Original Investigation

Paraoxonase 1 Activity and Phenotype Distribution in Lumbar Disc Herniation Patients

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ABSTRACT

AIM: To compare the Paraoxonase 1 (PON1) activity and phenotype distribution between lumbar disc herniation (LDH) patients and healthy individuals.

MATERIAL and METHODS: This research included 40 LDH patients and 42 healthy individuals. Spectrophotometric assays were performed to determine the serum PON1 and arylesterase activities. The PON1 ratio, which represents the salt-stimulated PON/arylesterase level, demonstrated a trimodal distribution. This ratio was applied to identify the different phenotypes; QQ, QR, and RR of each subject.

RESULTS: The LDH patients had lower PON1 activity than the healthy individuals (p<0.05). LDH patients had a statistically significant QQ phenotype compared to the healthy subjects (p<0.05).

CONCLUSION: LDH patients had statistically lower PON1 activity, suggesting that the low PON1 activity and PON1 QQ phenotype may be a risk factor for LDH occurrence.

KEYWORDS: Lumbar disc herniation, Paraoxonase, Phenotype, PON1

ABBREVIATIONS: **LDH:** Lumbar disk herniation, **PON:** Paraoxonase, **ARE:** Arylesterase

■ INTRODUCTION

Lumbar disk herniation (LDH) is a common disorder and the primary cause of sciatica in adults (11). Recent investigations have demonstrated that specific inflammation, in addition to mechanical problems, is an important cause of disk degeneration and LDH (9,13,17). While oxidative stress is known to cause chronic inflammation, we have limited information about its effect on disk degeneration and LDH.

Human serum paraoxonase (PON) is an antioxidant enzyme produced by the liver, and there are three different PON isoforms in humans: PON1, PON2, and PON3 (5,18). The most well-known and well-characterized isoform in the PON family is PON1. Human PON1 is a glycoprotein with a molecular weight of 43000 Daltons and contains approximately 354 amino acids. PON1 contains two calcium ions, one of which is structural and the other is responsible for catalytic activity (16). PON1 exhibits paroxanase, arylesterase (ARE), and lactonase activity. In addition, PON1 can hydrolyze several insecticides (such as paration, diazinon, and chlorpyrifos) and nerve agents (such as samon and sarin); and this hydrolysis ability reduces the toxin effects (1,4,14).

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Previous studies have examined the PON1 activity in LDH patients, but the PON1 phenotypes and their effect on LDH formation remain unestablished. This study investigated the distribution and activity of PON1 phenotypes in LDH patients and the control group. Three different PON1 phenotypes were determined by comparison of PON and arylesterase activities of PON1.

■ MATERIAL and METHODS

Study Groups

This prostective study included 82 patients who applied to the Neurosurgery Department at Balıkesir University Faculty of Medicine. (40 of them had LDH (mean age: 43.42 ± 6.7 years, age range: 22-70 years) and 42 were healthy individuals).

This study included 40 patients with LDH (mean age: 43.42 ± 6.7 years, age range: 22–70 years) who had applied to the Neurosurgery Department, Balikesir University Faculty of Medicine, and 42 healthy individuals. The inclusion criteria for LDH patients were ipsilateral radicular pain and MRI confirmation of extruded or sequestered LDH. The exclusion criteria for subject selection were degenerative spondylolisthesis; sagittal vertebral translation >3 mm and angulation of >10° on dynamic radiography; the presence of accompanying inflammatory diseases such as infectious diseases and autoimmune disorders; neoplastic diseases; familial hypercholesterolemia; liver, pulmonary, kidney, and heart diseases. Smoking habit was also an exclusion criterion.

The control group for this study consisted of 42 healthy individuals (mean age: 39.06 ± 4.8 years, age range: 20–70 years) who did not have a history of LDH or meet any of the exclusion criteria. Performing consistent hard manual work and lifting heavy objects are considered risk factors for LDH in humans. To ensure appropriate comparison, we selected a control group with individuals having similar lifestyles and working statuses as the LDH patients. This study was approved by the Clinical Research Ethics Committee, Balikesir University Faculty of Medicine, Balikesir, Turkey (Decision No. 2022/93, Date: 07/09/2022). Verbal and written informed consent was obtained from all LDH patients and control individuals.

Blood Samples

Venous blood samples (6 mL) were collected from 40 LDH patients and 42 control subjects who had fasted overnight; the samples were centrifugated (10 min at 3,000 xg), and the collected sera were maintained in tubes at −30°C until further analyses.

