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# Endoplasmic reticulum stress-related protein GRP78 and CHOP levels in synovial fluid correlate with disease progression of primary knee osteoarthritis: A cross-sectional study

Dan-Dan Liu [1 a], Yuan-Chuang Zhao [2 a], Hai-Hong Li [1], Lian-Jun Yin [1], Jun-Qi Chen [1 *], Gang Liu [3 *]

[1] *Southern Medical University, The Third Affiliated Hospital, Department of Rehabilitation, 510630 Guangzhou, Guangdong Province, China*
[2] *Guangzhou Special Service Recuperation Center of PLA Rocket Force, The Fourth Recuperate Area, Department of Rehabilitation, 510515 Guangzhou, Guangdong Province, China*
[3] *Southern Medical University, Nanfang Hospital, Department of Rehabilitation Medicine, 510515 Guangzhou, Guangdong Province, China*

**Abstract**
*Background:* Endoplasmic reticulum (ER) stress has been shown to play an important role in osteoarthritis (OA).
*Objective:* This study was aimed at assessing the relationship of endoplasmic reticulum (ER) stress-related glucose-regulated protein 78 (GRP78) and CCAAT/enhancer-binding protein homologous protein (CHOP) concentrations in the serum/synovial fluid (SF) with disease severity of primary knee osteoarthritis (pkOA).
*Methods:* Patients with pkOA together with healthy individuals were consecutively recruited from our hospital. The levels of GRP78 and CHOP in serum / SF were detected using enzyme-linked immunosorbent assay. The levels of IL-6 and MMP-3 were also examined. Radiographic progression of pkOA was evaluated based on Kellgren–Lawrence (K–L) grades. Receiver Operating Characteristic (ROC) curves were used to assess the diagnostic value of GRP78/CHOP levels with regard to K–L grades. The assessment of clinical severity was conducted using the visual analogue scale (VAS), Oxford knee score (OKS), and Lequesne algofunctional index (LAI).
*Results:* A total of 140 pkOA patients and 140 healthy individuals were included. Serum GRP78 and CHOP levels in pkOA patients were not significantly different from those in healthy individuals. The SF GRP78 and CHOP levels in healthy controls were not detected due to ethical reasons. Compared to those with K–L grade 2 and 3, the pkOA patients with K–L grade 4 had higher GRP78 and CHOP levels in the SF with statistical significance. In addition, the pkOA patients with K–L grade 3 exhibited drastically upregulated GRP78 and CHOP concentrations in the SF compared to those with K–L grade 2. Positive correlations of GRP78 and CHOP levels with K–L grades, IL-6, and MMP-3 levels in the SF were observed. ROC curve analysis indicated that both GRP78 and CHOP levels may act as decent indicators with regard to OA. GRP78 and CHOP concentrations in the SF were positively correlated with VAS/LAI score and negatively associated with OKS score.
*Conclusion:* The study indicated that GRP78 and CHOP levels in the SF but not the serum were positively correlated with disease severity of pkOA.

**Keywords:** CCAAT/enhancer-binding protein homologous protein; Disease severity; Glucose-regulated protein 78; Knee osteoarthritis

**Highlights:**
- Both GRP78 and CHOP levels in SF were positively correlated with K–L grade, SF IL-6, and MMP-3 levels.
- ROC curve analysis indicated that both GRP78 and CHOP levels may act as decent indicators with regard to OA.
- SF GRP78 and CHOP concentrations were positively related to VAS score and LAI score and negatively associated with OKS score.

## Introduction

As one of the most common diseases in degenerative joints, osteoarthritis (OA) mainly influences older people. OA is defined by damage to articular cartilage, accompanied by synovial inflammation, the narrowing of joint space, and the formation of osteophytes and the sclerosis of subchondral bone, resulting in constant pain, joint swelling, incremental physical disability, and finally joint arthroplasty (Mandl, 2019). So far, the diagnosis of OA has relied on clinical manifestations together with radiographic changes. However, radiographic assessment

* **Corresponding authors:** Gang Liu, Southern Medical University, Nanfang Hospital, Department of Rehabilitation Medicine, GuangZhou, GuangDong Province, China, 510630; e-mail: lgnfyy@sina.com; Jun-Qi Chen, Southern Medical University, The Third Affiliated Hospital, Department of Rehabilitation, 10515 Guangzhou, Guangdong Province, China; e-mail: meixibao@126.com
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[a] Dan-Dan Liu and Yuan-Chuang Zhao contributed equally to this work.

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might not correspond to the presence of symptoms (Oo et al., 2017). In addition, when pkOA patients develop severe symptoms, the radiographic signs may indicate a late stage of OA (Wang et al., 2018). Current available conservative treatments for OA are mainly for pain alleviation and may not be beneficial for the reversal of joint deterioration (Rychel, 2010).

