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Levels of circulating GRP78 and CHOP in endoplasmic reticulum stress pathways in Chinese type 2 diabetic kidney disease patients

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Abstract

The current study aimed to investigate circulating glucose-regulated protein 78 (GRP78) as well as CCAAT/enhancer-binding protein homologous protein (CHOP) concentrations in Chinese type 2 diabetes mellitus (T2DM) patients, especially those with microalbuminuria. We recruited 67 patients with T2DM and 63 control subjects. We determined circulating GRP78 and CHOP concentrations by ELISA, collected anthropometric data, and measured biochemical parameters in a clinical laboratory. Compared with control groups, patients with T2DM showed decreased circulating levels of GRP78 (0.21 [0.16–0.24] vs 0.16 [0.16–0.19] ng/mL, $P < .01$) and CHOP ([0.29 ± 0.02] vs [0.27 ± 0.03]ng/mL, $P < .01$). Reduction in circulating GRP78 and CHOP levels was more pronounced in patients with more severe categories of albuminuria. Amounts of circulating 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. Circulating CHOP level was positively correlated with age, Cr, BUN, Cys-c, and urinary microalbumin/creatinine (UmALB/Cr). Circulating GRP78 was predicted independently by Cr, BUN, serum uric acid, estimated glomerular filtration rate, and Cys-c, while CHOP depended on age, Cr, BUN, estimated glomerular filtration rate, UmALB/Cr, and Cys-c. After controlling for confounding factors, circulating GRP78 and CHOP expression were significantly associated with diabetic kidney disease (binary logistic regression, $P < .01$). Patients with T2DM showed increased circulating GRP78 and CHOP concentrations. Receiver operating characteristic areas under the curve for predicting diabetic kidney disease based on GRP78 and CHOP were 0.686 (95% CI: 0.558–0.813) and 0.670 (0.524–0.816), respectively.

Abbreviations: BUN = blood urea nitrogen, CHOP = CCAAT/enhancer binding protein homologous protein, Cr = creatinine, Cys-c = cystatin-c, DKD = diabetic kidney disease, DM = diabetes mellitus, DN = diabetic nephropathy, eGFR = estimated glomerular filtration rate, ER = endoplasmic reticulum, GRP78 = glucose-regulated protein 78, HC = hip circumference, HOMA = homeostasis model assessment, PERL = protein kinase R-like ER kinase, QUICKI = quantitative insulin check index, ROC = receiver operating characteristic, ROS = reactive oxygen species, T2DM = type 2 diabetes mellitus, UmALB/Cr = urinary micro albumin/creatinine, UPR = unfolded protein response, WHR = waist-to-hip ratio.

Keywords: CCAAT/enhancer-binding protein homology protein (CHOP), diabetic kidney disease (DKD), endoplasmic reticulum (ER) stress, glucose-regulated protein (GRP) 78, type 2 diabetes

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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 No. 1 People's Hospital (Protocol number: 2018-0522).

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

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 to protect patient privacy. Data are available from Ning Ma, lygmaning@163.com for researchers who meet the criteria for access to confidential data.

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The datasets generated during and/or analyzed during the present study are available from the corresponding author on reasonable request.

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1. Introduction

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 a 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% to 47% of cases of end-stage renal disorders and is a major cause of death in patients with DM.[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, 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]

Type 2 DM (T2DM) is characterized by renal hypoxia, oxidative and endoplasmic reticulum (ER) stress, and defective nutrient deprivation signaling.[4] In the attempt to counteract numerous environmental stressors and preserve normal cell function, kidney cells in patients with DM develop delicate signaling systems, such as a homeostatic pathway for regulating membrane structure and secretory activity of ER (unfolded protein response [UPR]). It was reported that the activation of UPR in DM kidneys contributed to ER functional restoration and preserved cell viability.[5] Growing evidence now suggests that ER stress plays a critical role 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.[6]

