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Evaluation of Serum Superoxide Dismutase Levels in Lumbar Degenerative Spinal Diseases: A Prospective Meta-analysis

Lomber Dejeneratif Omurga Hastalıklarında Serum Süperoksit Dismutaz Düzeylerinin Değerlendirilmesi: Prospektif Bir Metaanaliz

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ABSTRACT

Background: The aim of this study was to evaluate the correlation between serum superoxide dismutase (SOD) enzyme levels and lumbar degenerative spinal diseases (LDSD).
Materials and Methods: Ninety-four patients with LDSD and 64 patients without LDSD were investigated. Human SOD ELISA kits were used to measure the amount of enzymes in the samples. Serum SOD enzyme levels were determined by Student-t and Mann Whitney-U tests to determine differences between groups.
Results: The patient group was classified according to the characteristics of the disease, clinical symptoms, Visual Analog Scale (VAS) values, and Oswestry Disability Index (ODI) scores. Along with these parameters, serum SOD levels were evaluated statistically. There was no statistically significant difference in serum SOD levels in both groups. However, serum SOD levels were relatively lower in the patient group ($p>0.05$).
Conclusions: Our study could supply objective value for future researchers investigating specific lumbar diseases, should they attempt to find a serum biomarker for the disease. More studies with an increasing number of patients

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are needed to support the results of our study. Doing so may offer more specific insights on the mechanisms of LDSD and its features, which could contribute to the literature.

Keywords: Lumbar degenerative spinal disease, superoxide dismutase enzyme, serum biomarker

ÖZ

Amaç: Bu çalışmanın amacı, serum süperoksit dismutaz (SOD) enzim düzeyleri ile lomber dejeneratif omurga hastalıkları (LDOH) arasındaki ilişkiyi değerlendirmektir.
Gereç ve Yöntem: LDOH'li 94 hasta ve LDOH'siz 64 hasta incelendi. Örneklerdeki enzim miktarını ölçmek için insan SOD ELISA kitleri kullanıldı. Gruplar arasındaki farklılıkları belirlemek için serum SOD enzim düzeyleri Student-t ve Mann Whitney-U testleri ile belirlendi.
Bulgular: Hasta grubu, hastalığın özelliklerine, klinik semptomlara, Visual Analog Scale (VAS) değerlerine ve Oswestry Disability Index (ODI) skorlarına göre sınıflandırıldı. Bu parametrelerle birlikte serum SOD düzeyleri istatistiksel olarak değerlendirildi. Her iki grupta serum SOD düzeylerinde istatistiksel olarak anlamlı fark yoktu, ancak serum SOD düzeyleri hasta grubunda göreceli olarak daha düşüktü (p>0,05).
Sonuçlar: Gelecekte araştırmacılar spesifik lomber hastalıkları incelemek için bir serum biyobelirteci bulmaya çalışırlarsa, çalışmamız nesnel değerler sağlayabilir. Sonuçların desteklenmesi için artan hasta sayısı ile daha fazla çalışmaya ihtiyaç vardır. Bu bilgiler ışığında hastalıkların veya özelliklerin daha spesifikleştirilmesi ile denenebilecek araştırmalar için çalışmamızın sonuçlarının literatüre katkıda bulunacağı düşüncesindeyiz.

Anahtar Kelimeler: Lomber dejeneratif omurga hastalıkları, süperoksit dismutaz enzimi, serum biyobelirteci

INTRODUCTION

Lumbar degenerative spinal diseases (LDSD) are the most common cause of loss in the workforce today. Recently, the average lifetime for someone with LDSD has increased with the advancement of technology and medical information and the frequency of occurrence of degenerative spinal diseases has increased in parallel with this. The spinal degeneration process is multifactorial and irreversible, leading to mechanical dysfunction [(1)]. Thus, progressive intervertebral disc degeneration results in decreased disc height, affecting the biomechanics of the spine, which results in degenerative diseases such as disc herniation, spinal stenosis, and spondylolisthesis [(2)].

