (CHOP) is another essential player in ERS-induced apoptotic cell death [[18]]. CHOP contains a C-terminal alkaline zinc finger (bZIP) domain and an N-terminal transcriptional activation threshold. The expression of CHOP can significantly affect cell survival. It is also known as growth arrest and DNA damage inducible gene 153 (GADD153) [[19]]. Each of the three ERS pathways can induce C/EBP source protein CHOP – is a translocation factor unique to ERS [[19]]. CHOP mainly exists in the cytoplasm, and its expression level is very low. When ERS is induced, the expression of CHOP increases substantially and is activated and translocated into the nucleus [[20]]. Overexpressed CHOP promotes cell cycle stagnation or apoptosis [[21]], but CHOP can also protect cells from apoptosis [[22]].

Acute kidney injury leads to perturbations of kidney cell pathways, which causes ER accumulation of unfolded/misfolded proteins and subsequent UPR or ERS. The cell fate in ERS depends on the balance between the UPR adaptive and apoptotic pathway [[23]]. The involvement of serum GRP78 and/or CHOP in the development of DKD through ERS pathway has not yet been elucidated. Although some observations have been made in animal studies, few have yet explored ERS in humans with DKD. Therefore, here we investigated the relationships of serum concentrations of GRP78 and CHOP in T2DM patients from China, particularly in patients with different severity categories of microalbuminuria, to test the hypothesis that ERS potently affects the pathophysiological mechanisms of DKD.

Methods

Subjects

We enrolled 67 T2DM patients hospitalized at Lianyungang No. 1 People's Hospital and 63 healthy patients from a medical examination center that were included as controls. T2DM was diagnosed as per American Diabetes Association diagnostic criteria from 2014 [[24]]: fasting glucose of 7.0 mmol/L or higher, glycosylated hemoglobin of 6.5% or higher, or oral glucose tolerance test (OGTT) showing plasma glucose of 11.1 mmol/L or higher at 2 hours after glucose load. Based on cystatin-c levels, two groups were defined, including Group A (Cys-c $\leq$ 1.03) and Group B (Cys-c > 1.03). Standard OGTT was also conducted in control group to confirm normal glucose tolerance. Histories of disease, smoking, and alcohol consumption were collected via a detailed questionnaire. The following were considered as exclusion: type 1 DM, secondary diabetes, pregnancy, thyroid diseases, endogenous or exogenous corticosteroid excess, acute or chronic virus hepatitis, malignant tumor, failure of major organs (such as heart, liver, kidneys), infection or inflammation. The study was performed in accordance with the Helsinki Declaration, and the Ethics Committee of our hospital approved the study. Each subject provided a written informed consent after understanding the study details. We followed the methods of Xing-bo Cheng et al 2017 [25].

Anthropometric data collection

Based on hospital case files, the data on body height, body weight, waist circumference (WC), and hip circumference (HC) were obtained. We calculated waist-to-hip ratio (WHR) by dividing WC by HC. After resting in a sitting position for 10 minutes, before blood pressure was measured using Omron electronic sphygmomanometer. The average of three measurements of blood pressure was calculated.

Biochemical measurements

Patients had fasted overnight not less than 10 hours before venous blood samples were taken. Blood samples were taken at 07:00–08:00 in the morning, and centrifuged. Blood was tested for the following fasting parameters: fasting glucose, fasting c-peptide, fasting insulin (FIns), glycosylated hemoglobin (HbA1c), serum uric acid, CA19-9, carcinoembryonic antigen (CEA), alpha-fetoprotein (AFP), neuron-specific enolase (NSE), total homocysteine (tHcy), D-dimer. Serum lipidogram included total cholesterol

(TC) and cholesterol fractions (high-density- and low-density lipoprotein cholesterol - HDL-C and LDL-C, respectively) and level of triglycerides (TG). For measuring insulin concentration we used an automated immunoassay analyzer (Beckman Coulter AU5800).

