The Relation of Lumbar Disc Herniation With Increased Lipid Hydroperoxide, Paraoxonase 1 and Total Oxidative Status

Hamza Karabag1*, Sezen Hatice2

Abstract
Objective: Lumbar disc herniation (LDH) is well known low back disorder. Beside a lot of reason, the chronic inflammation is stated to have a role in the importance of LDH. As known, there is a complex connection between inflammation and oxidative stress. The relationship between LDH and the levels of lipid hydroperoxide (LOOH), total oxidative status (TOS), paraoxonase 1 (PON1) and total antioxidative status (TAS) has not been studied until now. The purpose of this study was to evaluate the levels of oxidative markers, such as LOOH, TOS, PON1 and TAS in patients with preoperative stage of disc herniation.

Materials and Methods: Fifty consecutive patients (8 patients were excluded, n = 42) with LDH and 50 healthy controls were subjected in this prospective study. Serum PON1, LOOH, TAS and TOS levels were determined.

Results: Serum PON-1 level was found to be significantly lower ($P = 0.008$), serum TAS and LOOH levels were measured as significantly higher (both $P < 0.001$) in the LDH group than in the control group. Positive significant correlations were detected between LOOH and TAS, TOS, and oxidative stress index (OSI); then again between OSI and low-density lipoprotein cholesterol (LDL-C).

Conclusion: The inflammatory-oxidant environment decreased PON1 and increased LOOH- may be the cause or reason of disc herniation. This result suggests that LDH may be related with atherosclerosis.

Keywords: Lumbar disc herniation, lipid hydro-peroxide, paraoxonase 1.

Introduction
Lumbar disc herniation (LDH) is well known low back disorder and the main reason of sciatica in adults (1). Recent investigations have showed that inflammation have a significant role in the course of disc degeneration (2-4). It is believed that not only mechanic irritation but specific inflammation may contribute to the low back pain syndromes (5-9).

Connection between the inflammation and oxidative stress are complicated (10). In spite of inflammation being a process associated with LDH, we have limited information about the effect of oxidative stress on the disc degeneration (11).

Lipid peroxidation end products in oxidative damage are pathogenic in many diseases including inflammation (12,13). Otherwise, human serum paraoxonase 1 (PON1) is mainly related with serum high-density lipoprotein cholesterol (HDL-C) and arrest lipid peroxidation via the inhibition oxidation of low-density lipoprotein cholesterol (LDL-C), showing antioxidant and anti-inflammatory properties (14, 15).

The role of lipid hydroperoxide (LOOH) and PON1 in the course of disc hernia is not clear. Therefore, our purpose was to show the relation of serum levels of LOOH and PON1, and total oxidative and antioxidative status in patients with preoperative stage of disc herniation.

Materials and Methods
Fifty consecutively patients with LDH referring to neurosurgery clinic between March 2014 and May 2015 were enrolled. However, 4 patients for active infection and 4 patients for personal reason were excluded from study. Active inflammatory and infectious disease, schizophrenia, Alzheimer disease, obsessive-compulsive disorder, major organ failure, diabetes mellitus, hypertension, atherosclerotic heart disease and receiving statin, angiotensin converting enzyme inhibitor, beta-blocker and antioxidant vitamin supplement were exclusion criteria of our study. The control group included 50 age-paired healthy volunteers.

Antecubital venous blood samples were collected after the overnight fasting. The tubes were centrifuged at 1,500 rpm for 10 minutes. The LOOH, PON1, total oxidant status (TOS) and total antioxidative status (TAS) were measured.

Measurement of Lipids
Plasma triglycerides (TG), total cholesterol (TC) and HDL-C were measured by an automated chemistry analyzer (Abbott Aeroset® system; Abbott Laboratories, USA). Friedewald formula has been used to calculate High-density lipoprotein cholesterol (HDL-C).
density lipoprotein cholesterol (HDL-C) (16).

Measurement of LOOH
Xylenol orange assay were used to measure the serum LOOH levels with the ferrous oxidation methods (17). The origin of the assay based on the oxidation of ferrous ion to ferric ion via various oxidants and the produced ferric ion measurement with xylenol orange. The serum LOOH’s reduces by triphenyl phosphine which is a specific reductant for lipids. The LOOH levels can be predicted by the diversity in values that seem because of the absence or presence of TPP.

Measurement of Paraoxonase Activities
A paraoxonase activity measurement depends on the absence (basal activity) and presence of NaCl (salt-stimulated activity). The amount of paraoxon hydrolysis (diethyl-p-nitrophenylphosphate) was calculated by monitoring the increase of absorbance at 412 nm at 25°C. Generated p-nitrophenol level was calculated usage of molar absorbitivity coefficient at pH 10.5 (18). Paraoxonase activity was expressed as U/L serum.