As an important organelle, endoplasmic reticulum (ER) participates in protein synthesis, modification, and secretion (Schwarz and Blower, 2016). Physiological or pathological processes that contribute to the misfolded/unfolded protein accumulation in the ER are recognized as ER stress. Under the persistent ER stress, cells would be programmed and change from pro-survival to pro-apoptosis state (Almanza et al., 2019).

Recently, ER stress has been found to exert a great impact on OA progress. ER stress increases with age and disrupts the homeostasis of cartilage in various ways (Rasheed and Haqqi, 2012). It participates in cell apoptosis and cartilage degeneration (Feng et al., 2019). The number of dead chondrocytes, the expressions of MMP1 and MMP13, and inflammatory cytokines were reported to be increased in ER stress-induced OA (Hamamura et al., 2009). Moreover, Yang et al. (2005) have identified that ER stress could lead to chondrocyte apoptosis and reduce the mRNA expression of extracellular matrix proteins such as aggrecan and type II collagen in articular cartilage.

Glucose-regulated protein 78 (GRP78) and CCAAT/enhancer-binding protein homologous protein (CHOP) are two of the main ER stress-related markers (Zheng et al., 2014). As the immunoglobulin heavy chain binding protein (Elfiky et al., 2020), GRP78 is one of heat-shock proteins (HSP70 family) (Ibrahim et al., 2019). ER stress-induced GRP78 expression facilitates cell death (Elfiky et al., 2020). CHOP also plays an essential role in ER stress-induced cell apoptosis (Li et al., 2014), consisting of a C-terminal alkaline zinc finger domain as well as an N-terminal transcriptional activation threshold. In addition, CHOP expression has a potential influence on cell survival. ER stress-induced expression of CHOP substantially increases, followed by the activation and translocation into the nucleus (Nishitoh, 2012).

It is reported that both GRP78 and CHOP were upregulated in OA cartilage in contrast to non-OA cartilage (Husa et al., 2013; Nugent et al., 2009). Cartilage degeneration in CHOP-knockout mice has also been found to be lower compared to the wild-type mice (Uehara et al., 2014). Although some studies on GRP78 and CHOP have been conducted *in vitro* and *in vivo*, the relationship of the two proteins with disease severity of primary knee osteoarthritis (pkOA) remains unavailable.

Therefore, we detected the concentrations of GRP78 and CHOP in the serum or synovial fluid (SF) in pkOA patients, and assessed their association with disease severity of pkOA to verify whether ER stress potentially affects the pathophysiological mechanisms of pkOA.

## Materials and methods

### *Study patients*

Patients with pkOA were diagnosed based on American College of Rheumatology criteria (Kolasinski et al., 2020) and consecutively recruited from the third affiliated hospital of Southern Medical University between May 2021 and May 2022.

Exclusion criteria were as follows: (1) Patients with traumatic OA; (2) Patients with rheumatoid arthritis (RA), gout, calcium pyrophosphate deposition disease (CPDD); (3) Patients with septic arthritis; (4) Patients with systemic inflammatory or autoimmune disorders, malignant disease, advanced renal disease; (5) Patients with haemophilia, congenital patellar dysplasia, and other congenital disease or knee congenital deformity; (6) Patients who were on medication for hypertension, diabetes, and lipidemia; (7) Patients with a previous history of nonsteroidal anti-inflammatory drugs (NSAID) or a pure analgesic medication within 1 month; (8) Patients participating in intense or competitive activity of more than 1 hour per/week within the last 3 months.

The healthy individuals receiving regular body check were selected as the control group, with age and sex matched with the pkOA patients. All participants are southern Chinese population. The demographic information, including gender, age, body height, body weight and BMI was recorded. The study was approved by the institutional review board of the local ethical committee of the Third Affiliated Hospital, Southern Medical University (Ethical No. 20220033). Informed consent was obtained from all participating subjects.

### *ELISA*

Samples of fasting blood were collected from participants in the morning, with the serum separated and aliquoted into Eppendorf tubes (1.5 ml). SF samples were collected from the most affected knee. The serum was obtained by centrifugation at 3,000 × g for 10 min, both serum and SF samples were stored at −80 °C until analysis. GRP78 and CHOP levels in the serum and SF were examined by the kits of enzyme linked immunosorbent assay (ELISA) (GRP78: LifeSpan BioSciences, Inc., USA; Catalog No. LS-F6280; CHOP: LifeSpan BioSciences, Inc., USA; Catalog No. LS-F11284) complying with the instructions of the manufacturer. MMP-3 (JiangLai Biotech, China; Catalog No. JL10218-48T) and IL-6 levels (R&D Systems, Minneapolis, MN, USA; Catalog No. D6050) were also examined. In addition, the coefficients of variation (CV) were as follows: (1) inter-assay: <12%, intra-assay: <10% for GRP78; (2) inter-assay: CV<8.6%, intra-assay: CV<4.2% for CHOP; (3) inter-assay: 10.4%, intra-assay: 9.2% for MMP-3; (4) inter-assay: 8.1%, intra-assay: 7.5% for IL-6. To avoid or correct the false positive or false negative data. The whole ELISA experiment procedure was conducted by an experienced laboratory technician. Each step of the test was strictly controlled. The experiments were carried out in triplicate, with the results averaged.