Cells are protected against oxidative damage by antioxidant defense systems under normal physiological conditions. These systems include enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and non-enzymatic antioxidants such as vitamin C and vitamin E.

SOD is a metalloenzyme that catalyzes the reaction of the superoxide radical to hydrogen peroxide and molecular oxygen. The catalyzed reaction of this enzyme is known as the first defense against oxidative stress. Because it is a powerful initiator of superoxide chain radical reactions, oxygen radical levels in cellular compartments are kept under control.

The aim of our study was to evaluate the correlation between the serum SOD enzyme level and LDSD. Since the degeneration processes correlate with the increase of free radicals in terms of a causal relationship, our hypothesis was that an individual's SOD level could be used as a biomarker in the diagnosis and clinical follow-up of LDSD.

METHODOLOGY

A control group of 94 patients with LDSD and 64 patients without LDSD were enrolled in our study. Patient numbers were determined by the power analysis method. The number of patients in our study was determined by the power analysis method as follows:

In studies evaluating the effects of SOD and reactive oxygen radicals in patients with LDSD, the quantitative determination of free

oxygen radicals in the serum of all patients was performed. According to the hypothesis, the SOD levels of patients with LDSD were expected to be lower than those of patients without LDSD.

In the literature, when the calculated effect size (d) for the difference between the level of free oxygen radicals between the patient and control groups is 0.5, the working power of at least 80% according to the design of 5% type-1 error and bidirectional statistical hypothesis testing. In order to achieve this, it was calculated that a total of 128 participants, 64 people in each of the study arms, should be included in the study. See Fig. 1.

Included in this study were adult patients with lumbar disc herniation, spinal stenosis, lumbar spondylolisthesis, and related spinal deformities as well as those who had previously undergone similar degenerative sequelae. Excluded were those who had suffered some form of trauma or who had been diagnosed with a malignancy. The control group consisted of volunteers who had images of lumbar vertebrae in the archive; no complaints of problems with the lumbar spine; no findings of neurological importance; and no lumbar pathology.

Blood samples were taken from patients and control groups; neurological examinations were performed; and radiologic findings and anamnesis were taken into consideration. Serum samples were centrifuged at 5,000 rpm for 15 minutes. SOD levels were determined using ELISA test kits. This was carried out at the central laboratory of molecular medicine.

The amount of SOD enzyme in the samples was measured with human SOD-ELISA kits. The kit, which works on the sandwich ELISA principle, contains two different antibodies, one for fixing the target molecule to the lots on the microplate, and the second for the assay for enzymatic labeling. Serum samples and standards were pipetted into wells on a microplate coated with a specific antibody for surface SOD and ligated with any available SOD immobilized antibody. After removal of unbound material, the HRP-Conjugate Human SOD detection antibody was added to the lots. A chromogen solution was added to the batch after washing to remove unbound HRP reagent. It was observed that the color changed in parallel with the amount of SOD. Color intensity was measured at a wavelength of 450 nm.

Obstacles and limitations during experiments

Reagents from other lots or sources were not mixed. A check was conducted to ensure that the selected calibrator diluent for the standard curve was consistent with the tested samples. Since the samples were expected to produce higher values than the highest standard, the samples were replicated with the diluent under the appropriate calibrator. This analysis was designed to remove the interference of other factors found in biological samples. ELISA did not rule out the possibility of interference until all the factors were tested.

Storage conditions

Kit reagents were stored at (+2) - (+8) ºC. Immediately after use, the remaining reagents were kept in cold storage at +4 °C.