Indices of insulin secretion and insulin sensitivity/resistance

Insulin resistance status was assessed based on homeostasis model assessment of insulin resistance index (HOMA-IR) which was calculated as a product of fasting glucose (mmol/L) and fasting insulin (mIU/L), divided by 22.5 [[26]]. The following formulate was used to calculate insulin secretion index HOMA-β [[26, 27]]: HOMA-β = fasting insulin (mIU/L) × 20 / [fasting glucose (mmol/L) − 3.5]. Insulin sensitivity index - quantitative insulin check index (QUICKI) was determined as follows [[27]]: QUICKI = 1 / [$\log_{10}$ fasting glucose (mg/dL) + $\log_{10}$ fasting insulin (mIU/L)].

Different severity category of renal function

Urinary micro albumin/creatinine (UmALB/Cr), blood urea nitrogen (BUN), creatinine (Cr), and cys-c were determined using a Beckman coulter AU5800 analyzer (Beckman coulter, Inc, USA). estimated glomerular filtration rate(eGFR)was used to evaluate the status of renal function by CKD-EPI formula [[28]].

Measurements of serum GRP78 and CHOP

After centrifuging of blood samples, serum samples were preserved at − 80 °C for further analyses. Commercial ELISA kits (Cloud-Clone Corp., Wuhan, China) were used to determine serum concentrations of GRP78 and CHOP proteins, strictly complying with the instruction manual. The detection ranges of the GRP78 and CHOP assays were 0.312–20 ng/mL and 0.156–10 ng/mL, respectively, while minimum detectable doses were typically lower than 0.129 ng/mL (GRP78) and lower than 0.065 ng/mL (CHOP). The interassay and intraassay coefficients of variation were < 12% and 10% for both proteins.

Statistical analysis

Statistical analyses were conducted by means of SPSS v22.0 (SPSS Inc., Chicago, IL, USA). To test data distribution, Kolmogorov–Smirnov test was used. Mean with standard deviation (SD) was used for presenting normally distributed data, while median with interquartile range (IQR, 25th–75th) was used for non-normally distributed data (skewed distribution). Comparisons among categorical variables were conducted using Chi-square test. Differences in continuous variables between two groups were done using Kruskal–Wallis H test or one-way analysis of variance (ANOVA). For multiple comparisons among groups, Bonferroni correction was used after one way ANOVA or Kruskal–Wallis H test. To analyze correlations between GRP78, CHOP, and other variables, we used bivariate correlations. For identification of factors independently associated with GRP78 and CHOP and control for covariates, we performed multiple stepwise regression. Data not fitting to normal distribution underwent log-transformation (log-GRP78, CHOP) before correlation and regression analyses. A receiver operating characteristic (ROC) curve analysis was applied to determine the area under curve and cutoff value for potential of serum GRP78 and CHOP levels as biomarkers for DKD. A two-tailed *p* value below 0.05 was considered as significant. In

addition, the power analysis showed that the effect size for GRP78 concentrations was 0.686 [95% CI 0.558–0.813] and for CHOP concentrations was 0.670 [95% CI 0.524–0.816].

Results

Characteristics of study participants

Table 1 shows the clinical parameters of the 67 T2DM and 63 health control patients. The groups did not differ in age, sex, and BMI. Serum GRP78 and CHOP concentrations were significantly lower ($p < 0.01$) in T2DM than in control group.