Measurement of PON1 and ARE Activities

The PON1 enzyme activity was measured by the method developed by Eckerson et al. (6). This method involved the determination of the hydrolysis rate of PON1 spectrophotometrically by assessing the absorbance increase at 412 nm due to the formation of p-nitrophenol, using paraoxon (p-nitrophenyl phosphate, Sigma Chemical Co.) as a substrate at 37°C for 1 min. The PON1 enzyme activity was determined from the molar extinction coefficient of 17100 m^{-1} cm^{-1}. One unit (U) of PON activity was defined as 1 μmol of p-nitrophenol formed in 1 min. Fresh paraoxon substrate was prepared daily. For phenotype distribution, the PON catalysis was determined in 1 M NaCl-containing pH:10.5 fosfat buffer.

The arylesterase activity was measured spectrophotometrically by measuring the absorbance increase at 270 nm at 25°C for 1 min with a phenylacetate substrate. The enzyme activity was determined from the molar extinction coefficient of 1310 m^{-1} cm^{-1}. One unit (U) of arylesterase activity was defined as 1 μmol of phenylacetate formed in 1 min. Fresh phenylacetate substrate was prepared daily.

PON1 Phenotype Distribution

The dual-substrate method was employed to understand the phenotypic distribution of PON1 (12). The 192 Q/R polymorphism determines the phenotypic distribution of the PON1 enzyme. Individuals with the Q allele have lower PON1 enzyme activity compared to those with the R allele. Blood paraoxon hydrolysis capacity reveals the PON1 enzyme activity, which reflects the 192 Q/R polymorphism and the changes in the concentration of the PON1 enzyme. The genetic polymorphism at codon 192Q/R is present as two isotypes: Q (low activity) and R (high activity). The ratio of PON catalysis 1 M NaCl-containing buffer to phenylacetate catalysis was used to identify the presence of the 3 phenotypes (i.e., QQ, QR, and RR). The paraoxon hydrolysis activity encoded by the R allele of PON1 is eight times bigger than that encoded by the Q allele. The PON Q/R polymorphism has been demonstrated to affect serum concentration and enzyme activity. A single amino acid change determines the structure of the enzyme and the enzyme's activity. Compared to those with R (arginine), individuals with Q (glutamine) at position 192 have lower serum PON enzyme activity. Homozygous individuals for the R allele have a higher enzyme concentration compared to homozygous Q individuals. Those with the R allele have high PON activity, while individuals with the Q polymorphism have low PON activity. Q/Q is associated with low activity, while R/R and Q/R are associated with high activity.

The cut-off values for the different phenotypes are as follows: slow enzyme activity, type QQ, ratio <3.0; intermediate enzyme activity, type QR, ratio 3.0–7.0; high enzyme activity, type RR, ratio >7.0 (12).

Statistical Analysis

Kolmogorov—Smirnov test was performed to determine the normality character of the parametric variables. An independent sample t-test was performed to compare the age of the patients between the two study groups. To compare the gender distribution of the groups, a Chi-square test was performed. The PON1 and ARE activities in LDH and control groups were compared using the Mann–Whitney U-test. PON1 phenotype distribution in patient and control subjects was also measured by Chi-square test. p<0.05 was considered to indicate statistical significance. SP-SS 20.0 statistical software was used for all statistical analyses.

Table I: Clinical Parameters of the Study Subjects

	LDH (n=40)	Control (n=42)	p-value*
Sex (M/F)	19/21	19/23	>0.05
Age	43.42 ± 6.7	39.06 ± 4.8	>0.05
LDH Level			
L2-L3	5		
L3-L4	8		
L4-L5	17		
L5-S1	12		

*Independent sample t test, chi-square test , *p<0.05, statistically significant.*

RESULTS

The study included 40 LDH patients and 42 healthy individuals. Table I reports the age, sex, and clinical features of the LDH and control subjects. The mean age and gender distribution of LDH patients and the control subjects were not statistically different, and none of the subjects met the exclusion criteria.