Test procedure

All reagents were prepared before testing. All standards and samples were added to the microplate in duplicate. Sample wells were tested by setting standard lots. A standard 50 µL kit diluted in standard wells was added. A sample

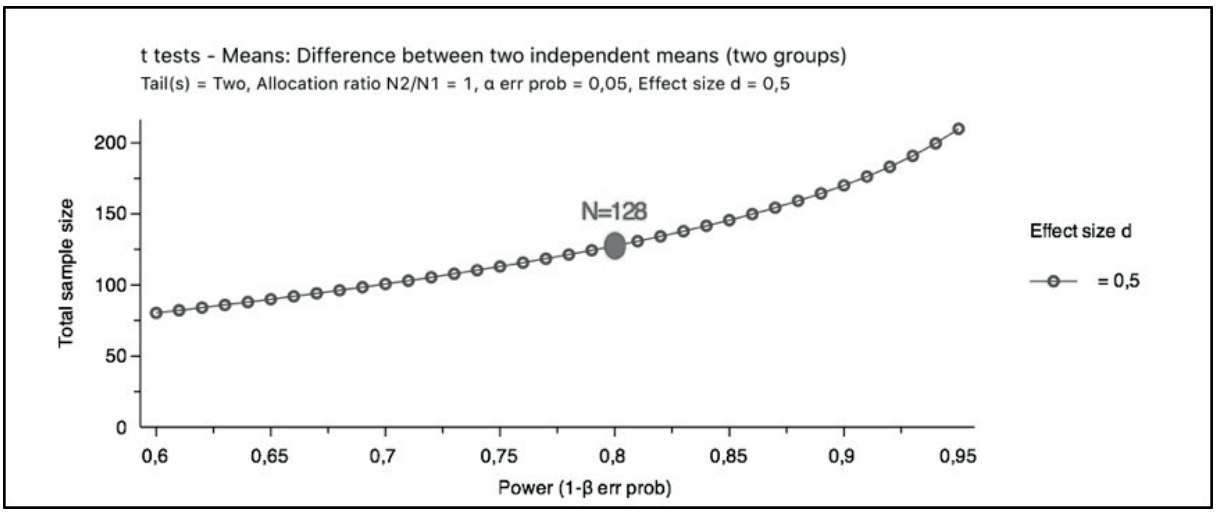

Figure 1. Sample size calculation

diluent of 40 µL was added to test the sample. Then 10 µL samples were added. These were incubated at 37 ° C for 45 minutes after being covered with a plate lid. The procedure was repeated four times for a total of five washes, with each well aspirated and washed. Each hopper was filled with the washing buffer (250 µL) using a bladder, manifold dispenser, and automatic washer. For good performance, care was taken to completely remove the liquid at each step. The remaining buffer after washing was cleaned by aspiration and filtration. After the plate was inverted, it was cleaned with a clean paper towel. Except empty lots (blind), each lota HRP-conjugate detection antibody (50 µL) was added and incubated for 30 minutes at 37°C. The aspiration/washing process was repeated five times. To each lota, chromogenic solution (50 µL) and chromogenic solution B (50 µL) were added and incubated for 15 minutes at 37°C with gentle mixing. Care was taken to protect the system from light. Fifty µL of stop solution were added to each lota. The color in the wells was expected to turn yellow. The solutions in the wells were allowed to mix well when they were green or when the color change was not correct. The optical density at 450 nm was read within 15 minutes using a microtiter plate reader.

Data analysis and calculation of results

For each standard, control and sample, double readings were averaged and the average zero standard optical density was subtracted. A standard curve was generated as a logarithmic curve fit with four parameters, reducing the data. Separately, a curve was drawn from the points on the graph based on the average absorbance for each standard in the x and y axes; a double control was applied. In the diluted samples, the concentration read from the standard curve was multiplied by the dilution factor. A standard curve with a detection range of 12.5 to 200 pg/ml was generated for each sample group tested.

Measures taken for standardization during the experiment

Care was taken to not put the reagents from one kit lot into another. Standard, conjugate,

and microtiter plates were matched for optimal performance. Kit reagents and materials were allowed to reach room temperature (20-25ºC) before use. No water bottles were used to dissolve samples or reagents. Only diluted or distilled water was used to dilute the reagents. Unused strips were stored at (+2) - (+8) ºC in the provided dryer and sachets. New disposable pipette tips were used for each transfer to prevent contamination. Acid and sodium hypochlorite solutions were not mixed. All samples were processed to inactivate viruses. Sodium hypochlorite was added to the liquid waste to a final concentration of 1.0%. The wastes were left for at least 30 minutes to neutralize the viruses before being thrown away.