Table 1
General clinical and laboratory parameters of study participants

Variable	Normal control group	T2DM group	*P* value
N	63	67	
Sex (M/F)	34/29	37/30	0.886
Age (y)$_a$	54.76 ± 18.77	59.34 ± 12.94	0.110
BMI (kg/m^2)$_b$	26.27 ± 3.85	24.54 ± 4.32	0.018
SBP (mmHg) $_a$	127.83 ± 21.294	147.45 ± 23.92	0.000
DBP (mmHg)$_a$	72.05 ± 14.463	83.97 ± 12.691	0.000
Fasting glucose (mmol/L)$_a$	5.27 ± 0.45	10.57 ± 5.30	0.000
HbA1c (%)$_a$	5.48 ± 0.50	9.09 ± 2.24	0.000
Creatinine$_b$	66.00 (53.00–73.00)	60.60 (50.20–85.60)	0.939
Blood urea nitrogen$_b$	5.00 (4.00–7.00)	6.34 (4.96–8.79)	0.002
TC (mmol/L)$_a$	4.25 ± 0.88	4.93 ± 1.68	0.004
TG (mmol/L)$_b$	1.00 (1.00–1.00)	1.63 (1.17–2.94)	0.000
LDL-C (mmol/L)$_b$	2.00(2.00–3.00)	2.87(2.07–3.15)	0.041
HDL-C (mmol/L)$_a$	1.37 ± 0.49	1.10 ± 0.34	0.000
Serum uric acid$_a$	294.11 ± 69.12	349.09 ± 138.05	0.005
CA19-9$_a$	8.86 ± 5.96	21.82 ± 13.16	0.000
AFP$_a$	1.63 ± 0.79	3.25 ± 1.73	0.000
CEA$_b$	1.00 (1.00–2.00)	2.89 (2.14–4.61)	0.000
GRP78$_b$	0.21(0.16–0.24)	0.16 (0.16–0.19)	0.000
CHOP$_a$	0.29 ± 0.02	0.27 ± 0.03	0.000

Variable	Normal control group	T2DM group	*P* value
BMI body mass index; WC waist circumference; WHR waist–hip ratio; SBP systolic blood pressure; DBP diastolic blood pressure; QUICKI: Quantitative Insulin Check Index. The enumeration data were compared with χ^2 test.			
a: Data normally distributed are shown as mean ± SD. Independent sample T test was performed.			
b: Data with skewed distributions are shown as median (IQR, 25th–75th). Mann–Whitney U test was performed.			

Serum GRP78 and CHOP concentrations

As shown in Fig. 1a, according to the cys-c, serum GRP78 level was significantly higher in DKD (p = 0.008). As shown in Fig. 1b, serum CHOP concentrations also showed significant differences (p = 0.011). The biochemical and clinical parameters and of patients with DKD are shown in Table 2.

Table 2
General clinical and laboratory parameters in patients with DKD

Variable	Group A	Group B	*p* value
N	42	25	
Sex (M/F)	23/14	19/11	0.921
Age (y)$_a$	56.93 ± 11.39	63.4 ± 14.53	0.064
BMI (kg/m2)$_a$	24.75 ± 4.96	24.20 ± 3.06	0.620
WC(cm)$_a$	93.15 ± 10.84	91.25 ± 6.81	0.477
WHR $_a$	0.95 ± 0.07	0.95 ± 0.04	0.009
SBP(mmHg) $_a$	143.83 ± 21.38	153.52 ± 27.05	0.109
DBP(mmHg) $_a$	83.71 ± 12.26	84.40 ± 13.63	0.833
Duration of DM(month) $_a$	113.63 ± 83.33	190.56 ± 115.43	0.003
Fasting glucose(mmol/l) $_a$	10.66 ± 3.89	10.43 ± 7.16	0.882
Fasting insulin(mIU/l)$_b$	8.41(5.12–13.12)	5.00(3.86–15.63)	0.422
Fasting c-peptide(pmol/l)$_b$	553.45(366.75–812.38)	766.30(547.79–1182.00)	0.086
HbA1c(%)$_a$	9.54 ± 2.17	8.33 ± 2.19	0.032
Creatinine$_b$	52.45(47.83–60.30)	140.10(75.80–164.00)	0.000
Blood urea nitrogen$_b$	5.35(4.68–6.40)	9.80(7.07–12.92)	0.000
UmALB/Cr$_b$	52.05(6.80–183.20)	665.00(175.15–834.80)	0.000
TC(mmol/l)$_a$	5.02 ± 1.84	4.79 ± 1.40	0.591
TG(mmol/l)$_b$	1.60(1.08–3.02)	1.76(1.18–2.29)	1.000
LDL-C(mmol/l)$_b$	2.86(2.27–3.14)	2.89(2.00–3.15)	0.932
HDL-C(mmol/l)$_a$	1.11 ± 0.32	1.07 ± 0.37	0.672
THcy $_a$	7.20 ± 4.65	13.27 ± 7.77	0.000
Serum uric acid $_a$	304.46 ± 81.56	420.51 ± 177.12	0.001
AFP $_a$	3.47 ± 1.63	2.90 ± 1.87	0.202

