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ORIGINAL RESEARCH

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Assessment of oxidative and endoplasmic reticulum stress markers in women with uterine myomas

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Abstract

The most frequent benign gynecological tumor in women who are fertile, uterine myoma significantly lowers quality of life. Endoplasmic reticulum stress (ERS) and oxidative stress (OS) play a role in the development of numerous disease states, including gynecological disorders. In order to determine the ERS and OS levels activated in women with uterine myoma, we sought to measure Total Oxidant Status (TOS) level, Glucose regulatory protein 78 (GRP78), C/EBP homologous protein (CHOP) expression, and protein levels. A total of 40 women with uterine myomas and 40 healthy women were included in the study. Serum levels of TOS, GRP78, CHOP, and protein levels were determined using an enzyme-linked immunosorbent assay (ELISA) kit. Reverse transcription polymerase chain reaction (RT-PCR) performed the mRNA levels of endoplasmic reticulum stress-associated molecules GRP78 and CHOP. The TOS levels, GRP78 and CHOP protein levels were significantly higher in women with myomas than in the controls, with values of $p = 0.009$, $p = 0.001$, and $p = 0.0001$, respectively. GRP78 and CHOP expression increased in women with myomas and were significantly higher than the control group used ($p < 0.05$). ROC curve analysis showed that GRP78 and CHOP are promising biomarkers for uterine myomas (area under the curve (AUC): 0.953, AUC: 0.969, $p < 0.001$, respectively). Oxidative and ERS may play a vital role in the pathophysiology of the disease due to the increased levels of oxidative and ERS markers in women with uterine myomas.

Keywords

CHOP; ER stress; GRP78; Uterine myoma

1. Introduction

Uterine leiomyomas (uterine fibroids or fibroids) are the most common benign female reproductive system tumors containing varying amounts of fibrous tissue. They occur predominantly in women of reproductive age (20–25%) and during pregnancy (2%). It is difficult to determine the incidence of fibroids, which are asymptomatic [1]. Although they are typically not fatal, they have an impact on the affected women's economy, quality of life, and morbidity [2]. They cause significant medical problems, including heavy menstrual bleeding, anemia, pelvic pain, and infertility [3]. Uterine myomas are usually single or multiple (leiomyomata), smoothly circumscribed or irregular, lobulated, millimetrically adherent or can be seen in a way that they can fill the entire abdominal cavity [4]. Uterine myomas are more common, bigger, and more numerous as women age until menopause, at which point they start to decline in postmenopausal women. A family history of fibroids constitutes another risk factor for myomas development [5]. Although racial and genetic predisposition has been suggested in its etiology, oxidative stress (OS) has been shown to play a role in the onset and progression of uterine myomas. Studies in both epidemiology and experimentation indicate that OS might be crucial in the etiology of gynecological disorders, such as uterine myomas [2]. Reactive oxygen species (ROS) are responsible for the pathogenesis of many diseases [6]. OS develops due to increased ROS production and accumulation in cells/tissues [7]. OS is thought to be involved in the pathogenesis of many disorders in the female genital system [8]. Studies on the role of OS in gynecological diseases have yet to receive sufficient attention, and knowledge of the mechanisms underlying these diseases is minimal. Research has shown that maintenance of pro-anti-oxidant balance is vital in reproductive physiology, embryopathies, and pregnancy [9]. Clinical research suggests that women with some common benign gynecological diseases may be at increased risk of developing malignant tumors.

Studies indicate that OS markers may trigger the formation of myomas tissue [10]. Many physiopathological processes between OS and ERS have been demonstrated [11]. The organelle responsible for the synthesis, folding and modification of transmembrane and secreted proteins is called the endoplasmic reticulum (ER). The ability of this organelle to fold proteins can be compromised by dysregulated gene transcrip-

tion, the expression of shortened or altered proteins encoded by mutant genes, and the presence of damaged proteins brought on by radiation, chemotherapy, and oxidative stress. It can trigger an ERS state characterized by the accumulation of misfolded or unfolded proteins [12]. The unfolded protein response (UPR) is a set of signaling pathways that the cell initiates. Its primary goal is to restore homeostasis, but if the stress is not lessened, it can also cause apoptosis. Restoring ER equilibrium and relieving stress are the goals of the UPR [13].