The main ER function in normal conditions is related to the folding, modification, and degradation of secretory binding proteins of the plasma membrane.[7,8] We know that disrupted homeostasis in DM due to various factors, such as ROS, high serum glucose, free fatty acids, etc lead to ER stress, as reflected in ER accumulation of unfolded proteins.[9] Previous studies showed that ER stress plays a key role in diabetes.[10–14] Still other studies have identified disease-causing mutations in epithelial-restricted genes, indicating the significance of severe or prolonged ER stress in degenerative diseases and fibrosis in multiple organs, including the fibrosis-promoting role of UPR signaling in different cell types.[15] Stressors encountered upon kidney injury may trigger ER stress. In the kidney, despite its involvement in both acute and chronic histological damage, ER stress may have nephroprotective effects and promote cellular adaptation.[16] Nevertheless, pathological ER stress activation may lead to inflammatory response, cell apoptosis, and alteration in protective processes, such as autophagy and mammalian target of rapamycin complex activation.[5] However, the way ER stress participates in the promotion 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 . It is an ER lumen protein whose expression is induced during ER stress and plays a novel protective role in preventing ER stress-induced cell death.[17] As an ER chaperone, GRP78 modulates the UPR signaling network. In conditions of ER stress, it dissociates from protein kinase R-like ER kinase (PERK) and binds to unfolded or misfolded proteins.[18]

CCAAT/enhancer-binding protein homologous protein (CHOP) is another essential player in ER stress-induced apoptotic cell death.[19] 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. CHOP is also known as growth arrest and DNA damage-inducible gene 153.[20] Each of the 3 ER stress pathways can induce CCAAT/enhancer-binding protein source protein CHOP – is a translocation factor unique to ER stress.[20] CHOP mainly exists in the cytoplasm, and its expression level is very low. When ER stress is induced, the expression of CHOP increases substantially and is activated and translocated into the nucleus.[21] Overexpression of CHOP promotes cell cycle stagnation or apoptosis,[22] but CHOP can also protect cells from apoptosis.[23]

The cell fate in ER stress depends on the balance between the UPR adaptive and apoptotic pathways.[24] The involvement of circulating GRP78 and/or CHOP in the development of DKD through ER stress pathway has not yet been elucidated. Although some observations have been made in animal studies, few have yet explored ER stress in humans with DKD. Therefore, here we investigated the relationships of serum concentrations of GRP78 and CHOP in patients with T2DM from China, particularly in patients with different severity categories of microalbuminuria, to test the hypothesis that ER stress potently affects the pathophysiological mechanisms of DKD.

2. Methods

2.1. Subjects

We enrolled 67 patients with T2DM, hospitalized at Lianyungang No. 1 People's Hospital and 63 healthy patients from a medical examination center that were included as controls. All patients received treatment at the Department of Endocrinology and Metabolism at our hospital from July 2019 to December 2019. T2DM was diagnosed as per American Diabetes Association diagnostic criteria from 2014[25]: fasting glucose of 7.0 mmol/L or higher, glycosylated hemoglobin of 6.5% or higher, or oral glucose tolerance test showing plasma glucose of 11.1 mmol/L or higher at 2 hours after the glucose load. According to the different clinical stages of kidney disease, DKD meets the diagnostic criteria of the 2017 Chinese Diabetes Guidelines and diagnoses are made according to the Kidney Disease Outcomes Quality Initiative formulated by the National Kidney Foundation of the United States in 2007. Based on UmALB/Cr levels, 2 groups were defined, including Group A ([UmALB/Cr] < 300 mg/g) and Group B ([UmALB/Cr] ≥ 300 mg/g). Standard oral glucose tolerance test was also conducted in the 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 viral hepatitis, malignant tumor, failure of major organs (such as heart, liver, and 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 written

informed consent after understanding the study details. We followed the methods of Xing-bo Cheng et al 2017.[26]

2.2. Anthropometric data collection

Based on hospital case files, the data on body height, body weight, waist circumference, and hip circumference were obtained. We calculated waist-to-hip ratio by dividing waist circumference by hip circumference. After resting in a sitting position for 10 minutes, before blood pressure was measured using Omron electronic sphygmomanometer. The average of 3 measurements of blood pressure was calculated.

2.3. Biochemical measurements

Patients had fasted overnight not less than 12 hours before venous blood samples were taken. Blood samples were taken at 07:00 to 08:00 in the morning, and centrifuged. Blood was tested for the following fasting parameters: fasting glucose, fasting c-peptide, fasting insulin, glycosylated hemoglobin, serum uric acid, CA19-9, carcinoembryonic antigen, alpha-fetoprotein, neuron-specific enolase, total homocysteine, and D-dimer. Serum lipidogram included total cholesterol and cholesterol fractions (high-density and low-density lipoprotein cholesterol) and level of triglycerides. For measuring insulin concentration, we used an automated immunoassay analyzer (Beckman Coulter AU5800).