Measurement of Total Antioxidant Status
Erel method has been used to determine the Serum TAS levels which is a new measurement technique (19). Due to this method, hydroxyl radical the most potent radical, was produced via Fenton reaction. In this reaction, the hydroxyl radical is occurred by mixing of hydrogen peroxide solution and ferrous ion. Due to assay, the ferrous ion solution in the reagent 1, is mixed with reagent 2 (includes hydrogen peroxide). Oxidizing radicals such as brown coloured diansidinyl radical cation, which triggered by the hydroxyl radical, are also potent radicals (13).

In this assay, antioxidative effect of the sample over the potent free radical reactions started by the produced hydroxyl radical is calculated. The method gives perfect precision values, which are lower than 3%. The results are defined as mmol Trolox equivalents/L.

Measurement of Total Oxidant Status
Erel method has been used to measurement of serum TOS levels (20). Oxidants present in the sample oxidize the ferrous ion-o-diansidine complex to ferric ion. The oxidation reaction is enhanced by glycerol molecules present in the reaction medium. In an acidic medium, the ferric ion makes a coloured complex with xylenol orange. The density of colour, which can be measured spectrophotometrically, is belonging to the total amount of oxidant molecules exist in the sample. Calibration of assay has been carried out by hydrogen peroxide. Results are defined as μmol H₂O₂ equivalents/L (13).

Oxidative Stress Index
Percent of TOS to TAS level was accepted as OSI. The purpose of this computation is to specify the direction of the oxidative balance in the body. The 1 value indicates to equilibrium with the total antioxidant effect and total oxidant effect. The OSI value was calculated due to the following formula: OSI = TOS (mmol H₂O₂ Equiv./L)/TAS (mmol Trolox Equiv./L) (21). The OSI value was defined as arbitrary unit (13).

Statistical Analysis
Due to data distribution analysis Kolmogorov-Smirnov test has been used. Independent sample t test has been used for comparison of continuous variables between two groups. The chi-square test was used to compare the categorical variables between groups. Pearson and Spearman correlation analyses were used to examine the association of the oxidant and antioxidant levels in LDH patients. SPSS for Windows version 20.0 (SPSS Inc., Chicago, IL, USA) was used for All statistical analyses. Variables defined as mean ± standard deviation (SD) and categorical variables were expressed as percentages. Two-sided P value <0.05 was considered statistically significant.

Results
Table 1 represents the demographic and laboratory data of the patients. There are no statistically significant differences between the groups regarding demographic data and laboratory findings.

In epitome, the serum PON-1 levels were found to be reduced importantly in the LDH group than in the control group (P = 0.008, Figure 1A). Serum TAS and LOOH levels were found to be significantly higher in cases of LDH as compared to the control group (P<0.001, Figure 1B and 1C). There were no important differences between the serum TOS and OSI levels among the patients with LDH and healthy controls (both P>0.05, Figure 1D and 1E). Positive significant relations were detected between LOOH and TAS, TOS, and OSI; between OSI and LDL-C as to Pearson and Spearman correlation analyses.

Discussion
In this research, our results have demonstrate that (i) PON1 levels, which have anti-oxidant and anti-inflammatory function, were lower, (ii) LOOH levels, as a well-known marker of oxidative stress were higher in patients with LDH as compared to healthy controls. Interestingly,

Table 1. Presents the Demographic Characteristics and Laboratory Data of the Patients With LDH and the Controls

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Patients n = 42</th>
<th>Controls n = 50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, male %</td>
<td>50</td>
<td>64</td>
</tr>
<tr>
<td>TG</td>
<td>259.3 ± 110.4</td>
<td>198.3 ± 48.5</td>
</tr>
<tr>
<td>TK</td>
<td>214.5 ± 58.7</td>
<td>212.9 ± 46.4</td>
</tr>
<tr>
<td>LDL</td>
<td>120.66 ± 32.5</td>
<td>119.66 ± 39.0</td>
</tr>
<tr>
<td>HDL</td>
<td>47.6 ± 18.10</td>
<td>48.6 ± 16.3</td>
</tr>
<tr>
<td>Age. year</td>
<td>35 ± 14</td>
<td>36 ± 12</td>
</tr>
<tr>
<td>PON.</td>
<td>108.1 ± 35.2</td>
<td>127.9 ± 34.9*</td>
</tr>
<tr>
<td>LOOH</td>
<td>13.78 ± 5.38</td>
<td>8.88 ± 2.34*</td>
</tr>
<tr>
<td>TAS</td>
<td>1.26 ± 0.18</td>
<td>1.07 ± 0.17*</td>
</tr>
<tr>
<td>TOS</td>
<td>31.58 ± 12.19</td>
<td>28.80 ± 8.34</td>
</tr>
<tr>
<td>OSI</td>
<td>2.56 ± 1.00</td>
<td>2.70 ± 0.70</td>
</tr>
</tbody>
</table>

*P value lower than 0.01.
(iii) serum TAS levels of LDH group, which reflects the anti-oxidant balance of metabolism, were also higher than control group, and (iv) LOOH levels were positively correlated with TAS, TOS and OSI-the parameters that predict the oxidative status accurately.