### *Radiographic evaluation*

Standard weightbearing anteroposterior radiography was performed for the affected knees at full extension based on the Kellgren–Lawrence (K–L) grades (Schiphof et al., 2008). The radiographic findings of pkOA were divided into 5 grades, with grade 0 indicating normal joint space, no osteophyte, and no obvious deformity; grade 1 indicating that the joint space is suspected to be narrowed, and there may be osteophytic lipping; grade 2 indicating obvious osteophytes together with possible narrowing of joint space; grade 3 indicating a moderate amount of osteophytes, clear narrowing of joint space, as well as sclerotic changes; grade 4 indicating large osteophytes, severe narrowing of joint space, bone sclerosis along with clear deformity of bone contour. Patients with pkOA were delineated by the radiographic findings with K–L grade 2–5. The control group was delineated with no radiographic OA in the knee. Assessment of radiographic changes was performed by two experienced radiologists blinded to the result of the K–L grading, with the consistency of their findings determined by the *kappa* value.

### *Assessment of clinical severity*

Knee pain was evaluated using a 100 mm visual analogue scale (VAS), Oxford Knee Score (OKS), and Lequesne Algofunctional Index (LAI). For VAS assessment, a straight line was drawn on a piece of paper, with one end representing 0 points and the other representing 10 points. The patient was asked to choose the score according to their subjective feelings: (1) 0 point: no pain; (2) <3 points: slight pain; (3) 4–6 points: obvious pain; (4) 7–10 points: very severe and unbearable pain (Heller et al., 2016). The Oxford Knee Joint Score (OKS) is one of the most common patient-reported prognostic indicators. OKS is specifically developed and validated for measuring the prognosis of knee joint replacement surgery, designed for both preoperative and postoperative use. There are 12 items in OKS, and the score of each ranges from 0 to 4, with the total score of 0 points suggesting the worst outcome and 48 points suggesting the best outcome (Booij et al., 2021). This LAI scale is a modified version of the Lequesneindex and is a commonly used scoring standard for international osteoarthritis. It was first proposed by Lequesne MG (Nadrian et al., 2012) for the evaluation of the severity of hip and knee osteoarthritis. LAI was carried out to assess clinical symptoms of OA based on 3 scales, including pain/discomfort, maximum walking distance, and the activities of daily living, with a maximum score of 24 (a higher score indicates more severe symptoms) (Nadrian et al., 2012).

### *Statistical analysis*

Statistical analysis was carried out utilizing Graphpad 8.0 prism software (San Diego, CA, US). All data were described with mean ± standard deviation (SEM) or the median and interquartiles (IQR). Shapiro–Wilk test was employed to examine whether data were normally distributed. Unpaired Student's *t* test or Mann–Whitney *U* test was used to compare the differences between two independent groups, and one-way analysis of variance (ANOVA) or Kruskal–Wallis test was applicated to compare the differences among ≥3 independent groups. Coefficients from Pearson or Spearman correlation were used to examine the association between the levels of GRP78 and CHOP in the serum/SF and other indexes. Statistical significance for differences and correlations was set at $P < 0.05$.

## Results

### *Basic characteristics of pkOA patients and healthy controls*

The basic characteristics of pkOA patients and healthy controls are compared in Table 1. In total, 140 patients with pkOA aged from 56 to 72 years (65.4 ± 6.5 years), and 140 healthy individuals aged between 57 and 73 years (65.6 ± 6.6 years) were enrolled in the study. The age, sex, and BMI between pkOA between patients and healthy controls did not reach significantly difference. The serum GRP78 concentration was 178.3 ± 22.6 pg/ml in pkOA patients and 175.7 ± 23.7 pg/ml in healthy controls, and no significant differences were found between them ($P = 0.277$); the serum CHOP concentration was 269.4 ± 15.3 pg/ml in pkOA patients and 271.2 ± 17.8 pg/ml in healthy controls, and no significant differences were found between them ($P = 0.326$) (Table 1). In addition, the levels of GRP78 and CHOP in the SF were much higher in contrast to the serum samples with statistical significance (GRP78: 225.5 ± 44.7 pg/ml vs. 178.3 ± 22.6 pg/ml, $P < 0.001$; CHOP: 298.4 ± 37.1 pg/ml vs. 269.4 ± 15.3 pg/ml, $P < 0.001$) (Table 1).