The PON1 activity of LDH patients was statistically lower than that of the control subjects (24.37 ± 10.7 vs. 59.18 ± 13.7 IU) (p<0.05) (Figure 1). In addition, the arylesterase activity was calculated to determine the phenotype frequency in both the LDH and control groups, respectively (86.89 ± 13.4 vs. 97.23 ± 15.8 IU) (p > 0.05) (Table II). LDH patients showed a significantly higher frequency of the QQ phenotype relative to the control subjects (Figure 2), this difference was statistically significant (p<0.05) (Table III).
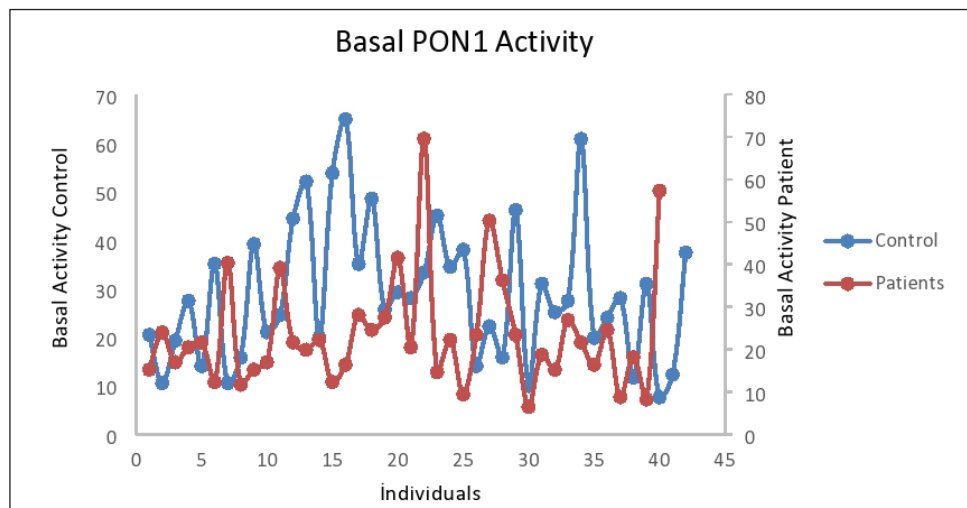
Table II: Biochemical Parameters of the Study Subjects

	LDH (n=40)	Control (n=42)	*p-value
PON1 activity (U ml^{-1})	24.37 ± 10.7	59.18 ± 13.7	**p<0.05**
ARE activity (U ml^{-1})	86.89 ± 13.4	97.23 ± 15.8	**p>0.05**

*Mann-Whitney U test, **PON1:** Paraoxonase, **ARE:** Arylesterase, *p<0.05, statistically significant.*

Table III: PON1 Phenotype Distribution in the Patients and Control Subjects

			Phenotypes			Total	p-value*
			QQ	QR	RR		
Groups	**LDH**	Count	22	8	10	40	
		% within Group	55.0	20.0	25.0	100.0	
	Control	Count	12	18	12	42	**p<0.05**
		% within Group	28.6	42.9	28.6	100.0	
Total		Count	34	26	22	82	
		% within Group	41.5	31.7	26.8	100.0	

Chi-square test, QQ phenotype distribution was more common in LDH group than in control group (p<0.05).

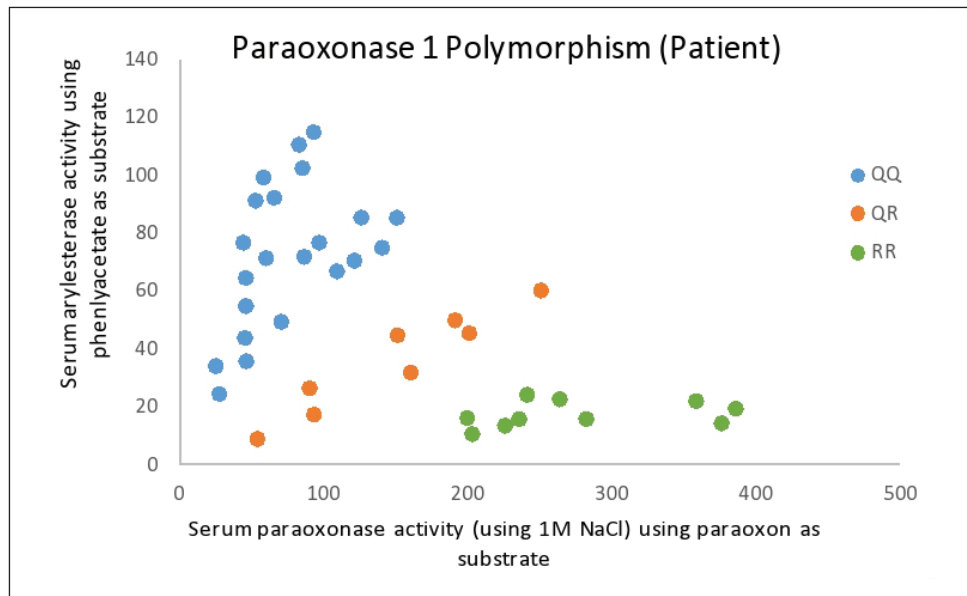**Figure 1:** Serum paraoxonase (PON1) activity in the LDH and control groups.