2.4. Indices of insulin secretion and insulin sensitivity/resistance

Insulin resistance status was assessed based on the homeostasis model assessment of insulin resistance index, which was calculated as a product of fasting glucose (mmol/L) and fasting insulin (mIU/L) divided by 22.5.[27] The following formula was used to calculate insulin secretion index (HOMA-β)[27,28]: 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 [28]: QUICKI = 1/[$\log_{10}$ fasting glucose (mg/dL) + $\log_{10}$ fasting insulin (mIU/L)].

2.5. Different severity category of renal function

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 using the Chronic Kidney Disease Epidemiology Collaboration formula.[29]

2.6. 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 to 20 ng/mL and 0.156 to 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 intra-assay coefficients of variation were <12% and 10% for both proteins.

2.7. Statistical analysis

Statistical analyses were conducted by means of SPSS v22.0 (SPSS Inc., Chicago, IL, USA) and Graphpad Prism 8 (GraphPad Software Inc., San Diego, CA, 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 the Chi-square test. Differences in continuous variables between 2 groups were done using Kruskal–Wallis *H* test or one-way analysis of variance. For multiple comparisons among groups, Bonferroni correction was used after one-way analysis of variance 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 curve analysis was applied to determine the area under curve and cutoff value for the potential of serum GRP78 and CHOP levels as biomarkers for DKD. A two-tailed *P* value below .05 was considered 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).

3. Results

3.1. 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. Circulating GRP78 and CHOP concentrations were significantly lower ($P < .01$) in T2DM than in the control group.

3.2. Circulating GRP78 and CHOP concentrations

As shown in Figure 1A, according to the UmALB/Cr, the circulating GRP78 level was significantly higher in DKD ($P = .008$). As shown in Figure 1B, circulating CHOP concentrations also showed significant differences ($P = .011$). The biochemical and clinical parameters and of patients with DKD are shown in Table 2.

3.3. Correlations and regression analysis between circulating GRP78 and CHOP concentrations and clinical parameters

Circulating GRP78 level was negatively correlated with eGFR and positively correlated with fasting c-peptide, Cr, BUN, Cys-c, and serum uric acid. Circulating CHOP level was positively correlated with age, Cr, BUN, Cys-c, UmALB/Cr, and eGFR. Circulating GRP78 was predicted independently by Cr, BUN, serum uric acid, eGFR, and Cys-c, while CHOP depended on age, Cr, BUN, eGFR, UmALB/Cr, and Cys-c. (Tables 3–6).

3.4. Serum GRP78 and CHOP concentrations and DKD

As shown in Figure 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).

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	.886
Age (yrs)*	54.76 ± 18.77	59.34 ± 12.94	.110
BMI (kg/m^2)†	26.27 ± 3.85	24.54 ± 4.32	.018
SBP (mmHg)*	127.83 ± 21.294	147.45 ± 23.92	≤.001
DBP (mmHg)*	72.05 ± 14.463	83.97 ± 12.691	≤.001
Fasting glucose (mmol/L)*	5.27 ± 0.45	10.57 ± 5.30	≤.001
HbA1c (%)*	5.48 ± 0.50	9.09 ± 2.24	≤.001
Creatinine (umol/L)†	66.00 (53.00–73.00)	60.60 (50.20–85.60)	.939
Blood urea nitrogen (mmol/L)†	5.00 (4.00–7.00)	6.34 (4.96–8.79)	.002
TC (mmol/L)*	4.25 ± 0.88	4.93 ± 1.68	.004
TG (mmol/L)†	1.00 (1.00–1.00)	1.63 (1.17–2.94)	≤.001
LDL-C (mmol/L)†	2.00 (2.00–3.00)	2.87 (2.07–3.15)	.041
HDL-C (mmol/L)*	1.37 ± 0.49	1.10 ± 0.34	≤.001
Serum uric acid (umol/L)*	294.11 ± 69.12	349.09 ± 138.05	.005
CA19-9 (U/mL)*	8.86 ± 5.96	21.82 ± 13.16	≤.001
AFP (ng/mL)*	1.63 ± 0.79	3.25 ± 1.73	≤.001
CEA (ng/mL)†	1.00 (1.00–2.00)	2.89 (2.14–4.61)	≤.001
GRP78†	0.21 (0.16–0.24)	0.16 (0.16–0.19)	≤.001
CHOP*	0.29 ± 0.02	0.27 ± 0.03	≤.001

The enumeration data were compared with χ^2 test.