Statistical analysis

Statistical analyzes of this study were performed using the SPSS 24.0 package program. The statistical significance limit was taken as $p<0.05$. Student-t and Mann Whitney-U tests were used to determine the differences of serum SOD enzyme levels between groups.

RESULTS

The mean age of 94 patients in our study was calculated as 50.11 ± 12.20. In the group of patients with LDSD, the number of males was 39 and the number of females was 55. The mean body mass index (BMI) of this group was 30.79 ± 27.18. The mean age of 64 people in the control group was 41.23 ± 10.36. The number of males in this group was 39 while the number of females was 25. The mean value of BMI in the control group was calculated as 26.68 ± 4.48 (Table 1).

SOD levels were measured and compared by sera from patients and control groups using the ELISA method. No statistically significant difference was found between both groups (Figure 2). After comparing SOD levels of both groups, the group with LDSD was separated according to some features. These features were compared statistically by looking at the SOD levels. The features examined were bulging-protrusion-extrusion number, black disc count, facet joint hypertrophy, listezis, flavum hypertrophy, muscular atrophy, Modic degeneration, and lordosis characteristics in the lumbar disc. These properties are described in detail in Table 2. Based on these characteristics, there was no significant difference in statistical calculations made within patient groups.

Patients' clinical symptoms were classified as back pain (axial pain), leg pain (radicular pain), waist pain, and leg pain. With reference to these symptoms, patient groups were statistically evaluated according to their SOD levels. Results obtained according to clinical features were not statistically significant (Table 3). Patients were compared statistically with SOD level values between Visual Analog Scale (VAS) values and Oswestry Disability Index (ODI) scores

Table 1. Demographic datas, BMI and smoking habits of each patient and control groups

Parameter	Patient (n=94)	Control (n=64)	p value
Age	50,11 ± 12,20	41,23 ± 10,36	0,000*
Gender			
Female/Male	55 / 39	25 / 39	0,016*
BMI	30,79 ± 27,18	26,68 ± 4,48	0,241
Smoking			
Yes/No	23 /71	20 / 44	0,367
Smoking			
(package year)	4,79 ±12,52	5,96 ± 10,06	0,534

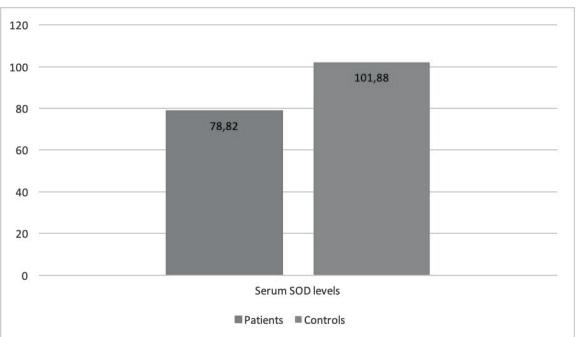

Figure 2. Comparison of serum SOD levels of patients and control groups

Table 2. Patient group, according to the characteristics of the disease

Parameters	Patient Group (n=94)					
Modic Degeneration	**Absent**		**Type 1**		**Type 2**	
(n)	77		5		12	
Lordosis	**Normal**		**Straight**		**Scoliosis**	
(n)	65		28		1	
Facet Joint Hypertrophy	**Absent**			**Exist**		
(n)	18			76		
Lysthesis	**Absent**			**Exist**		
(n)	24			70		
Flavum Hypertrophy	**Absent**			**Exist**		
(n)	30			64		
Muscle Atrophy	**Absent**			**Exist**		
(n)	75			19		
Number of Bulging	**0**	**1**	**2**	**3**	**4**	
(n)	31	39	18	4	2	
Number of Protrusion	**0**	**1**	**2**	**3**		
(n)	37	42	10	5		
Number of Extrusion	**0**	**1**	**2**			
(n)	58	35	1			
Number of Black Disc	**0**	**1**	**2**	**3**	**4**	**5**
(n)	20	43	21	6	3	1