Figure 2: Paraoxonase polymorphism by two substrate hydrolysis assays at LDH.

◼ DISCUSSION

LDH is a very common lower back disorder caused by intervertebral disk degeneration (IVDD), and it is the main reason for sciatica in adults. In addition to mechanical problems, specific inflammation plays a significant role in the course of IVDD and LDH. IVDD is a multifactorial pathology characterized by extracellular matrix degradation and a reduction in the nucleus pulposus cells. Past studies have identified that inflammatory reactions and oxidative stress are the leading causes of extracellular matrix degradation and a reduction in nucleus pulposus cells. Reducing inflammatory reactions and oxidative stress may alleviate the progression of IVDD and LDH (3).

PON1 is synthesized in different tissues, mainly in the liver, and then distributed throughout the body. The PON1 activity may be influenced by dietary intake. Some studies have reported that consuming butter, fruit, and tea can increase PON1 activity. Patients who used medication for anemia, diabetes mellitus, hypertension, rheumatoid arthritis, neurological, liver, and cardiovascular diseases were not included in the study. Individuals with a history of cancer or hepatitis, and those using antipsychotic, serum lipid-lowering, and antioxidant drugs were also excluded from the study.

Numerous studies have reported the anti-inflammatory or anti-oxidative properties of PON1. In addition, several studies have investigated the correlation between PON1 phenotype and different diseases. Soran et al. reported that decreased levels of PON1 are involved with osteoarthritis, while Erturk et al. found that PON1 has some negative effect on knee osteoarthritis by mediating the oxidative status (7,19). The pathological characteristics of IVDD are not different from those of knee osteoarthritis. Although there are some studies on the relationship between PON1 activity and IVDD or LDH (3,10), no study has yet investigated the correlation between PON1 phenotype distribution and LDH. Chen et al. (3) clarified the role of PON1 in the gene expression process associated

with IVDD. They identified multiple polymorphisms in *PON1* and found that the coding region contained two common polymorphisms, at amino acid codons 55 and 192. Moreover, five single nucleotide polymorphisms have been identified in the promoter region, at positions −108, −126, −162, −832, and −909. In this study, the relationship between IVDD and PON1 was investigated through PCR analysis. Karabag and Sezer (10) investigated the relationship between PON1 and LD. The authors also studied the relationship among the levels of lipid hydroperoxide (LOOH), total oxidative status, total antioxidative status markers, and LDH. They used the Eckerson method to measure the PON1 activity, but phenotype classification was not performed and the relationship with LDH was not compared. In our study, the PON1 activity of the patient and control groups was compared in accordance with the Gan method by measuring the absorbance increase at 412 nm using paraoxon as a substrate. In addition, the PON1 phenotype distribution of the patient and control groups was measured by the dual-substrate method. In this method, the phenotype distribution is determined by using phenyl acetate and paraoxon as substrates, and the equation presented in the material–method section is used for calculation. Therefore, no study has yet evaluated the correlation between PON1 phenotype distribution and LDH by the method proposed in this study.

Accordingly, we speculated that the PON1 activity and arylesterase activity/phenotype distribution of PON1 may be related to LDH disease. In this study, we compared the activities of PON1 and arylesterase and hence the phenotype distribution of PON1 between LDH and healthy individuals. PON1 has anti-inflammatory and anti-oxidative properties (2,8,15). Our results showed that the activity of PON1 was statistically lower in LDH patients than in control individuals, and there was an association between low PON1 activity and LDH. In addition to comparing the PON1 activity, we compared the arylesterase activity to determine the phenotype

distribution of PON1 between LDH and healthy individuals. We measured and compared the three PON1 phenotypes (i.e., QQ, QR, RR) in LDH and healthy subjects and found that the LDH group showed a significantly higher frequency of the QQ phenotype than the control subjects. This study is the first to investigate PON1 phenotypes in LDH. Our findings demonstrated that the LDH group had a significantly higher frequency of the QQ phenotype compared to control subjects.

■ CONCLUSION

In our study, the LDH patient group showed lower PON activity compared to the control group. In addition, the Q allele was more prevalent in the LDH patient group. Previous literature has suggested that individuals with Q alleles had lower PON1 activity, which supports the validity of our results. Our study demonstrated that the LDH group had a significantly higher frequency of the QQ phenotype compared to the control group. However, further work is warranted across multiple centers to verify whether the QQ phenotype is a risk factor for LDH disease. One of the strengths of our study is that it is the first to investigate PON1 phenotypes in LDH and healthy individuals. However, this is a single-center study with a small sample size, which forms the limitations of our study.

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