**Table 1. Basic characteristics of patients with primary knee OA and healthy controls**

| | OA patients ($n = 140$) | Healthy controls ($n = 140$) | *P* value |
|---|---|---|---|
| Age (Y) | 65.4 ± 6.5 | 65.6 ± 6.6 | 0.307 |
| Sex distribution (F/M) | 88/52 | 90/50 | |
| BMI (kg/m$^2$) | 24.6 ± 3.2 | 23.8 ± 3.7 | |
| VAS scores | 4.9 ± 1.9 | / | |
| OKS Scores | 13.8 ± 4.7 | / | |
| LAI Scores | | | |
| K–L grade (2/3/4) | 45/55/40 | | |
| Serum GRP78 levels (pg/ml) | 178.3 ± 22.6 | 175.7 ± 23.7 | 0.277 |
| SF GRP78 levels (pg/ml) | 225.5 ± 44.7 | / | / |
| Serum CHOP levels (pg/ml) | 269.4 ± 15.3 | 271.2 ± 17.8 | 0.326 |
| SF CHOP levels (pg/ml) | 298.4 ± 37.1 | / | |

### *Relationship between GRP78 / CHOP concentrations in the SF and clinical severity based on radiographic evaluation*

With the *kappa* value of 0.92, 140 patients with pkOA were divided into 3 groups according to K–L grades, including 45 (32.14%) at grade 2, 55 (39.29%) at K–L grade 3, and 40 (28.57%) at grade 4. The GRP78 level in the SF was 210.8 ± 23.8, 231.1 ± 32.2, and 247.6 ± 22.3 pg/ml in pkOA patients with grade 2–4, respectively. There were statistically differences between groups ($P < 0.001$ between group 2 and 3, $P = 0.006$ between group 3 and 4) (Fig. 1A). As shown in Fig. 1C, a positive correlation was found between the levels of GRP78 in the SF and K–L grades ($r = 0.473$, $P < 0.001$).

On the other hand, CHOP levels in the SF were 284.7 ± 19.7, 294.2 ± 21.7, and 311.2 ± 18.1 pg/ml in K–L grade 2–4. There were statistically differences between groups ($P = 0.025$ between group 2 and 3; $P < 0.001$ between 3 and 4) (Fig. 1B). As shown in Fig. 1D, a positive correlation was found between CHOP levels in the SF and K–L grades as well ($r = 0.457$, $P < 0.001$).

As shown in Fig. 2, ROC curve analysis of GRP78 levels in the SF for K–L grade 2 vs 3 (AUC = 0.688, $P < 0.001$) as well as

K–L grade 3 vs 4 (AUC = 0.652, $P < 0.001$) both showed significant AUCs (Fig. 2A, B). ROC curve analysis of CHOP levels in the SF for K–L grade 2 vs 3 (AUC = 0.631, $P < 0.025$) and K–L grade 3 vs 4 (AUC = 0.731, $P < 0.001$) also showed significant AUCs (Fig. 2C, D). These findings indicate that both SF GRP78 and CHOP might have potential as markers for radiographic progression at both early and medium-late stages.

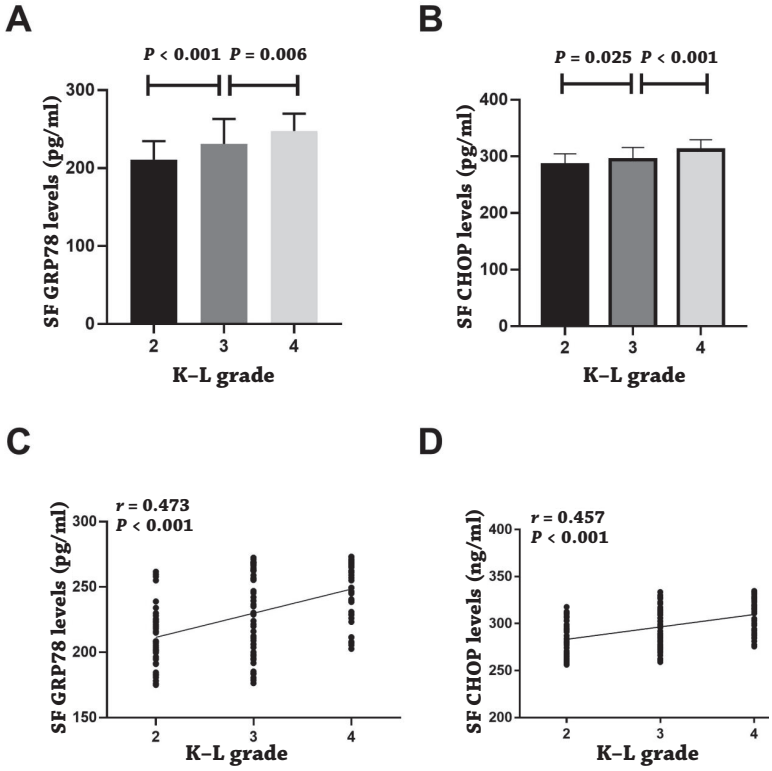


**Fig. 1.** (**A**) Comparison of GRP78 levels in the synovial fluid (SF) among K–L grade 2–4; (**B**) CHOP levels in the SF among K–L grade 2–4; (One-way ANOVA followed by Tukey's test) (**C**) Correlation between GRP78 levels in the SF and K–L grade 2–4 (Pearson); (**D**) Correlation between CHOP levels in the SF and K–L grade 2–4. (Spearman analysis)