Table 3. SOD levels in patient group according to clinical features

Parameter	Back Pain (n=17)	Leg Pain (n=11)	Back and Leg Pain (n=66)	p Value
SOD level values	54,47 ± 54,92	55,65 ± 27,73	88,96 ± 137,11	p > 0,05

Table 4. ODI values of patients group

ODI	1	2	3	4	5
Number of patients	0	16	32	27	19

ODI Scala: 0% to 20% - minimal disability 20% to 40% - moderate disability 40% to 60% - severe disability 60% to 80% - crippled 80% to 100% - bed bound (or exaggerating symptoms).

themselves. There was no statistically significant difference for either VAS or ODI values (Table 4). In group of patients with LDSD, the average VAS value was 6,5.

DISCUSSION

Symptoms of lumbar disc herniation occur most frequently in people ages 30-50 years and the prevalence is about 1-3% [3]. It is estimated that every year, 2,75 out of every 1000 people with back pain will have a severe attack requiring hospitalization [4]. However, the number of lumbar spine surgeries has also increased over the past two decades. This increase has increased hospital costs and surgical complications [5]. When conservative treatment is unsuccessful, the indication for surgery is on the agenda [6]. Traditionally, the surgical procedure is discectomy [7].

Hou et al. reported that serum and intervertebral disc SOD activity decreased gradually with age. In the geriatric group, the intervertebral disc SOD activity was significantly lower than the young and adult group, and the serum SOD activity in the young group was significantly higher than in the adult and geriatric group [8]. This support that, if the SOD activity is high the disc is healthy.

Ho et al. investigated the effects of age, gender and smoking habits on enzymatic activities. They explained that erythrocytes have antioxidant enzyme activities such as CAT, SOD, GPx. A significant decrease in erythrocyte GPx

activity was detected in smokers compared to non-smokers, while significant increases were observed in erythrocyte CAT and SOD activities. There was no age-related difference in erythrocyte GPx activity between the groups. Erythrocyte CAT and GPx activities were significantly lower at the age of 60 in the smoker group. It was found that women had higher erythrocyte GPx activity than men [9]. In our study, smokers had lower serum SOD levels than non-smokers, but this was not statistically significant.

Silig et al. reported the genotyping of the polymorphism of the SOD1 gene of 494 healthy Turkish individuals. The distribution of SOD1 A251G polymorphism in this population was investigated using a PCR-RFLP method. Genotype and allele frequencies were counted. The expected and observed genotype distributions were assessed using the Hardy-Weinberg equilibrium X2 test. In this study, 494 (278 females, 56.3% and 216 males, 43.7%) A251G polymorphisms in the SOD1 gene were investigated. The average age of the study group was 38.4. The observed genotype frequencies of SOD1 were AA: 86.2%; AG: 13.4%; and GG: 0.4%, respectively (A: Adenine, G: Guanine). This is important because it was the first study on SOD1 A251G polymorphism in the Turkish population [10]. We believe that research on antioxidant systems in different areas, such as our work, will provide values specific to the Turkish community as well as the medical literature as a whole.

Andersen et al. defined the methodological conditions suitable for the analysis of copper-zinc-SOD (CuZn-SOD), GPx, CAT and glutathione reductase (GR) in highly reproducible human erythrocytes. 220 people randomly selected from 20-89 age group were included in the study. CuZn-SOD and GR activities were associated with an age-related decrease, while no significant changes in age were observed for GPx and CAT. These results were consistent with previous studies showing that CuZn-SOD activity in erythrocytes decreases with age [11]. However, age-related changes in SOD activity are controversial. In our study, no statistically significant correlation was found between age and serum SOD levels.

CONCLUSION

Enzyme levels were relatively low in patients with LDSD (p=0.302) as expected. However, there was no statistically significant difference in serum SOD levels between the patient and control groups. We believe that the results of our study will shed light on future studies, so more specific information about LDSD will contribute to the literature.

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Ethical Approval: This study was approved by the Taksim Training and Research Hospital Clinical Research Ethics Committee (No: 2018/123 / 24.01.2018).
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