Variable	Group A	Group B	*p* value
CEA $_b$	3.46 ± 2.3	3.99 ± 1.96	0.343
CA199$_a$	21.78 ± 12.3	23.79 ± 13.7	0.553
NSE$_a$	12.66 ± 2.69	13.00 ± 5.40	0.760
D-Dimer$_b$	62.00(32.00–87.50)	175.00(93.50–292.25)	0.000
eGFR $_a$	107.76 ± 11.54	75.13 ± 14.54	0.000
HOMA-IR$_b$	3.50(2.00–6.00)	3.00(2.00–8.00)	0.487
HOMA-β$_b$	12.50(7.00–24.00)	14.00(6.25–33.50)	0.650
QUICKI$_b$	0.52(0.47–0.59)	0.56(0.44–0.64)	0.394
GRP78$_b$	0.16(0.15–0.17)	0.17(0.16–0.19)	0.011
CHOP$_a$	0.28 ± 0.02	0.25 ± 0.036	0.008

Group A (T2DM group, Cystatin-C ≤ 1.03)

Group B (T2DM group, Cystatin-C > 1.03)

BMI, body mass index; WC, waist circumference; WHR, waist-hip ratio; SBP, systolic blood pressure; DBP, diastolic blood pressure; QUICKI: Quantitative Insulin Check Index.

The enumeration data were compared with χ^2 test.

a: Data normally distributed are shown as mean ± SD. Independent sample T test was performed.

b:Data with skewed distribution are shown as median (IQR, 25th–75th). Mann-Whitney U test was performed.

Correlations and regression analysis between serum GRP78 and CHOP concentrations and clinical parameters

Serum GRP78 level was negatively correlated with eGFR and positively correlated with fasting c-peptide, Cr, BUN, cys-c, and serum uric acid. Serum CHOP level was positively correlated with age, Cr, BUN, cys-c, UmALB/Cr, and eGFR (Tables 3–6).

Table 3
Bivariate correlation between GRP78 levels and other variables.

GRP78	*r*	*P*
Fasting c-peptide(pmol/l)	0.258*	0.045
Creatinine	0.401**	0.001
Blood urea nitrogen	0.244*	0.047
Cys-c	0.426**	0.000
Serum uric acid	0.360**	0.003
eGFR	−0.319**	0.009
CHOP	−0.256*	0.037
Pearson correlation analysis was used. *P* value < 0.05 was considered significant. ** significant differences ($p < 0.01$).		

Table 4
Bivariate correlation between CHOP levels and other variables.

CHOP	*r*	*P*
Age (y)	−0.309*	0.011
Creatinine	−0.282*	0.021
Blood urea nitrogen	−0.383**	0.001
Cys-c	−0.462**	0.000
UmALB/Cr	−0.319**	0.008
eGFR	0.451**	0.000
GRP78	−0.256*	0.037
Pearson correlation analysis was used. *P* value < 0.05 was considered significant. ** significant differences ($p < 0.01$)		

Table 5
Multiple stepwise regression analysis: independent factors associated with GRP78 levels in patients with T2DM.

Independent factors	β (unstandardized coefficient)	Std. error	t	*P* value
Fasting c-peptide(pmol/l)	< 0.001	0.000	2.053	0.045
Creatinine	0.000	0.000	3.532	0.001
Blood urea nitrogen	0.002	0.001	2.024	0.047
Cys-c	0.018	0.005	3.792	0.000
Serum uric acid	< 0.001	0.000	3.063	0.003
eGFR	0.000	0.000	−2.709	0.009
CHOP	−0.243	0.114	−2.132	0.037

Table 6
Multiple stepwise regression analysis: independent factors associated with CHOP levels in patients with T2DM.