BMI = body mass index, CHOP = CCAAT/enhancer-binding protein homologous protein, DBP = diastolic blood pressure, DKD = diabetic kidney disease, GRP78 = glucose-regulated protein 78, SBP = systolic blood pressure, T2DM = type 2 diabetes mellitus, WC = waist circumference, WHR = waist–hip ratio.

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

† Data with skewed distributions are shown as median (IQR, 25th–75th). Mann–Whitney *U* test was performed.

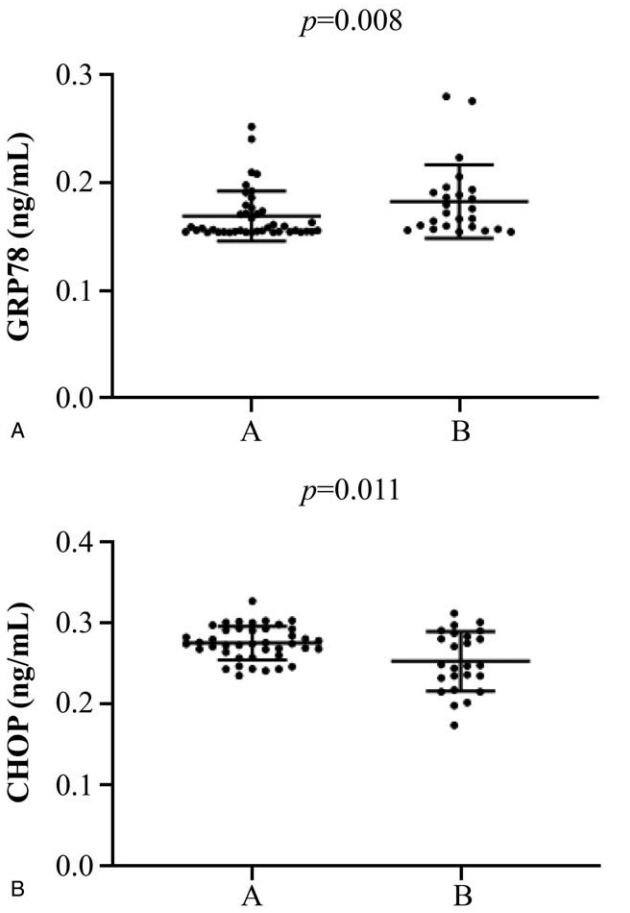

Figure 1. (A) Median (IQR) plasma GRP78 levels in Chinese type 2 diabetic patients and (B) mean ± SD plasma CHOP levels in Chinese type 2 diabetic patients. A Group A (UmALB/Cr < 300 mg/g). B Group B (UmALB/Cr ≥ 300 mg/g). CHOP = CCAAT/enhancer-binding protein homologous protein, GRP78 = glucose-regulated protein 78.

4. Discussion

ER stress is a central link in the development of a variety of systemic chronic metabolic diseases, including T2DM. It is also coupled with inflammatory response, oxidative stress, autophagy, apoptosis, and other signaling pathways.[30] In this study, we found higher serum concentrations of GRP78 and CHOP in the T2DM group than in the control subjects ($P < .05$). ER stress is evoked in various kidney diseases, including diabetic nephropathy (DN), renal fibrosis, inflammation or osmolar contrast-induced renal injury, ischemia-reperfusion, genetic mutations of renal proteins, and proteinuria and cyclosporine A treatment. The ER stress response provides protection against some kidney diseases, although the PERK–ATF4–CHOP pathway is pro-apoptotic in some kidney diseases.[31] ER stress upregulates GRP78 expression, activates the CHOP and caspase-12 pathways, and causes apoptosis of mouse podocytes, which may be related to the development of DKD.[32]

