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EVALUATION OF SERUM THIOL/DISULFIDE HOMEOSTASIS AND ISCHEMIA-MODIFIED ALBUMIN LEVELS IN LUMBAR DISC HERNIATION

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ABSTRACT

Objective: Lumbar disc herniation (LDH) is a socio-economic burden. The physiopathology of the disease is not clarified completely. This study aimed to investigate the oxidation-reduction balance in LDH via serum thiol-disulfide and ischemia-modified albumin (IMA) levels.

Materials and Methods: This prospective case-control study included 30 patients with LDH and a control group with 30 healthy volunteers. Blood samples were analyzed for total thiol (-SH+ -S-S-), native thiol (-SH), and IMA levels. Disulfide levels and native thiol/total thiol ratio were calculated. The results of the two groups were compared.

Results: Native and total thiol levels were significantly higher in the LDH group than in the control group (p=0.007 and p=0.008, respectively). IMA levels were significantly higher in the LDH group than in the control group (p=0.000). The receiver operating characteristic curve demonstrated that the IMA value of 1.41 could predict the LDH with 80% sensitivity and 80% specificity (area under curve=0.888, confidence interval: 0.802-0.974).

Conclusion: LDH influences the thiol-disulfide balance, and increased IMA levels can predict LDH.

Keywords: Lumbar disc herniation, thiol-disulfide, IMA

Introduction

Lumbar disc herniation (LDH) is a common cause of low back pain (LBP) and lumbar radiculopathy[1], resulting in a socio-economic burden. The incidence of symptomatic LDH was reported as 1-3%[2]. Despite its high incidence, the cause and physiopathology of the LDH are unclear. Two possible mechanisms underlying the LDH are increased intranuclear pressure resulting in the prolapsed intervertebral disc (IVD) and degeneration of IVD. Although there were mechanical properties of the pathology, different arguments against the mechanical theory were proposed to explain the mechanism of the pain in LDH[3-5]. The severity of the symptoms does not always correlate with the size of the defect[6]. Therefore mechanical compression can not explain the clinical symptoms adequately.

The leading cause of LDH is intervertebral disc degeneration (IDD). IDD increases with age due to the changes in collagen integrity and oxidative stress.

Previously, radiculopathy was highly correlated with biochemical mediators of inflammation, and various antioxidant molecules have been shown to increase during degenerative LDHs[7-9]. Currently, it is shown that IDD has a high association with oxidative stress and reactive oxygen species (ROS)[6,10,11].

Erel and Neselioglu[11] first described thiol-disulfide homeostasis to assess the oxidation-reduction reactions. Thiols have sulfhydryl groups that become disulfide molecules under oxidative stress conditions. The inflammation, which is correlated with IDD in LDH, is an environment characterised by oxidative stress. Ischemic events also influence the metal binding capacity of the albumin. IMA, an oxidatively modified protein, is a new marker used to detect tissue ischemia[12].

Oxidative stress is a significant factor regulating aging and degeneration; both conditions have an extensive role in the pathogenesis of radiculopathy[6,10,13]. Therefore, we aimed to evaluate the thiol-disulfide homeostasis and IMA levels in patients with LDH for the first time in the literature and compare them with healthy people.

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MATERIALS AND METHODS

This prospective case-control study was performed between November 2019- April 2020 in Ankara City Hospital, Turkey. Ethical approval was obtained from Ankara City Hospital, No. 1 Clinical Research Ethics Committee Presidency (no: E1-20-819). The study protocol was performed according to the principles of the Declaration of Helsinki, and the written informed consent containing of the details of the study was obtained from all the participants.

Among outpatient individuals, 30 patients between the ages 25-62 with LBP due to LDH resistant to conservative treatment and surgery planned, included in the study. Patients who did not benefit from medical and local analgesic treatment during six months were determined as resistant to conservative treatment. As the control group, 30 age-matched healthy people were included in the study.

Patient Selection

LDH diagnosis was made depending on the clinical symptoms and radiographic findings. A total of 30 patients between 25-62 age years old who were included operation list due to spinal magnetic resonance imaging findings (extruded or sequestrated) were included in the study (Figure 1). Among the healthy people working in the neurosurgery clinic, age and gender-matched 30 volunteer people were randomly selected for the control group. Those patients who had the systemic disease (i.e., diabetes mellitus, hypothyroidism), a detectable cause of LDH (i.e., tumor, infection), previous lumbar surgery history, severe spinal stenosis, or lumbar fracture were excluded from the study.

Blood Sampling

The antecuboidal venous blood samples were taken into the ethylenediaminetetraacetic acid including tubes, at the time of admission. Blood samples were centrifuged at 2000×g for 10 minutes. Plasma at the top of the tubes separated and kept at -80 °C until the analyzing day.