Independent factors	β (unstandardized coefficient)	Std. error	t	*P* value
Age (y)	−0.001	0.000	0.000	0.011
Creatinine	0.000	0.000	−2.372	0.021
Blood urea nitrogen	−0.003	0.001	−3.341	0.001
UmALB/Cr	<-0.001	0.000	−2.717	0.008
Cys-c	−0.020	0.005	−4.198	0.000
AFP	0.004	0.002	1.957	0.055
eGFR	0.001	0.000	4.075	0.000
GRP78	−0.268	0.126	−2.132	0.037

T**Serum GRP78 and CHOP concentrations and DKD**

As shown in Fig. 2, the area under the curve of GRP78 for DKD prediction was 0.686 [95% CI 0.558–0.813], and that of CHOP was 0.670 [95% CI 0.524–0.816].

Discussion

Recent studies suggest that development of diabetic nephropathy (DN) is partly caused by ER dysfunction [[29]]. High glucose may induce ERS in podocytes. ERS upregulates GRP78 expression, activates the CHOP pathway and caspase-12 pathway, and causes apoptosis of mouse podocytes, which

may be related to the development process of DKD [[30]]. There is already evidence for the involvement of ERS-mediated apoptosis in development of diabetic complications in eyes and kidneys, but also in pathogenesis of non-diabetic neurodegenerative changes. For instance, a study in hippocampal neurons of diabetic mice induced by streptozocin (STZ) showed a reduced expression of GRP78 along with higher expression of the UPR-associated pro-apoptotic regulator CHOP [[31]]. Wu et al. have shown that GRP78 levels in renal tissue are higher than CHOP, JUK, and the caspase-12 pathway. The parallel relationship between expression and transcription of death signals suggests that excessive ERS promotes progressive damage of DKD by increasing apoptosis [[18]]. Expression of nuclear transcription factor rBp65, CHOP, and GRP78 were increased in DN rats with myocardial infarction compared with control rats with myocardial infarction. In addition, the degree of podocyte damage caused by high-glucose-mediated ERS was more severe, which deformed the structure and function of the glomerulus [[32]].Cao et al.. induced a DN model by unilateral nephrectomy combined with single STZ (65 mg/kg) injection intraperitoneally in rats. GRP78 was found by histochemical staining in diabetic rats compared with controls, and the expression levels of renal glomerular and tubular epithelial cells were upregulated[[33]]. Lindenmeye and colleagues confirmed that, compared with mild diabetes, mRNA expression of GRP78, oxyregulatory protein 150, and transcription molecule X-box binding protein-1(Xbp-1) of diabetic patients increased in the kidneys, indicating that ERS was stimulated in human DN [[34]]. These studies suggest that ERS is a central link in the development of a variety of systemic chronic metabolic diseases including T2DM, and it is also coupled with inflammatory responses, oxidative stress, autophagy, apoptosis, and other signaling pathways [[35]].

In this study, the classic proteins of ERS, GRP78, and CHOP, were measured and compared with cys-c, urinary microalbumin, eGFR, and other indicators for prediction of DKD. We found higher serum concentrations of GRP78 and CHOP in T2DM group than in controls ($p < 0.01$). GRP78 and CHOP concentrations were significantly increased during DKD (GRP78: $p = 0.008$; CHOP: $p = 0.011$). Urinary micro albumin creatinine ratio (UACR) or eGFR are usually chosen as a standard, but in this study cystatin-c (Cys-c) was used as a grouping indicator. Increased UACR and decreased eGFR are closely related to higher risk of adverse cardiovascular events and death. UmALB/Cr is usually used as the evaluation index of DKD, but UACR measures the influence of various factors, e.g., hypertension, heart failure, infection, hyperglycemia. Microalbuminuria as a marker of DKD progression has been challenged [[36, 37]].Early DKD is often associated with eGFR, a phenomenon known as high glomerular hyperfiltration. A cross-sectional survey showed that some diabetic patients did not have abnormal urinary albumin excretion but had decreased eGFR [[38, 39]]. Calculation of eGFR requires information on patient age and sex, as well as serum Cr level. When a patient's eGFR < 60 mL, a decrease in eGFR can be diagnosed. However, the eGFR value may fluctuate and should be reviewed when a decrease occurs to determine the DKD stage. eGFR decline is closely associated with a higher cardiovascular risk and risk of death. Recent studies from China have shown that even mild eGFR decline can increase cardiovascular risk [[40]]. Cys-c, a low molecular weight protein that can be produced by all nucleated cells in the body, was not glycosylated, and its production rate is constant and is not affected by the patient's age, gender, etc. Therefore, serum Cys-c level mainly depends on the filtration rate of the glomerulus, which, together