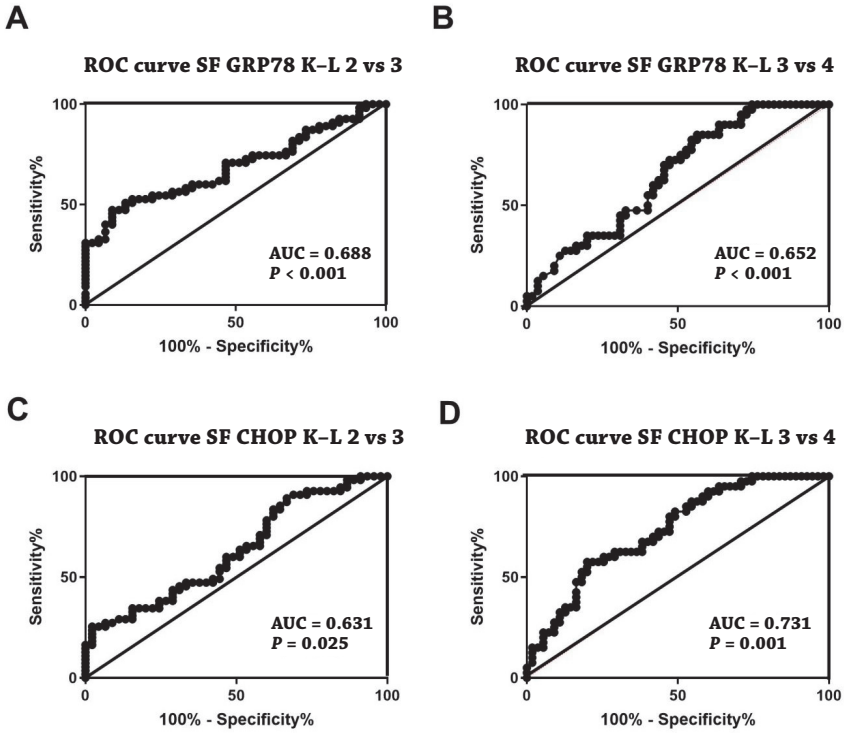


**Fig. 2.** (**A**) ROC curve of GRP78 levels in the synovial fluid (SF) for K–L grade 2 versus 3; (**B**) ROC curve of GRP78 levels in the SF for K–L grade 3 versus 4; (**C**) ROC curve of CHOP levels in the SF for K–L grade 2 versus 3; (**D**) ROC curve of CHOP levels in the SF for K–L grade 3 versus 4. AUC: Areas under curve.

### *Correlation of GRP78 and CHOP levels with clinical severity*

Next we explored the association between the levels of GRP78 and CHOP in the SF and clinical severity of pkOA. We first found a positive association between GRP78/CHOP levels in the SF and VAS scores (GRP78: $r = 0.498$, P < 0.001; CHOP: $r = 0.509$, $P < 0.001$) (Fig. 3A, B), indicating that patients with higher GRP78 and CHOP levels in the SF suffered more pain. Subsequently, we used OKS and LAI scores to define the functional ability, and found GRP78 and CHOP levels in the SF were negatively related to OKS (GRP78: $r = –0.479$, $P < 0.001$; CHOP: $r = –0.505$, $P < 0.001$) (Fig. 3C, D), but positively correlated with LAI score (GRP78: $r = 0.416$, $P < 0.001$; CHOP: $r = 0.428$, $P < 0.001$) (Fig. 3E, F). This suggests that patients with lower GRP78 and CHOP levels in the SF would have better functional ability. After adjustment for age and BMI, the above correlations remained significant (Table 2, 3).