Glucose regulatory protein 78 (GRP78) is a calcium-dependent chaperone involved in protein folding and transport in the ER. The ability of the ER to rapidly respond to cellular stress is essential for maintaining homeostasis in the cell [14, 15]. In the early stages of ER stress, the cell primarily increases GRP78 to reduce the accumulation of misfolded proteins by the UPR and to support cell survival by restoring normal ER functions. If all this fails and ERS is prolonged, the cell-protective signaling of the UPR becomes pro-apoptotic and results in CHOP induction [13]. CHOP protein, involved in the transcriptional regulation of the apoptotic cell pathway, initiates apoptotic cell death by increasing its expression when ER protein homeostasis is disrupted. CHOP is a molecule involved in ER stress-induced apoptosis and is synthesized at low levels in the absence of ERS [16]. There is a dearth of information on the role of ROS and antioxidants in gynecological disorders, as well as a paucity of studies on the impact of OS in these conditions. According to certain research, women with benign and malignant gynecological illnesses have peripheral circulations and tissues with elevated levels of lipid peroxidation and reduced antioxidant enzyme activities [17]. When the literature was examined, no study examined the levels of GRP78 and CHOP proteins to evaluate ERS status in myomas patients.

This study aimed to evaluate endoplasmic reticulum and OS levels in patients with uterine myomas. For this purpose, circulating GRP78, CHOP expression levels, and protein levels were determined to evaluate the ERS status of patients and the healthy control group. In addition, serum Total Oxidant Status (TOS) levels were determined, and the relationship between OS and ERS parameters was evaluated.

2. Material methods

2.1 Patients and preparation of samples

In this study, 40 volunteer Patients who underwent surgery with a preliminary diagnosis of uterine leiomyoma and whose diagnosis was confirmed histopathologically after surgery (myomectomy or hysterectomy) in Sivas Cumhuriyet University Faculty of Medicine, Gynecology and Obstetrics Clinic were included. The study group (patients with concomitant malignancy and chronic inflammatory diseases were excluded from the study), and 40 healthy volunteer patients (ultrasonographic uterus and ovarian pathology was shown to be absent) were included as the control group.

The patients included in the study were aged between 30–55 years on average. During the patients' examination and control, 10 cc of blood was collected in EDTA and serum tubes for serum and plasma. After centrifuging the blood samples for ten minutes at 4000 rpm, the serum samples were collected and stored at −80 °C in microcentrifuge tubes until the pertinent parameters were examined. Samples of peripheral whole blood were used to isolate RNA.

2.2 Determination of TOS

The method developed by Erel was used to determine serum TOS values [18]. The measurements used the Rel Assay Diagnostic Assay Kit (Total Oxidant Status, Lot: ST21136O, Gaziantep, Turkey). The method is based on oxidant molecules in the samples oxidizing the iron ion chelating complex to ferric ion. H_2O_2 (Hydrogen Peroxide) was standard, and the results were calculated as μmol H_2O_2 Equiv./L. TOS = ΔAbs Sample/ΔAbs Standard.

2.3 GRP78 and CHOP protein measurements

Glucose regulatory protein 78 (Sunredbio, REF: DZE SRB-T-88720, China) and CHOP (Sunredbio, REF: DZE201125342, China) levels in serum samples were determined by Enzyme-Linked Immunosorbent Assay (ELISA) method using commercial kits. GRP78 and CHOP protein measurements were performed at 450 nm using the protocols of ELISA kits. Results were given as ng/mL.