In this study, the classic proteins of ER stress, GRP78 and CHOP, were measured and compared with cys-c, urinary microalbumin, eGFR, and other indicators for the prediction of DKD. We found GRP78 and CHOP concentrations were significantly increased during DKD ($P = .008$ and.011, respectively). There is already evidence for the involvement of ER stress-mediated apoptosis in the development of diabetic complications in kidneys. For instance, a study on hippocampal neurons of diabetic mice induced by streptozocin showed a reduced expression of GRP78 along with a higher expression of the UPR-associated, pro-apoptotic regulator CHOP.[33] Wu et al have shown that GRP78 levels in renal tissue are higher than CHOP, JUK (c-JUN NH2-terminal kinase), and the caspase-12 pathway. The parallel relationship between the expression and transcription of cell death signals suggests that excessive ER stress promotes progressive damage of DKD by increasing apoptosis.[19] The expression of nuclear transcription factors 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 ER stress was more severe, which deformed the structure and function of the glomerulus.[34] Other studies suggest that the development of diabetic DN is partly caused by ER dysfunction.[35] Cao et al induced a DN model by unilateral nephrectomy combined with a one-time, intraperitoneal streptozocin (65 mg/kg) injection in rats. Furthermore, a study demonstrated the presence of GRP78 by histochemical staining in diabetic rats and found the expression levels of renal glomerular and tubular epithelial cells were upregulated.[36] Lindenmeyer et al confirmed that, compared with mild diabetes, mRNA expression of GRP78, oxyregulatory protein 150, and transcription molecule X-box binding protein-1 increased in the kidneys of diabetic patients, indicating that ER stress response was activated in human DN.[37] These studies suggest that ER

Table 2

General clinical and laboratory parameters of patients with DKD.

Variable	Group A	Group B	*P* value
N	42	25	
Sex (M/F)	23/14	19/11	.921
Age (yrs)*	56.93 ± 11.39	63.4 ± 14.53	.064
BMI (kg/m^2)*	24.75 ± 4.96	24.20 ± 3.06	.620
WC (cm)*	93.15 ± 10.84	91.25 ± 6.81	.477
WHR*	0.95 ± 0.07	0.95 ± 0.04	.009
SBP (mmHg)*	143.83 ± 21.38	153.52 ± 27.05	.109
DBP (mmHg)*	83.71 ± 12.26	84.40 ± 13.63	.833
Duration of DM (month)*	113.63 ± 83.33	190.56 ± 115.43	.003
Fasting glucose (mmol/l)*	10.66 ± 3.89	10.43 ± 7.16	.882
Fasting insulin (mIU/l)†	8.41 (5.12–13.12)	5.00 (3.86–15.63)	.422
Fasting c-peptide (pmol/l)†	553.45 (366.75–812.38)	766.30 (547.79–1182.00)	.086
HbA1c (%)*	9.54 ± 2.17	8.33 ± 2.19	.032
Creatinine (umol/L)†	52.45 (47.83–60.30)	140.10 (75.80–164.00)	≤.001
Blood urea nitrogen (mmol/L)†	5.35 (4.68–6.40)	9.80 (7.07–12.92)	≤.001
TC (mmol/l)*	5.02 ± 1.84	4.79 ± 1.40	.591
TG (mmol/l)†	1.60 (1.08–3.02)	1.76 (1.18–2.29)	1.000
LDL-C (mmol/l)†	2.86 (2.27–3.14)	2.89 (2.00–3.15)	.932
HDL-C (mmol/l)*	1.11 ± 0.32	1.07 ± 0.37	.672
THcy (umol/L)*	7.20 ± 4.65	13.27 ± 7.77	≤.001
Serum uric acid (umol/L)*	304.46 ± 81.56	420.51 ± 177.12	.001
AFP (ng/mL)*	3.47 ± 1.63	2.90 ± 1.87	.202
CEA (ng/mL)†	3.46 ± 2.3	3.99 ± 1.96	.343
CA199 (U/mL)*	21.78 ± 12.3	23.79 ± 13.7	.553
NSE (ng/mL)*	12.66 ± 2.69	13.00 ± 5.40	.760
-Dimer (ng/mL)†	62.00 (32.00–87.50)	175.00 (93.50–292.25)	≤.001
eGFR*	107.76 ± 11.54	75.13 ± 14.54	≤.001
HOMA-IR†	3.50 (2.00–6.00)	3.00 (2.00–8.00)	.487
HOMA-β†	12.50 (7.00–24.00)	14.00 (6.25–33.50)	.650
QUICKI†	0.52 (0.47–0.59)	0.56 (0.44–0.64)	.394
GRP78†	0.16 (0.15–0.17)	0.17 (0.16–0.19)	.011
CHOP*	0.28 ± 0.02	0.25 ± 0.036	.008