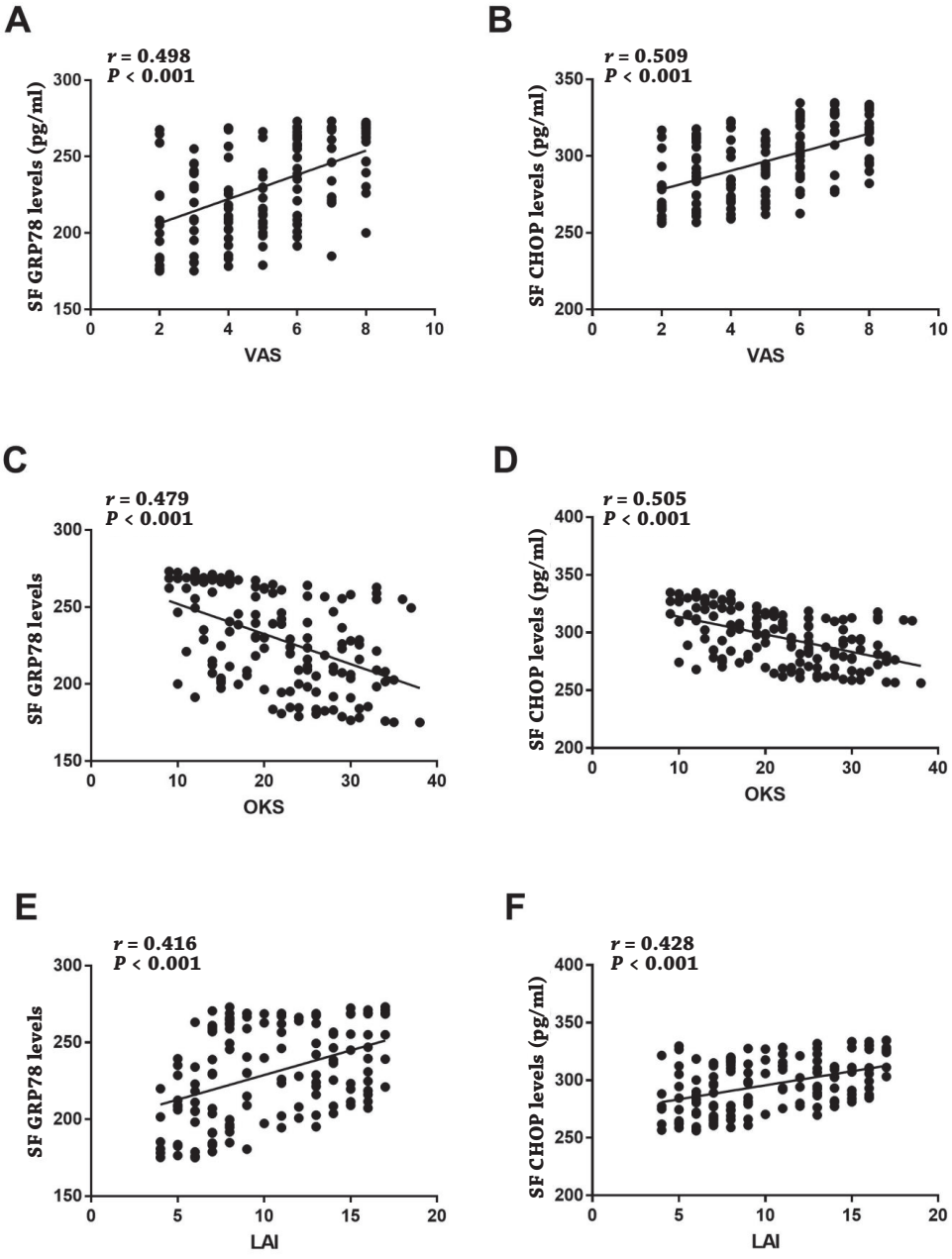


**Fig. 3.** (**A**) Correlation analysis between GRP78 levels in the synovial fluid (SF) with VAS score; (**B**) Correlation analysis between CHOP levels in the SF and VAS score; (**C**) Correlation analysis between GRP78 levels in the SF and OKS score; (**D**) Correlation analysis between CHOP levels in the SF and OKS score; (**E**) Correlation analysis between GRP78 levels in the SF and LAI score; (**F**) Correlation analysis between CHOP levels in the SF and LAI score. (All for Spearman correlation analysis)

### *Association of SF GRP78 and CHOP concentrations and biochemical indexes and ROC analysis*

We further investigated MMP-3 and IL-6 concentrations in the SF and their associations with GRP78 and CHOP. First, we found a positive correlation between the level of GRP78 in the SF and the concentration of IL-6 ($r$ = 0.381, $P$ < 0.001) and MMP-3 ($r$ = 0.481, $P$ < 0.001) (Fig. 4A, C). We also found a positive correlation between the levels of CHOP in the SF and the concentration of IL-6 ($r$ = 0.419, $P$ < 0.001) and MMP-3 ($r$ = 0.510, $P$ = 0.002) (Fig. 4B, D).