2.4 RNA isolation and RT-PCR

RNA isolation from whole blood samples was performed using a commercial kit (High Pure RNA isolation kit Cat. No. 11828665001, Roche Applied Science, Mannheim, Germany) according to the manufacturer's instructions. The purity and amount of RNAs obtained were measured using a nanodrop BioSpec-nano Shimadzu device. Preparation of cDNA from isolated RNA samples was performed using a commercial kit (Transcriptor First Strand cDNA Synthesis Kit, Cat. No. 04896866001, Roche Applied Science, Mannheim, Germany). Reaction mixtures for cDNA were incubated under the following conditions: 29 °C for 10 minutes, 48 °C for 60 minutes and 85 °C for 5 minutes. At the end of the time, the synthesized cDNA concentrations were measured spectrophotometrically. GRP78, CHOP and ACTB gene expression levels were determined using Roche Light Cycler 480-II (Rotkreuz, Switzerland). Syber green (Wizbio; WizPure™ qPCR Master (SYBR); W1711) was used to determine gene expression levels. Expression levels of ACTB were determined as a reference gene. The primer design of the genes used is as follows. Forward and reverse 5′-CAGAGCTGGAACCTGAGGAG-3′ and 5′-CTGCAGTTGGATCAGTCTGG-3′, GRP78 F: AAACCGCTGAGGCTTATTTGG R: CTTGGCGTTGGGCATCATT, Beta actin Forward and reverse 5′-GGCACCACACCTTCTACAATG-3′, 5′-TGGATGGCTACGTACATGGCTG-3′. Reaction mixtures were incubated under the following conditions: 95 °C for 5 min, 1 cycle (pre-incubation); 95 °C for 10 s, 55 °C for 15 s, 72 °C for 20 s, 40 cycles (amplification); 72 °C for 2 min, 1 cycle (elongation). The obtained Ct (threshold cycle) values are analyzed by $2^{-\Delta\Delta Ct}$ calculation [19].

2.5 Statistical

Statistical data analysis was performed in the SPSS (Statistical Package for the Social Sciences) program (IBM SPSS 23, Chicago, IL, USA). The "Shapiro Wilk" test evaluated whether the parameters conformed to normal distribution. "Mann-Whitney U" test was used to compare the parameters that did not conform to a normal distribution with independent binary variables. The values of each group were expressed as arithmetic mean, standard deviation (SD) (X ± SD), and median (interquartile range (IQR) 25–75% (IQR)). $p < 0.05$ values were considered statistically significant. Receiver Operating Characteristics (ROC) curve analysis was performed using the MedCalc program (MedCalc 19.1 software BVBA, Ostend, Belgium). Spearman's correlation coefficient was applied to assess the relationships between serum GRP78 and serum CHOP levels in the myoma group.

3. Results

The anthropometric and biochemical parameters of the myoma patient and control groups included in our study are given in Table 1. The average age of the myoma group was 47.7 ± 8.6 years (min. 24, max. 72), and the average age of the control group was 46.1 ± 7.09 (min. 34, max. 63, $p = 0.224$). The biochemistry values of the control and the myoma groups are compared: Follicle-stimulating hormone (FSH) (8.59 (4.03–44.7) mIU/mL, $p = 0.260$), luteinizing hormone (LH) (8.90 (4.77–31.1) mIU/mL, $p = 0.160$), Estradiol (E2) (65.6 (17.7–131) pg/mL, $p = 0.764$), Prostaglandins (PG) (1.21 (0.225–2.42) ng/mL, $p = 0.801$), Prolactin (PRL) (80.6 (19.6–193) μg/L, $p = 0.958$), Hemoglobin (Hb) (12.7 (±2.32) g/dL, $p = 0.057$) concentrations were found in critically ill patients with myoma. When these parameters were compared between the control and myoma groups, no statistically significant difference was found. Median (IQR) serum TOS levels in the groups as a marker of OS are shown in Table 2. Serum TOS level ($p = 0.009$) was significantly higher in the myoma group compared to myoma and control groups (Fig. 1). Median (IQR) serum GRP78 and CHOP levels in the groups are shown in Table 2. Serum GRP78 ($p = 0.001$) and CHOP ($p = 0.0001$) protein levels were significantly higher in the myomas group compared with the control group (Fig. 2).

GRP78 and CHOP gene expression levels are shown in Table 2 (Fig. 3). GRP78 ($p = 0.014$) and CHOP ($p = 0.011$) gene expression levels were significantly higher than those of the control group. ROC curves analysis of serum GRP78 and CHOP levels are shown in Fig. 4. The ROC analysis graph of GRP78 and CHOP gene expression is shown in Fig. 5. Cut-off points, area under the curve (AUC), sensitivity and specificity values for individual parameters are shown in Figs. 4,5. In addition, there is a significant and strong correlation between GRP78 and CHOP in the myoma group (Fig. 6).