with urine α-microglobulin, IgG or IgM, and IV collagen, are sensitive indicators for early diagnosis of DKD [[41]]. In this study, we therefore used Cys-c as a grouping indicator.

Hitherto, the mechanisms behind lower levels in DKD have not been clarified. Notably, GRP78 and CHOP are closely related to DKD as animal studies have shown; herein, we demonstrated that GRP78 and CHOP in human serum correlated with DKD. Hence, we assumed a possible variation in levels of GRP78 between the subgroups divided by cys-c. Together with previous studies, the results reported herein suggest that GRP78 and CHOP levels may have potential to be used as biomarkers of the DKD risk.

Nevertheless, this study had a few limitations. Based on cross-sectional design of the study the potential influence of increased GRP78 and CHOP levels on the development of T2DM could not be evaluated, and further studies are warranted to further clarify this issue. The strength of our conclusions and wide extrapolation to the general population is limited by a relatively small sample size and single center study design. In addition, the study encompassed single measurements of fasting serum GRP78 and CHOP levels. That approach was based on limited funds and does not reflect any time-dependent fluctuations in GRP78 and CHOP levels, which is of particular interest after macronutrient consumption. Therefore, further studies are still necessary. In summary, GRP78 and CHOP serum levels are increased in T2DM patients from China. Furthermore, DKD patients had greater reductions in GRP78 and CHOP levels.

Conclusions

Here we showed evidence for the importance of ERS as well as associations of GRP78 and CHOP with DKD, which may lead to new therapeutic directions for renal complications of diabetes. With consideration of the roles of GRP78 and CHOP and involvement of ERS in other diabetic microvascular complications, it will be needed to further analyze the exact roles of ERS/UPR in DM-related complications, as well as evaluate interactions of ERS and biochemical parameters and their relationship with DKD. Our data highlight the possibility of using serum indicators of ERS as biomarkers of DKD. Therefore, with further study to elucidate the underlying mechanisms behind these effects, there may be a chance to improve treatment of DKD through improved regulation of ERS.

Abbreviations

ERS:endoplasmic reticulum stress;diabetic kidney disease:DKD;glucose-regulated protein 78:GRP78;CCAAT/enhancer binding protein homologous protein:CHOP;type 2 diabetes mellitus:T2DM;cystatin-c:Cys-c;creatinine:Cr;blood urea nitrogen:BUN;urinary microalbumin/creatinine:UmALB/Cr;receiver operating characteristic:ROC; diabetes mellitus:DM;cases of end-stage renal disorders:ESRD;advanced glycation end products:AGEs;endoplasmic reticulum:ER;unfolded protein response:UPR;mammalian target of rapamycin complex:mTORC;immunoglobulin heavy chain binding protein:BiP;CCAAT/enhancer binding protein:C/EBP;growth arrest and DNA damage inducible gene 153:GADD153;oral glucose tolerance test:OGTT;waist circumference:WC;hip circumference:HC;waist-to-hip ratio:WHR;fasting

insulin:Fins;glycosylated hemoglobin:HbA1c;carcinoembryonic antigen:CEA;alpha-fetoprotein:AFP;neuron-specific enolase:NSE;total homocysteine:tHcy;total cholesterol:TC;high-density lipoprotein cholesterol:HDL-C;low-density lipoprotein cholesterol:LDL-C;triglycerides:TG;homeostasis model assessment of insulin resistance index:HOMA-IR;homeostasis model assessment of insulin secretion index:HOMA-β;quantitative insulin check index:QUICKI; estimated glomerular filtration rate:eGFR;diabetic nephropathy:DN;streptozocin:STZ;X-box binding protein-1:Xbp-1;micro albumin creatinine ratio:UACR