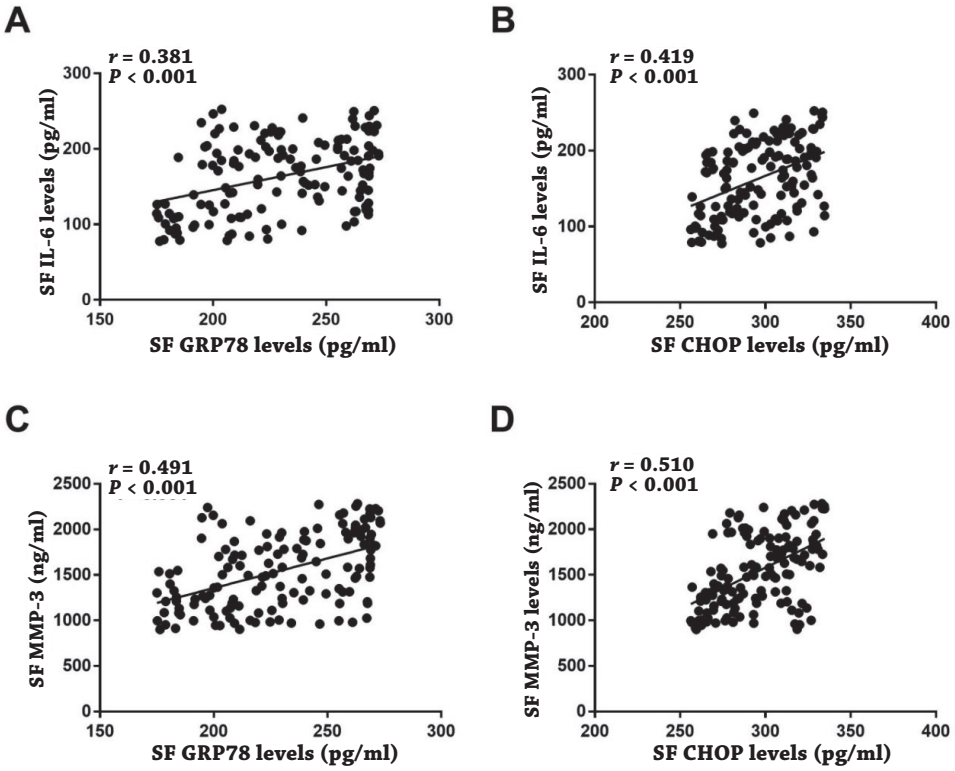


**Fig. 4.** (**A**) Correlation analysis between GRP78 levels and IL-6 levels in synovial fluid (SF) among primary knee OA (pkOA) patients; (**B**) Correlation analysis between CHOP levels and IL-6 levels in the SF among pkOA patients; (**C**) Correlation analysis between GRP78 levels and MMP-3 levels in the SF among pkOA patients; (**D**) Correlation analysis between CHOP levels and MMP-3 levels in the SF among pkOA patients. (All for Spearman correlation analysis)

**Table 2. Correlation between GRP78 levels in synovial fluid and K–L grade/VAS/OKS/LAI in elderly patients with primary knee OA**

| | Univariable | | Multivariable * | |
|---|---|---|---|---|
| Variables | *r* | *P* | *R* | *P* |
| BMI | 0.102 | >0.05 | / | / |
| Age | 0.017 | >0.05 | / | / |
| K–L grade | 0.473 | <0.001 | 0.423 | <0.001 |
| VAS | 0.498 | <0.001 | 0.444 | <0.001 |
| OKS | –0.479 | <0.001 | –0.421 | <0.001 |
| LAI | 0.416 | <0.001 | 0.361 | 0.001 |

*Note:* * Adjusted by age and BMI.

**Table 3. Correlation between CHOP levels in synovial fluid and K–L grade/VAS/OKS/LAI in elderly patients with primary knee OA**

| | Univariable | | Multivariable * | |
|---|---|---|---|---|
| Variables | *r* | *P* | *R* | *P* |
| BMI | 0.113 | >0.05 | / | / |
| Age | 0.025 | >0.05 | / | / |
| K–L grade | 0.467 | <0.001 | 0.405 | <0.001 |
| VAS | 0.509 | <0.001 | | |
| OKS | –0.505 | <0.001 | –0.454 | <0.001 |
| LAI | 0.428 | <0.001 | 0.376 | <0.001 |

*Note:* * Adjusted by age and BMI.

## Discussion

This study investigated the associations between the GRP78/CHOP level in the serum / SF and knee symptoms, radiographic changes, as well as cartilage/inflammatory biomarkers in patients with pkOA for the first time. After adjustment for age and BMI, positive associations of GRP78/CHOP levels in the SF with radiographic progression/clinical severity were found.

Due to the abnormal mechanical stress on the cartilages and the long-term inflammatory processes at a low level, OA has been a serious health issue for human beings, which can ultimately result in the degeneration of joints, or even disability (Berenbaum, 2013). As evidenced by previous studies, the apoptosis of chondrocytes is related to the degradation of cartilage matrix and has been recognized as the hallmark of OA. Therefore, the inhibition of chondrocyte apoptosis might be pivotal for OA treatment (Kim et al., 2002).

ER is significant for the survival and functions of cells (Hetz, 2012). ER stress induced by perturbations of intracellular calcium homeostasis has been reported to be linked to diabetes mellitus, ischemia reperfusion, as well as liver/kidney/neuro-degenerative diseases (Oakes and Papa, 2015). In addition, ER stress has also been demonstrated to be associated with the apoptosis of chondrocytes based on *in vivo* and *in vitro* experiments (Tan et al., 2020).

In this study, no significant differences were found between pkOA patients and healthy controls, indicating the local but not systematic occurrence of ER stress. Based on this, we noticed the positive correlation between the increased GRP78/CHOP levels and radiographic progression. The results of previous studies have revealed the enhanced apoptotic and reduced protective response in chondrocytes following ER stress in the OA-related cartilage (Takada et al., 2011). Consistent with the results of these studies, our findings demonstrated that pkOA would progress with the increase of GRP78 and CHOP levels.