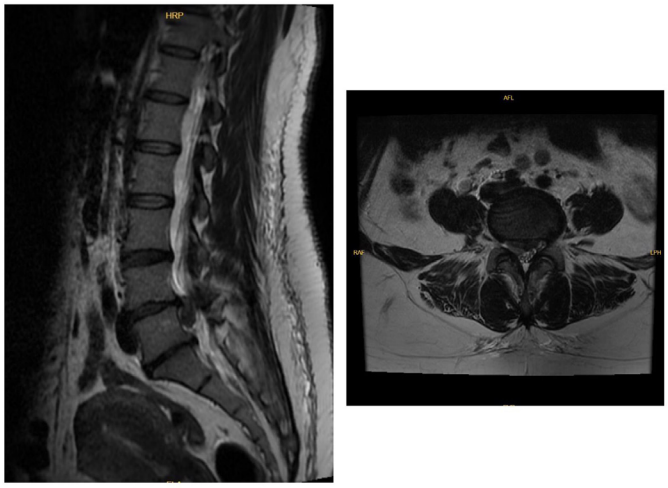

Figure 1. MRI findings of LDH
MRI: Magnetic resonance imaging, LDH: Lumbar disc herniation

Clinical chemistry analyser-Cobas 501 (Roche, Mannheim, Germany) was used to evaluate the dynamic plasma thiol/disulfide homeostasis by the automated procedure described by Erel and Neselioglu[11].

Total thiol (-SH+ -S-S-) and native thiol (-SH) were measured directly, and the disulfide levels, native thiol/total thiol ratio were calculated. With the method described by Erel and Neselioglu[11], the disulfide bonds were reduced to thiol groups containing sodium borohydride. Formaldehyde was used to remove the excess sodium borohydride to prevent further reduction of 5,5'-dithiobis (2-nitrobenzoic) acid (DTNB). DTNB reaction leads to identifying all thiol groups. The total and the native thiol levels were detected by Ellmann's and modified Ellmann's reagent. Half of the difference of the total and native thiol values was the dynamic disulfide bonds (-S-S). Additionally, albumin and IMA values were measured with the autoanalyzer (Roche, Cobas 501, Mannheim, Germany). Fifty mL of 0.1% cobalt was added to the serum samples. After 10 minutes of incubation, 50 mL 1.5 mg/mL dithiothreitol was added to the mixture. Subsequently, 1.0 mL of 0.9% sodium chloride solution was added after 2 minutes of incubation. The absorbance of the samples was measured with a spectrophotometer. The results were presented as absorbance units (kyn).

Statistical Analysis

The statistical analyses were done by using IBM SPSS Statistics version 21.0 (IBM Corp. Armonk, NY). Descriptive data were expressed as mean ± standard deviation and minimum, median, maximum values after evaluating the normality of variables by using the Kolmogorov-Smirnov test. For comparison of two groups, parametric data were estimated by using an independent sample t-test. The cut-off value for the IMA in predicting LDH was detected by the receiver operating characteristic (ROC) curve. Univariant logistic regression analyse was used to detect independent predictor factors of LDH. P-value <0.05 was interpreted as statistically significant.

RESULTS

There was no statistically significant difference between the groups in terms of age and gender. The mean ages (min-max) of the LDH and control group were 41.6 (25-62) and 40.97 (26-64), respectively (p=0.765). There were 18 women and 12 men in the LDH group, while there were 13 women and 17 men in the control group (p=0.301).

Table 1 shows the comparison of the thiol/disulfide homeostasis parameters and IMA levels of the groups. Accordingly, native and total thiol levels were significantly higher in the LDH group than in the control group (p=0.007 and p=0.008, respectively) (Figure 2). Although the disulfide/native thiol and disulfide/total thiol levels of the LDH group were higher than the control

group, the differences did not reach statistical significance (p>0.05). IMA levels were significantly higher in the LDH group than in the control group (p=0.000).

Univariate logistic regression analyse showed that native thiol, total thiol and IMA were predictors of LDH (p<0.05) (Table 2).

The ROC curve demonstrated IMA can predict the LDH with 80% and 80% specificity with the value of 1.41 (area under curve=0.888, confidence interval: 0.802-0.974) (Figure 3).