Low back disorders (LBD) are a main public health problem with individual comfort and health expenditure and LDH is one of the major causes of low back pain and sciatica. Inflammatory reactions have a significant role in the course of LDH (22). Furthermore, it has been known that patients with low back pain have severe oxidative stress (11). There is a complex relationship between oxidation and inflammation (10). Moreover, some evidence has been found between LBD and atherosclerosis. Atherosclerosis may play an important etiological role in the progression of disc degeneration and LDH. Nutrition of intervertebral disc was impaired because of reduced blood flow due to atherosclerosis. Furthermore, it has been known that the abdominal aorta, particularly the aortoiliac junction as a major blood supply of lumbar disc region, is the one of the most common sites of arteriosclerosis (23). Additionally, these two conditions paired common risk factors for obesity, smoking, aging and dyslipidemia (23-25).

Lipids are at essential target of radical attack and oxidative stress have critic role in the expansion of atherosclerosis by oxidation of LDL-C (26). Paraoxonase (PON) is a well-known antioxidant molecule which preserves LDL-C and HDL-C from oxidation with hydrolysing activated phospholipids and lipid peroxide products and therefore, inhibits atherosclerosis (14,15).

Protein oxidation has an important role in the pathogenesis of many degenerative diseases (27). Otherwise, it is well known that aging is a fundamental etiological factor in degeneration of disc and oxidative damage accumulates with aging in discs (27). In an experimental study, Nasto et al showed that the mitochondrial sourced reactive oxygen species play a pivotal role in aging-related changes of intervertebral disc degeneration (28). Moreover, Leon Fernandez et al reported that ozone therapy avoid the oxidation of proteins and reduced the pain in disc herniation. They concluded that the correlation between oxidative protein damage markers and pain, indicate the effect of oxidative stress in the pathological processes in disc herniation (11). Additionally, Hou et al found that serum and intervertebral malondialdehyde levels -as a secondary product of lipid peroxidation- increased with age and they hypothesized that lipid peroxidation develops during intervertebral disc aging (27). Besides all, Jhawar et al studied in a large population which was carried out over 16 years of follow-up and they concluded cardiovascular risk factors are independently related with LDH (25). Then again, it has been known that inflammation is an unfavourable factor and correlated with disease progression (29). Several studies showed that systemic inflammatory markers were elevated due to presence of disc herniation and their levels correlated with the pain (30-33).

Similarly, our results imply that the oxidative stress related lipid oxidation by LOOH was increased and antioxidant response by PON1 was decreased and these results may be indirectly evidence of subclinical atherosclerosis and increased inflammatory state in patients with LDH. There-withal, decreased TAS levels may support the consumption of antioxidant response of metabolism to accelerated oxidative environment. On the other aspects, positive correlation between LOOH and TAS, TOS, and OSI is a further evidence of impaired oxidant-antioxidant balance of metabolism related with LDH. Oxidation of lipids increases both oxidant and antioxidant response to protect

![Figure 1. Serum PON-1, LOOH, TAS, TOS and OSI Levels in the LDH and Control Groups.](www.SID.ir)
the organism possibly against to atherosclerotic process. There were a number of limitations of our study; firstly our study was a cross-sectional design, so that we could not evaluate our results with the prognosis of treatment. Secondly, pain of patients did not take into account and we did not know the connection between oxidant and antioxidant markers and degree of pain. Thirdly, we did not evaluate the atherosclerosis with a surrogate marker because of it is the outside of our aim and we only hypothesized that our results may demonstrate subclinical atherosclerosis.

**Conclusion**
LDH is related with atherosclerosis since they share common Etiopathological risk factors. We observed that PON1 decreased and LOOH raised in patients with LDH. This inflammatory-oxidant environment may be the cause or reason of disc herniation. The further studies are essential to evaluate the significance of this result.

**Ethical issues**
The study was approved by the local ethics committee and conducted in accordance with the 2008 Helsinki Declaration. All participants have been informed about the study and signed consent forms have been collected before the study.

**Conflict of interests**
Authors declare that, there is no conflict of interest.

**Financial support**
This research supported by Harran University Research Fund (HUBAK).

**Acknowledgements**
None.

**References**

Crescent Journal of Medical and Biological Sciences, Vol. 3, No. 3, July 2016


Th17 lymphocyte levels are higher in patients with ruptured than non-ruptured lumbar discs, and are correlated with pain intensity. Injury. 2013;44:1805-1810.