Group A (T2DM group, UmALB/Cr < 300 mg/g).

Group B (T2DM group, UmALB/Cr ≥ 300 mg/g).

BMI = body mass index, CHOP = CCAAT/enhancer-binding protein homologous protein, DBP = diastolic blood pressure, DKD = diabetic kidney disease, DM = diabetes mellitus, eGFR = estimated glomerular filtration rate, GRP78 = glucose-regulated protein 78, HOMA-IR = homeostasis model assessment of insulin resistance index, QUICKI = Quantitative Insulin Check Index, SBP = systolic blood pressure, T2DM = type 2 diabetes mellitus, WC = waist circumference, WHR = waist–hip ratio. The enumeration data were compared with χ^2 test.

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

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

stress is a central link in the development of a variety of systemic chronic metabolic diseases including T2DM, and that it is also concurrent with inflammatory responses, oxidative stress, autophagy, apoptosis, and other signaling pathways.[30] Hyperglycemia, proteinuria, advanced glycation end products, and free fatty acids have all been reported as inducers of ER stress and UPR in diabetic kidneys.[38] CHOP has cell-specific effects on the kidney.[35] Increased ER stress, renal CHOP expression, and albuminuria were reported in aged diabetic mice, and albuminuria was attenuated in diabetic CHOP-knockout mice compared to wild-type mice.[39] In the HG model, the expression of some key ER stress-associated genes, such as CHOP, X-box binding protein-1, and cleaved caspase 3, were significantly increased.[40] However, a study on myocardial infarction and ER stress in rats found the increased expression of nuclear transcription factor rBp65, CHOP, and GRP78.[34] Chop is decreased, the same as Wu et al.[41] Kang et al found that chrysin alleviated podocyte injury by downregulating the expression of the major ER stress and PERK–eukaryotic translation initiation factor 2α (eIF2α)–ATF4–CHOP pathways.[42] Elevated expressions of RTN1a and ERS markers, such as GRP78, p-PERK, and CHOP, were observed in the nephropathic mice.[43]

There were 3 crucial findings in the current study. First, we reported for the first time about the circulating GRP78 and CHOP with clinical parameters in all participants and observed a negative correlation between GRP78 and CHOP concentrations. After controlling for confounding factors, circulating GRP78 and CHOP expression were found significantly associated with DKD (binary logistic regression, $P < .01$). Furthermore, circulating GRP78 and CHOP levels were significantly increased in patients with T2DM who have UmALB/Cr > 300 ($P = .011$ and .008, respectively, $P < .05$). Second, the bivariate correlation between circulating GRP78 and CHOP levels and other variables demonstrated that circulating GRP78 positively correlated with fasting c-peptide, Cys-c, Cr, BUN, and uric acid ($r = 0.258$, 0.401, 0.244, 0.426, and 0.360, respectively; $P = .045$, .001, .047,

Table 3

Bivariate correlation between GRP78 levels and other variables.

GRP78	*R*	*P*
Fasting c-peptide	0.258*	.045
Creatinine	0.401**	.001
Blood urea nitrogen	0.244*	.047
Cys-c	0.426**	≤.001
Serum uric acid	0.360**	.003
eGFR	−0.319**	.009
CHOP	−0.256*	.037

Pearson correlation analysis was used.

CHOP = CCAAT/enhancer-binding protein homologous protein, Cys-c = cystatin-c, eGFR = estimated glomerular filtration rate, GRP78 = glucose-regulated protein 78.

* *P* value < .05 was considered significant.

** Significant differences ($P < .01$).

Table 4

Bivariate correlation between CHOP levels and other variables.