DISCUSSION

For the first time, this study evaluated the thiol-disulfide homeostasis and IMA levels in patients with LDH. Our results suggested that serum total and native thiol levels were significantly lower in patients with LDH. IMA levels were significantly higher in the LDH group indicating the hypoxic environment. IMA levels predicted the presence of LDH with

Table 1. Comparison of the groups according to the Thiol/disulfide homeostasis parameters and IMA levels

Groups Parameters	Lumbar disc hernia (n=30)		Control (n=30)		p-value*
		[Min - Max]		[Min - Max]	
Native thiol (µmol/L)	440.17±61.71	[315.0-528.0]	477.47±37.88	[387.0-574.0]	**0.007**
Total thiol (µmol/L)	481.87±66.90	[350.0-586.0]	522.37±43.17	[423.0-628.0]	**0.008**
Disulfide (µmol/L)	20.83±4.65	[12.0-29.4]	22.44±3.58	[12.5-30.0]	0.140
Disulfide/native thiol (%)	4.75±0.92	[2.8-6.7]	4.69±0.58	[3.0-5.9]	0.731
Disulfide/total thiol (%)	4.33±0.78	[2.6-5.9]	4.28±0.49	[2.8-5.2]	0.762
Native Thiol/total thiol (%)	91.34±1.55	[88.2-94.8]	91.44±0.97	[89.5-94.4]	0.768
IMA (U/mL)	1.60±0.29	[2.3-1.6]	1.18±0.25	[0.6-1.6]	**0.000**

*Independent Sample t-test
SD: Standard deviation, Min: Minimum, Max: Maximum, IMA: Ischemia-modified albumin

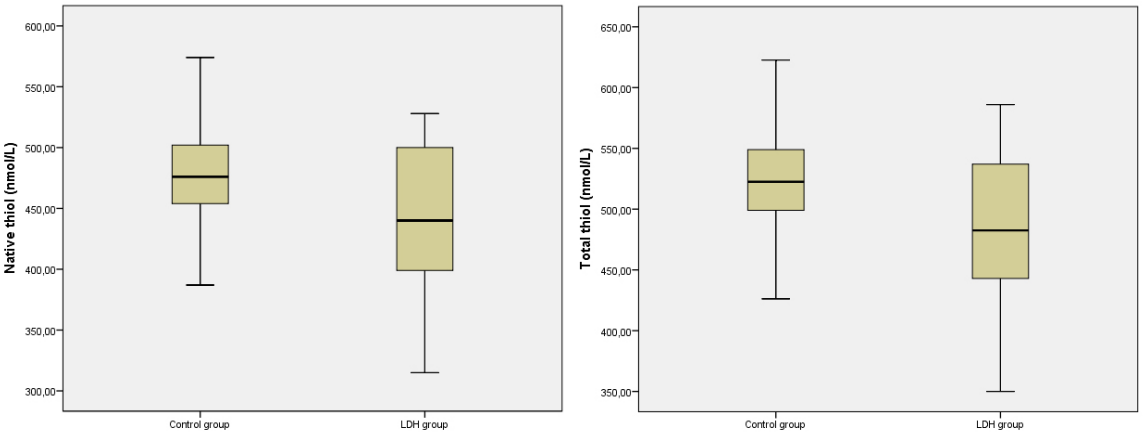

Figure 2. Native and total thiol levels of the groups
LDH: Lumbar disc herniation

Table 2. Univariate logistic regression analyse for LDH estimation

	Univariate analyses		
	p-value	Odds ratio	95% CI
Native thiol (mmol/L)	**0.012**	0.985	0.973-0.997
Total thiol (mmol/L)	**0.013**	0.987	0.976-0.997
Disulfide (mmol/L)	0.142	0.908	0.799-1.033
Disulfide/native thiol (%)	0.725	1.127	0.578-2,201
Disulfide/total thiol (%)	0.757	1.134	0.512-2,513
Native Thiol/total thiol (%)	0.763	0.941	0.632-1.401
IMA (U/mL)	**0.012**	6,016	2,294-15,780

LDH: Lumbar disc herniation, CI: Confidence interval, IMA: Ischemia-modified albumin

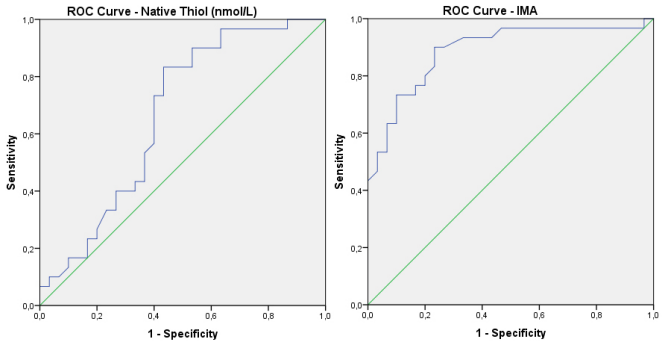

Figure 3. ROC curve of IMA levels
ROC: Receiver operating characteristic, IMA: Ischemia-modified albumin

high sensitivity and specificity (with 80% and 80% specificity with the value of 1.41).