4. Discussion

The most frequent benign gynecological tumor in women who are fertile is uterine myoma. It causes various problems, including menstrual abnormalities, recurrent pregnancy losses, gynecological diseases, and pelvic pain due to invasion [20].

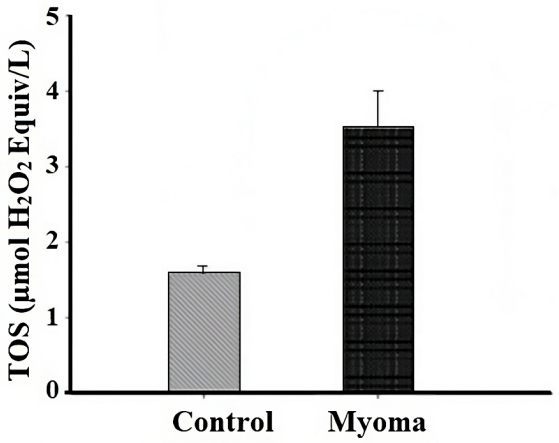


FIGURE 1. TOS levels of myoma oids group compared to the control group (results are presented as X ± SEM. $p < 0.05$). TOS: Total Oxidant Status; H_2O_2: Hydrogen Peroxide.

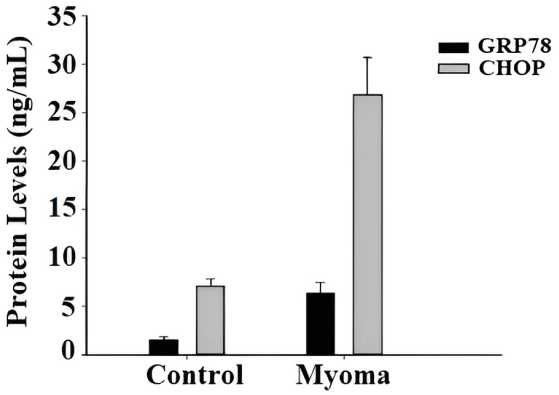


FIGURE 2. GRP78 and CHOP protein levels of the myoma group compared to the control group (results are given as X ± SEM. $p < 0.05$). GRP78: Glucose-regulated protein 78; CHOP: C/EBP homologous protein.

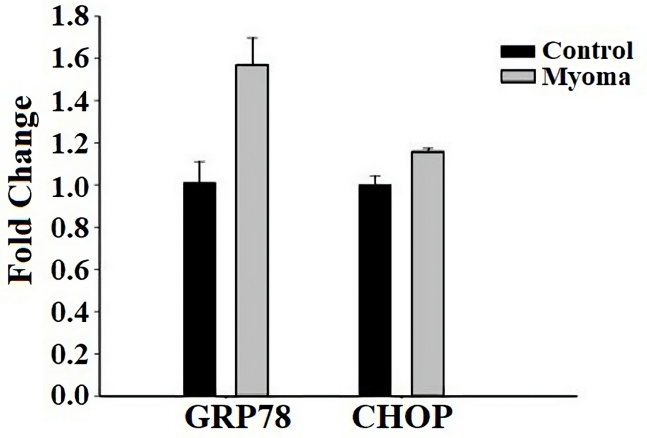


FIGURE 3. GRP78 and CHOP protein levels in the myoma group compared to the control group (results are expressed as X ± SEM. $p < 0.05$). GRP78: Glucose-regulated protein 78; CHOP: C/EBP homologous protein.

TABLE 1. Anthropometric and biochemical characteristics of the study group.