Declarations

Ethics approval and consent to participate

All procedures performed in studies involving human participants were in accordance with the 1964 Helsinki declaration. All participants gave their written informed consent prior to their participation in our study. The study was approved by the Ethics Committee of Lianyungang No1 People's Hospital (Protocol number: 2018–0522).

Consent for publication

The consent for publication is not required since no personal or identifying information of participants are contained within the manuscript or supplementary materials.

Availability of data and material

The serum expression data of GRP78 and CHOP of Chinese Type 2 Diabetic Kidney Disease patients used to support the findings of this study are restricted by the Ethics Committee of the First People's Hospital of Lianyungang in order to protect patient privacy. Data are available from Ning Ma, lygmaning@163.com for researchers who meet the criteria for access to confidential data.

Competing interests

The authors declare that there is no conflict of interest regarding the publication of this paper.

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Authors' contributions

All the authors engaged in the study. XBC and NM designed this article. WWL,PZ,NM,GFW and YH acquired and collected the data. NX,NM,DY,GJH and CHY organized all the data. NM,PZ and WWL analyzed all the information. NM,CXB,WWL and NX drafted the manuscript. XBC, NM and NX revised the article critically. All authors read and approved the final manuscript.

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Author details

[1] Department of Endocrinology and Metabolism, First Affiliated Hospital of Soochow University, 188 Shizi Road, Suzhou, Jiangsu, 215006, China.[2] Department of Endocrinology and Metabolism, Lianyungang No1 People's Hospital, 6 Zhenghua Road, Lianyungang, Jiangsu, 222002, China