CHOP	*R*	*P*
Age (yrs)	−0.309*	.011
Creatinine	−0.282*	.021
Blood urea nitrogen	−0.383**	.001
Cys-c	−0.462**	≤.001
UmALB/Cr	−0.319**	.008
eGFR	0.451**	≤.001
GRP78	−0.256*	.037

Pearson correlation analysis was used.

CHOP = CCAAT/enhancer-binding protein homologous protein, Cys-c = cystatin-c, eGFR = estimated glomerular filtration rate, GRP78 = glucose-regulated protein 78, UmALB/Cr = urinary micro albumin/creatinine.

* *P* value < .05 was considered significant.

** Significant differences ($P < .01$).

Table 5

Multiple stepwise regression analysis of independent factors associated with GRP78 levels in patients with T2DM.

Independent factors	β (unstandardized coefficient)	Std. error	*t*	*P* value
Fasting c-peptide	≤0.001	≤0.001	2.053	.045
Creatinine	≤0.001	≤0.001	3.532	.001
Blood urea nitrogen	0.002	0.001	2.024	.047
Cys-c	0.018	0.005	3.792	≤.001
Serum uric acid	≤0.001	≤0.001	3.063	.003
eGFR	≤0.001	≤0.001	−2.709	.009
CHOP	−0.243	0.114	−2.132	.037

CHOP = CCAAT/enhancer-binding protein homologous protein, Cys-c = cystatin-c, eGFR = estimated glomerular filtration rate, GRP78 = glucose-regulated protein 78, T2DM = type 2 diabetes mellitus.

Table 6

Multiple stepwise regression analysis of independent factors associated with CHOP levels in patients with T2DM.

Independent factors	β (unstandardized coefficient)	Std. error	*t*	*P* value
Age (yrs)	−0.001	≤0.001	≤0.001	.011
Creatinine	≤0.001	≤0.001	−2.372	.021
Blood urea nitrogen	−0.003	0.001	−3.341	.001
UmALB/Cr	<−0.001	≤0.001	−2.717	.008
Cys-c	−0.020	0.005	−4.198	≤.001
AFP	0.004	0.002	1.957	.055
eGFR	0.001	≤0.001	4.075	≤.001
GRP78	−0.268	0.126	−2.132	.037

CHOP = CCAAT/enhancer-binding protein homologous protein, Cys-c = cystatin-c, eGFR = estimated glomerular filtration rate, GRP78 = glucose-regulated protein 78, T2DM = type 2 diabetes mellitus, UmALB/Cr = urinary micro albumin/creatinine.

≤.001, and .003, respectively) and negatively correlated with eGFR and CHOP ($r = -0.319$ and -0.256, respectively; $P = .009$ and .037, respectively). Circulating CHOP levels were positively correlated with eGFR ($r = 0.451$, $P = \leq .001$) and negatively correlated with age, Cr, BUN, cys-c, and UmALB/Cr ($r = -0.309$, -0.282, -0.383, -0.462, and -0.256, respectively; $P = .011$, .021, .001, ≤.001, and .008, respectively). Finally, the receiver operating characteristic curve generated indicates that circulating GRP78 and CHOP levels could be a novel biomarker for distinguishing DKD, that of GRP78 was 0.686 (95% CI 0.558–0.813), and that of CHOP was 0.670 (95% CI 0.524–0.816).

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. Circulating GRP78 was predicted independently by Cr, BUN, serum uric acid, eGFR, and Cys-c, while CHOP depended on age, Cr, BUN, eGFR, UmALB/Cr, and Cys-c. Together with previous studies, the results reported herein suggest that GRP78 and CHOP levels may have the potential to be used as biomarkers of the DKD risk.