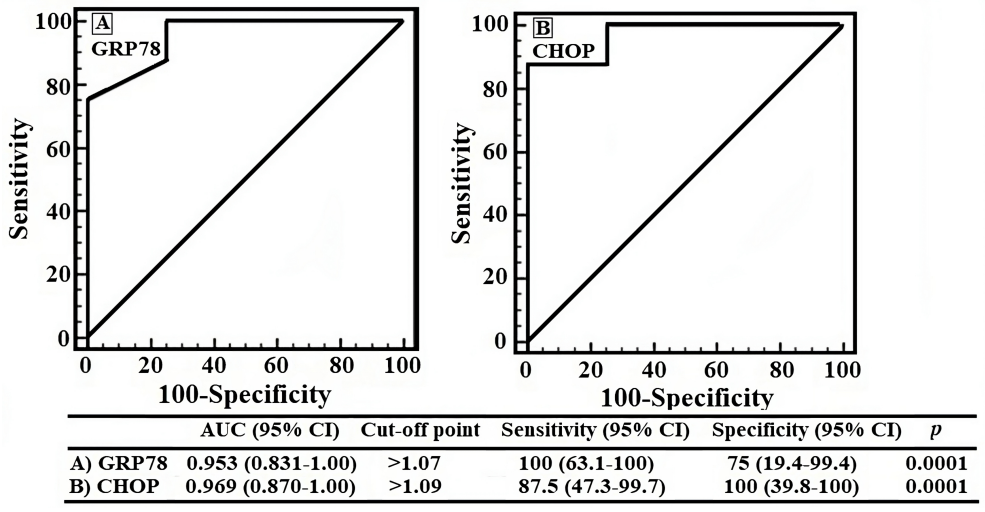
Anthropometric and biochemical parameters	Control n: 40	Myoma n: 40	*p*
Age (yr)	46.1 ± 7.1 (34–63)	47.7 ± 8.6 (24–72)	0.224*
FSH (mIU/mL)	7.8 (3.51–17.6)	8.6 (4.0–44.7)	0.260*
LH (mIU/mL)	7.5 (3.64–11.6)	8.9 (4.7–31.1)	0.160*
E2 (pg/mL)	45.0 (25.9–94.9)	65.6 (17.7–131)	0.764*
PG (ng/mL)	0.9 (0.29–2.30)	1.21 (0.225–2.42)	0.801*
PRL (µg/L)	105.0 (15.7–150)	80.6 (19.6–193)	0.958*
HB (g/dL)	12.7 ± 2.32	11.8 ± 1.89	0.057

*p shows differences between Control and Myoma according to student t-test. Data were expressed as mean ± SD. *p shows differences between Control and Myoma according to Mann Whitney U test. Data were expressed as median (interquartile range for 25–75%), p < 0.05. FSH: Follicle-stimulating hormone; LH: luteinizing hormone; E2: Estradiol; PG: Prostaglandins; PRL: Prolactin; HB: Hemoglobin.*

TABLE 2. OS and ERS biomarkers in myoma.

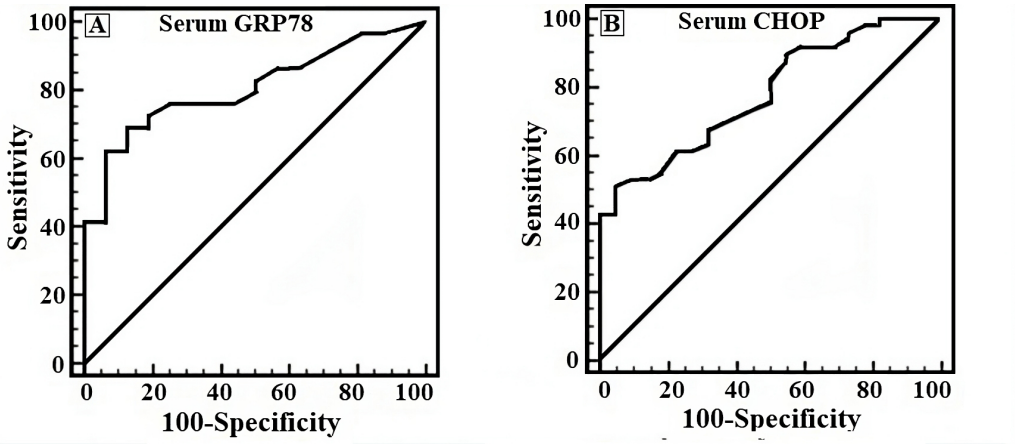
Parameters	Control (n: 40)	Myoma (n: 40)	*p*
ELISA and Colorimetric Biomarkers			
GRP78 (ng/mL)	1.56 ± 1.33 1.26 (0.79–1.73)	6.41 ± 5.54 3.96 (1.62–11.10)	0.0010
CHOP (ng/mL)	7.07 ± 3.68 7.26 (5.01–9.85)	26.92 ± 26.52 12.31 (7.35–38.90)	0.0001
TOS (µmol H_2O_2 Equiv./L)	1.59 ± 0.17 1.52 (1.44–1.77)	3.52 ± 1.09 3.39 (2.51–4.60)	0.0090
PCR Biomarkers			
GRP78 (fold change)	1.01 ± 0.20 0.97 (0.85–1.22)	1.57 ± 0.36 1.41 (1.28–1.99)	0.014
CHOP (fold change)	1.00 ± 0.09 1.01 (0.91–1.08)	1.16 ± 0.04 1.16 (1.15–1.19)	0.011