The human organism has a dynamic balance between prooxidants and antioxidant defence systems. In the disruption of this balance, ROS occur and lead to cellular degeneration via increased lipid peroxidation, oxidative DNA damage, and cellular death[14,15]. There are many oxidative and antioxidative molecules located in intra/extracellular areas. Dynamic thiol-disulfide homeostasis is one of these oxidation-reduction reactions, a part of the non-enzymatic antioxidant system of the human body[16-18]. -SH groups of sulfur-containing amino acids are exposed to oxidation reaction in the environment with free radicals. Thiol groups of proteins consist of SH groups at the active location and are converted to the disulfide bindings in case of oxidative stress[18]. This is a bidirectional reaction. In an oxygen-rich condition, disulfide bonds may convert to the thiols again. Therefore thiol-disulfide is a dynamic balance changing according to the oxygenation of the environment. Thiols are potent antioxidant molecules that create more than half of total antioxidant capacity[19]. Therefore it may be a guide for clinical applications to evaluate the serum level of the thiols.

The degeneration of the IVD is a prerequisite for LDH. The excessive apoptosis of the cells of the nucleus pulposus is the leading cause of IVD degeneration[20]. Apoptosis is triggered by hydrogen peroxide (H_2O_2)- mediated oxidative stress[7]. An experimental study demonstrated that invitro antioxidant treatment exerted a protective effect against H_2O_2 exposure[21]. Aging is in a strict relationship with ROS, and recent data proved that aging gives rise to degeneration of the intervertebral disc cells[10,13,14]. Previously studies showed that a critical trans factor which is regulating the antioxidant genes, decreases with age[10,13,14]. The increased rate of LDH with increasing age is probably related to oxidative stress and its regulatory role in aging and degeneration. Oxidative stress is shown to disrupt the catabolism balance in IVD[22-24].

Although many articles state the association between several pathologic conditions, including neurodegenerative diseases such as Alzheimer's disease, Parkinson disease, osteoarthritis[25-27] and thiol-disulfide homeostasis, no study has indicated LDH yet. Other studies demonstrated a strong relationship between oxidative stress and IVD. Many molecules, enzymatic activity, and pro-oxidant-antioxidant balance were evaluated. Some of them are carboxymethyl-lysine, pentosidine, and peroxynitrite[9,28-31]. However, there is no study evaluating thiol levels in LDH. Depending on the reports of these studies indicating that thiols are major antioxidant molecules in plasma, we hypothesized that the level of thiols might decrease in LDH. Our results showed that thiol levels were significantly lower in the study group, as expected. Disulfide/native thiol ratios were also higher in the patients with LDH.

IMA is an ischemia marker and is primarily investigated in pathological cardiac conditions[32,33]. Simultaneously, IMA is investigated in many conditions associated with oxidative stress and found as an essential ischemia marker. During the hypoxic status, the N-termination of albumin changes and loses the metal bonding function. This modified albumin is called IMA, which occurs as a response to ROS due to ischemia. In the current study, the serum IMA levels were significantly higher in the study group compared with the control group. Morever we found a cut-off value of IMA (1.41) predicting the LDH with 80% sensitivity and 80% specificity. Therefore serum IMA levels may be an essential indicator of LDH.

Study Limitations

This study's primary limitation was the small number of patients. All of the thiol-disulfide balance markers were evaluated from the patients' serum. Confirmation of the levels of these markers in the specimen obtained during surgery would strengthen the study's impact. However, it was not possible to evaluate the tissue levels of the thiol-disulfide balance. Further, it is reported that both systemic oxidative stress and local oxidative stress are influenced by the IDD[34].

Our study's strength is this is the first study that focuses on the thiol-disulfide balance and IMA in LDH and presents a cut-off value for predicting LDH.

CONCLUSION

The thiol-disulfide balance shifts through to oxidant way during the LDH pathogenesis. The increased IMA levels seem to be the best indicator of underlying acute ischemic pathology.

Ethics

Ethics Committee Approval: Ethical approval was obtained from Ankara City Hospital, No. 1 Clinical Research Ethics Committee Presidency (no: E1-20-819).

Informed Consent: Informed consent was obtained from patients.

Peer-review: Internally peer-reviewed.

Authorship Contributions

Concept: Ö.Ö., Design: Ö.Ö., D.D., G.G., Data Collection or Processing: Z.D., E.F.O., Ö.E., Analysis or Interpretation: Ö.Ö., A.D., Literature Search: Ö.Ö., A.E.S., Writing: Ö.Ö.

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Conflict of Interest: The authors have no conflicts of interest to declare.

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