Nevertheless, this study has a few limitations. First, the study is based on a cross-sectional design and the cohort was relatively small, as an observational study, The observational design of our study prevents any definite conclusions on the origin of circulating GRP78 and CHOP levels and on the pathogenic relationship between their serum levels with prevalent or incident diabetes. The association of T2DM and DKD with circulating GRP78 and CHOP levels may reflect causality, reverse causality, or an indirect bystander relationship. A cause-and-effect relationship could not be confirmed between circulating GRP78 and CHOP and the physiopathologic mechanisms of DKD. The cross-sectional design of the study prevents the evaluation of the potential influence of increased GRP78 and CHOP levels on the development of T2DM. Further studies are warranted to further clarify this. Furthermore, the strength of our conclusions and extrapolation of findings to the general population is limited by relatively small sample size and a single-center study design. This limitation calls for further validation in a larger, population-based prospective cohort study. Finally, 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, both in vitro and in vivo studies are necessary to elucidate the underlying mechanisms. Because of the study design and limited funds, we were only able to determine the circulating GRP78 and CHOP concentrations in DKD. Other data for the prediction of ER stress were not available. With consideration of the role of GRP78 and CHOP, future studies should dissect the role of ER stress/UPR in microvascular dysfunction associated with diabetes. Interactions among ER stress and other biochemical mechanisms implicated in the pathogenesis of DKD also need to be explored. Our work provides a rationale for the evaluation of variables of ER stress in easily accessible biological materials (circulating) as potential biomarkers of DKD with diagnostic and prognostic value. Therefore, further studies are required.

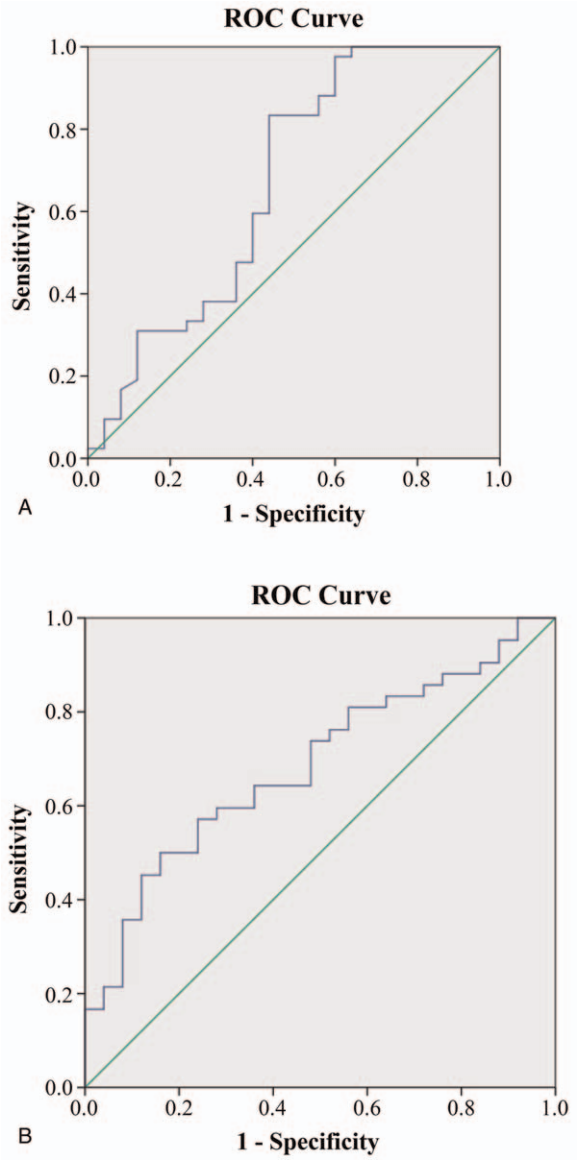

Figure 2. (A) Crude AUC of the ROC curve of plasma GRP78 levels and (B) plasma CHOP levels in Chinese type 2 diabetic patients for predicting the presence of DKD. CHOP = CCAAT/enhancer-binding protein homologous protein, DKD = diabetic kidney disease, GRP78 = glucose-regulated protein 78, ROC = receiver operating characteristic.

5. Conclusions

In this study, we demonstrated the importance of ER stress, as well as the association between 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 the involvement of ER stress in other diabetic microvascular complications, further analysis is needed to clarify the exact roles of ER stress/UPR in DM-related complications, as well as evaluate the interactions of ER stress and biochemical parameters and their relationship with DKD. Our data highlight the possibility of using serum indicators of ER stress as biomarkers of DKD. Therefore, with further studies to elucidate the underlying mechanisms behind these effects, the treatment of DKD may be improved through the improved regulation of ER stress.

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Author contributions

All the authors contributed to the study. XBC and NM wrote the final manuscript. WWL, PZ, NM, GFW, and YH 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 have read and approved the final manuscript.

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