p: determined according to "Mann-Whitney U" test.
Results are presented as X ± SD and median (Q1–Q3) (IQR-25–75%) (p < 0.05). ELISA: enzyme-linked immunosorbent assay; GRP: Glucose regulatory protein; CHOP: C/EBP homologous protein; TOS: Total Oxidant Status; PCR: polymerase chain reaction.



	AUC (95% CI)	Cut-off point	Sensitivity (95% CI)	Specificity (95% CI)	*p*
A) GRP78	0.953 (0.831-1.00)	>1.07	100 (63.1-100)	75 (19.4-99.4)	0.0001
B) CHOP	0.969 (0.870-1.00)	>1.09	87.5 (47.3-99.7)	100 (39.8-100)	0.0001

FIGURE 4. ROC curve for GRP78 and CHOP expression in myoma. GRP78: Glucose-regulated protein 78; CHOP: C/EBP homologous protein; AUC: area under the curve; CI: confidence interval.



	AUC (95% CI)	Cut-off point	Sensitivity (95% CI)	Specificity (95% CI)	*p*
A)Serum GRP78	0.806 (0.680-0.932)	>2.03	69.0 (49.2-84.7)	87.5 (61.7-98.4)	0.0001
B)Serum CHOP	0.780 (0.673-0.887)	>11.6	51.0 (36.3-65.6)	95.5 (77.2-99.9)	0.0001

FIGURE 5. ROC curve for serum GRP78 and CHOP in myoma disease. GRP78: Glucose-regulated protein 78; CHOP: C/EBP homologous protein; AUC: area under the curve; CI: confidence interval.

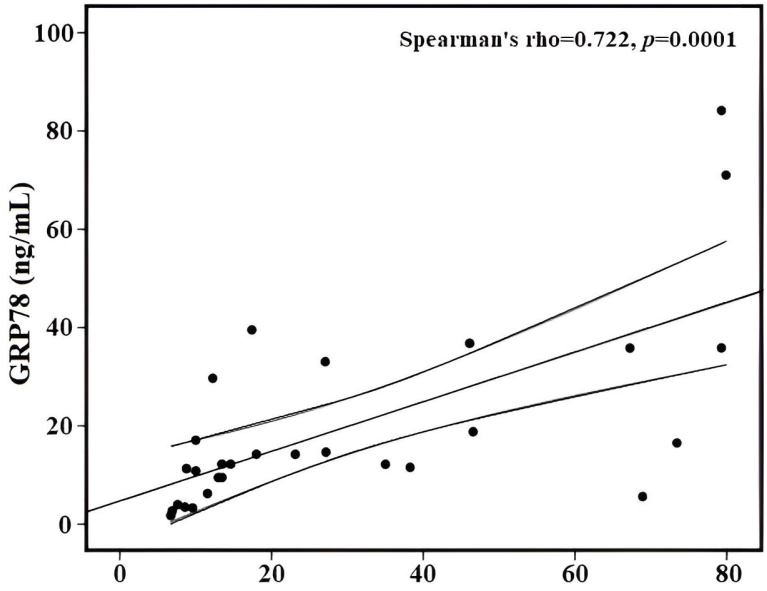


FIGURE 6. The correlation between GRP78 and CHOP in the myoma. GRP78: Glucose-regulated protein 78.