The abnormal GRP78 response to ER stress has been found to induce the inflammation of the synoviocytes, resulting in the destruction of cartilages (Yang et al., 2005). The upregulation of GRP78 has also been proved to be associated with OA (Nugent et al., 2009). In another study, GRP78 expression, p65 phosphorylation of primary cultured synoviocytes, along with M1/M2 polarization of macrophages were all increased by IL-1β (Mandl, 2019). Moreover, the knockdown of GRP78 significantly reversed the expression of its downstream molecules induced by IL-1β or macrophage polarization (Lee et al., 2021). Based on the above findings, it could be inferred that GRP78 takes part in the synovial inflammation in the progression of OA. On the other hand, as a pro-apoptotic transcription factor, CHOP is a downstream molecule of the protein kinase RNA-like ER kinase (PERK) and activating transcription factor-6 (ATF6) signalling during the unfolded protein response. As reported, the level of CHOP was increased in the cartilage of OA patients (Lee et al., 2021) and osteochondritis dissecans-induced cartilage degeneration of horses (Kornicka et al., 2019). Under abnormal levels of ER stress, the unfolded protein response would trigger a pro-apoptotic signaling cascade via inducing CHOP-dependent cascades (Ma et al., 2002), thereby inducing a detrimental influence on the phenotype of chondrocytes, including downregulated cell proliferation in the growth plate of long bones and cell death. The upregulation of CHOP resulting from the significant processes of OA progression, such as the mechanical, inflammatory, or oxidative stress in the cartilage, has also been demonstrated.

There were several limitations to this study. A major limitation is the lack of examination of GRP78 and CHOP in the synovial fluid of healthy participants due to ethical issues, thus weakening the relationship between ER markers and osteoarthritis progression. Second, this was a cross-sectional study, thus the causalities between the 2 ER stress-related factors and pkOA were unverified. Longitudinal or cohort studies would be necessary for further validation. For longitudinal studies, in addition to GRP78 and CHOP more potential ER related markers PERK, IRE1α, and ATF6 should be enrolled. These markers should be examined at baseline in year 2, 3, and 5 as a follow up. The K–L grade was also used. The alternation of potential enrolled ER markers is expected to be upregulated along with the K–L grade increase. Third, the subjects were recruited from two hospitals among Chinese racials and their representativeness was limited, thus the results may not be generalizable to those with general knee OA in the communities. Investigation into other races or countries would be necessary in future. Fourth, the sample size was not big enough, and a larger sample might introduce more significant results. Fifth, we only detected GRP78 and CHOP levels in this study; other ER stress-related proteins, including PERK, inositol-requiring protein-1 (IRE1α) and ATF6, were not involved, but may also provide valuable information. PERK is a protein kinase distributed on the endoplasmic reticulum membrane. When the protein folds normally, it binds to molecular chaperones such as BIP/GRP78 to form stable complexes; when a protein is not properly folded, the improperly folded protein binds to BIP/GRP78, competitively interfering with the interaction between BIP/GRp78 and PERK (Li et al., 2014). IREI is another protein kinase distributed on the endoplasmic reticulum membrane. The activation mode of this signaling pathway

is the same as that of PERK. When improperly folded proteins accumulate in the ER, the IREI-BIP/GRP78 complex dissociates, releasing IREI oligomerization and reverse self-phosphorylation, which are then activated (Li et al., 2014). Activated IREI can transmit signals of cell survival and apoptosis. The released PERK is activated through oligomerization and reverse self-phosphorylation. ATF6 is a type II transmembrane protein on the endoplasmic reticulum membrane. The N-terminal intracellular region of ATF6 contains the DNA transcription activation domain and nuclear localization signal of b-ZIP. Under non-stress conditions, it is distributed on the endoplasmic reticulum membrane in the form of an enzyme. When under ER stress, ATF6 transfers to the Golgi apparatus in the form of vesicles (Li et al., 2014). Activated by S1P and S2P cleavage in the Golgi apparatus, it then migrates to the nucleus under the traction of nuclear localization signals, inducing transcriptional expression of endoplasmic reticulum stress genes including CHOP/GADD153 in the nucleus (Li et al., 2014). The three signaling pathways PERK, IRE1, and ATF6 can all induce the production of CHOP/GADD153. The activation of CHOP/GADD153 is a direct result of ER stress response, and CHOP/GADD153 plays an important role in growth arrest and cell death. Last, we only commendably adjusted for age and BMI. Taking other factors into consideration including diet, physical activity, genetic factors, and medication use may create more objective findings.

## Conclusion

Collectively, positive associations between the level of GRP78/CHOP in the SF and radiographic severity of pkOA was demonstrated. This not only contributed to the aggravation of knee symptoms but also the upregulation of the levels of bone/cartilage biomarkers, suggesting that ER stress may have a significant impact on patients with pkOA. In future, longitudinal studies with larger sample sizes are necessary to further validate these findings.

***Ethical aspects and conflict of interest***

The authors have no conflict of interest to declare.

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