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Figures

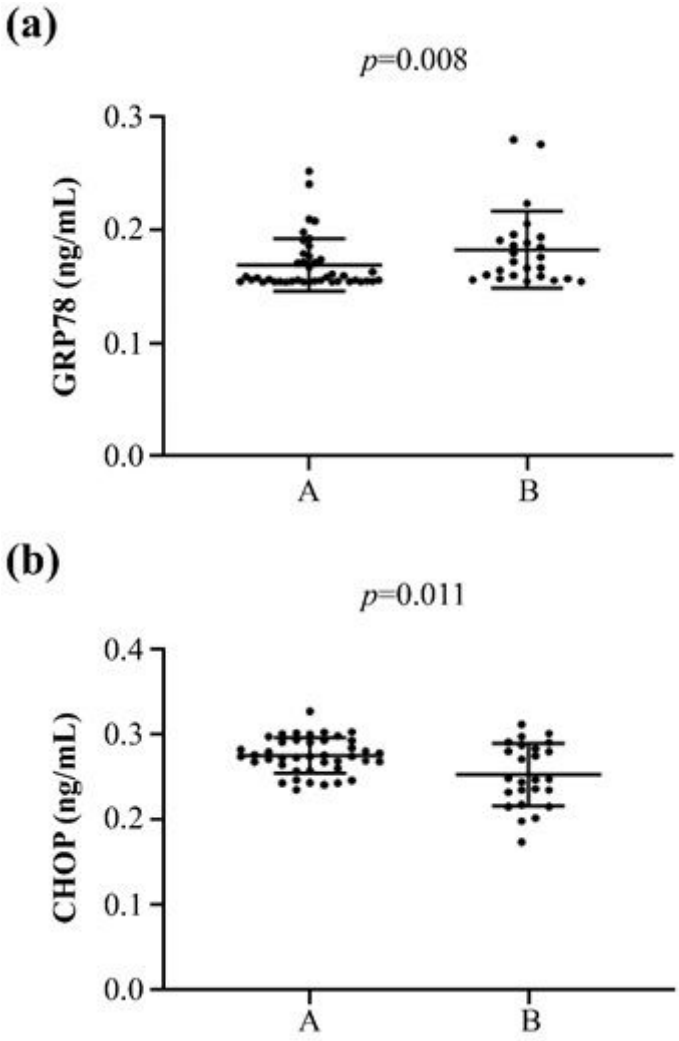

Figure 1

(a) Median (IQR) plasma GRP78 levels in Chinese type 2 diabetic patients. (b) Mean ± SD plasma CHOP levels in Chinese type 2 diabetic patients.

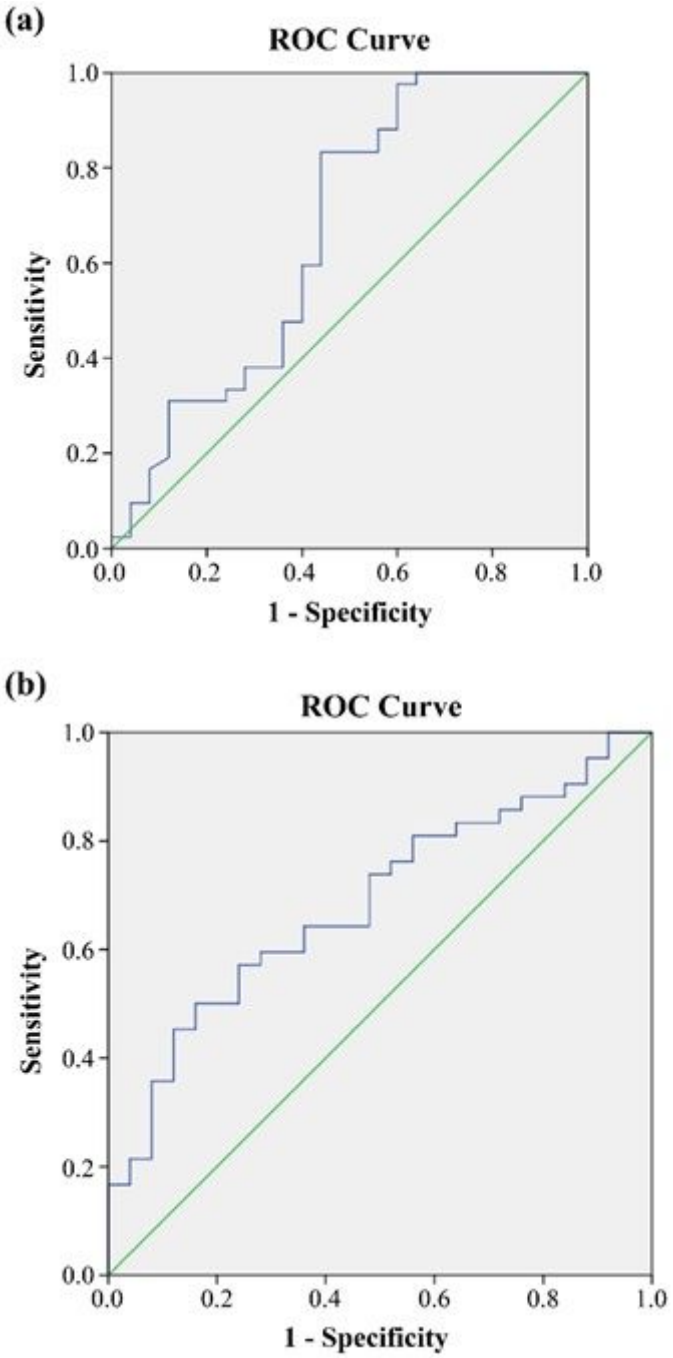

Figure 2

(a)The crude AUC of the ROC curve of plasma GRP78 levels in Chinese type 2 diabetic patients for predicting the presence of DKD. (b)The crude AUC of the ROC curve of plasma CHOP levels in Chinese type 2 diabetic patients for predicting the presence of DKD.