Although the occurrence and development of myomas have been investigated in depth, the etiology and pathogenesis remain unclear [21]. Despite significant advances in diagnosis and treatment, myomas are one of the leading causes of morbidity among women of reproductive age [22]. Although studies on the complex pathobiology of myomas development have increased in recent years, more studies are needed to understand these complex tumors' formation, growth and development mechanisms [14, 23]. It is known that myoma grows in a hypoxic microenvironment. Hypoxia induces the formation of ROS and OS [3]. Many studies have suggested the contribution of oxidative stress to myoma development [14, 23]. Extended and severe OS promotes the generation of reactive oxygen species (ROS), stimulates the consumption of antioxidants, and enhances the susceptibility of normal cells to free radical damage-induced malignant cell transformation. They have the power to interfere with the body's regular physiological processes and are essential to the etiology of many diseases, including cancer [24].

Oxidative stress has been associated with ERS in the pathophysiology of many diseases. According to recent research, OS can aggravate ERS by causing a reduction-oxidation (redox) imbalance, which lowers the effectiveness of protein folding processes and increases the amount of misfolded proteins produced [25]. It has been suggested that OS and ERS may play a role in the pathophysiology of a number of diseases conditions, including carcinogenesis [26]. This study aimed to evaluate TOS levels and GRP78, CHOP expression and protein levels to evaluate ERS and OS levels triggered by this stress in women diagnosed with uterine myomas. TOS, a marker of oxidative stress, consists of oxidants such as ROS, reactive nitrogen derivatives, malondialdehyde, and lipid peroxides produced in the body [27, 28]. In the literature, total antioxidant status (TAS), lipid peroxidation and antioxidant enzyme

levels were predominantly studied in studies related to OS and myoma [28–30]. In the study conducted by Hosnie *et al.* [29] malondialdehyde (MDA) and total antioxidant capacity (TAC) levels were evaluated in serum samples obtained from 50 myomas patients and 50 healthy individuals. It was reported that serum MDA levels increased significantly in myoma patients compared to healthy individuals (1.3 ± 0.65, 1.48 ± 0.5) and TAC level decreased significantly in the patient group compared to healthy individuals (466 ± 212, 321 ± 151) [29]. Another study showed that serum MDA levels increased and TAC levels decreased in patients with myomas. It was stated that high MDA levels in these patients may indicate oxidative and antioxidant imbalance [28]. In the study conducted by Nayki *et al.* [30] it was revealed that there were non-significant changes (decrease or increase) in antioxidant enzyme activities (decreased Catalase (CAT) activity and increased Superoxide dismutase (SOD) activity), TAS and TOS in the myomas group compared with the control group [30]. In our study, the TOS level was found to be 3.52 ± 1.09 in the patient group and 1.59 ± 0.174 in the control group. TOS level was statistically significant compared to the control group (p = 0.009). In the literature, the serum of women with myoma showed an increase in the level of ROS markers and a decrease in the general antioxidant level.

Severe/long-term ER stress-mediated stimulated UPR signaling pathways have been reported to play a role in many pathologies, including endometriosis, cancers, recurrent pregnancy loss and congenital anomalies, as well as being influential in the establishment of impaired ER homeostasis [31]. Glucose-regulated protein 78 and CHOP are used as markers of ERS [32]. While CHOP is a signal that controls regulated cell death (apoptosis) in response to protracted and persistent ERS, GRP78 is an ERS marker secreted to protect the cell in the early stages of ER stress [13]. In our study, when GRP78 protein and expression levels of myomas patients and the healthy control group were compared, GRP78 protein (6.41 ± 5.54) and expression (1.57 ± 0.358) levels were found to be significantly higher in the patient group compared to the control group (p = 0.001, p = 0.014) (Table 2, Figs. 2,3).

In the development of ER stress-associated apoptosis, the transcriptionally regulatory protein CHOP is either not synthesized or synthesized at a low level under physiological conditions. However, it is strongly stimulated at the transcription level in response to ERS [33]. Excessive stimulation of CHOP causes growth arrest and apoptosis. In cases where ERS persists chronically, loss of cellular function and subsequent cell death occurs [33, 34]. In our study, when CHOP protein and expression levels of myoma patients and the healthy control group were compared, CHOP protein (26.9 ± 26.5) and expression (1.16 ± 0.040) levels were found to be significantly higher in the patient group compared to the control group (p = 0.0001, p = 0.011) (Table 2, Figs. 2,3). ROC analysis was performed for serum GRP78 and CHOP to determine diagnostic prediction between the control and myomas groups. Serum GRP78 and CHOP AUC values were 0.806 and 0.780, respectively (Fig. 4). Serum GRP78 and CHOP may be potential biomarkers in the early stages of myoma. As a result of ROC analysis performed according to expression changes, GRP78 and CHOP AUC values were found to be 0.953 and 0.969, respectively (Fig. 5). These molecules may be potential biomarkers and increase their clinical use in myoma diagnosis. Moreover, the significant and influential correlation was present between GRP78 and CHOP levels of the ERS in myoma (Fig. 6). When the literature was examined, no study examined GRP78 and CHOP protein and gene expression levels in myoma patients' serum and whole blood samples to evaluate ERS status. When the studies were examined, Yan *et al.* [35] aimed to show the difference between ER stress-induced apoptosis in myoma and myometrium as a result of uterine artery occlusion (UAO) treatment and took samples from 25 patients with symptomatic myoma complaints and confirmed to have more than one intramural myoma by ultrasonography. They examined the mRNA and protein levels of ER stress-related molecules, including GRP78, CHOP, Jun N-terminal kinase (JNK), Bax, B-cell leukemia/lymphoma 2 protein (BCL-2) and Caspase4, in myomas and myometrial cells after incubation in low oxygen (1% O_2). They observed that myoma cells and tissues had higher expression levels of ER stress-related molecules (GRP78, CHOP, Caspase4 and Bax) but lower levels of BCL-2, an anti-apoptotic protein, compared to myometrial cells or tissues [35]. Another study on ERS and uterine myoma showed that selective progesterone receptor modulator asoprisnil induced ERS in uterine myoma cells. It has been shown that CHOP may play an important role in apoptotic mechanism as a result of asoprisnil-induced ERS in myoma cells by regulating upregulation of growth arrest and DNA damage inducible protein 34 (GADD34), Tribbles 3 (TRB3), Bax and Bak and downregulation of BCL-2 [36]. Lin *et al.* [37] reported that Manzamine (Manz) A, a β-carboline alkaloid, causes inhibition of cholesterol esterification in myoma cells, induces UPR sensors, protein kinase RNA (PKR)-like ER kinase (PERK), inositol-requiring enzyme-1 (IRE1) and activating transcription factor-6 (ATF6) and causes free cholesterol accumulation leading to ER stress-induced cell death. According to reports, an overabundance of free fatty acid buildup results in reactive oxygen species that interfere with mitochondrial oxidative phosphorylation and produce ERS through PERK/eukaryotic initiation factor 2 a (eIF2a)/CHOP signaling [37].

Although different pathways regarding the role of OS and ERS in uterine myomas have been investigated, most studies suggest that stress triggers ROS generation and ERS. Further investigation of the relationship between oxidative stress, ERS and myomas will improve our understanding of myomas pathobiology and provide information that may reveal various diagnostic and therapeutic implications.

5. Conclusions

Our study revealed elevated serum TOS, GRP78 and CHOP protein levels in patients with myoma; these proteins may be an important part in the early diagnosis of myoma and the identification of oxidative and ERS predisposing factors to the disease. Although the results of our preliminary study are promising, it has some limitations. There is a need to increase the sample size and to determine the disease stages more clearly (early-late). More studies on pathways are needed to determine the precise mechanisms of interactions between

ERS and OS.

AVAILABILITY OF DATA AND MATERIALS

Not applicable.

AUTHOR CONTRIBUTIONS

SM, SOY and EBY—study design, Manuscript writing. SM, EBY and AC—Analysis. SM, EBY and CH—The literature search. SM, CH and CY—Manuscript editing. All authors evaluated and approved the manuscript final version.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Before the study, approval was obtained from Sivas Cumhuriyet University Faculty of Medicine, Sivas Cumhuriyet University, Non-Interventional Clinical Research Ethics Committee, with decision numbered 2023-04/02. Consent was obtained from all patients included in this study.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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