Research Article

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Endocan; A Novel Inflammatory Indicator For Spinal Trauma?: An Experimental Study İn Rats

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1. Introduction

Spinal cord injury (SCI) is one of the most important public health problems today since it is a devastating neurologic condition that leads to a number of serious physical, psychological, and economic complications, and there is currently no universally accepted treatment protocol [1, 2]. Its incidence varies between 20-40/1000.000 and it is known that the mean age of these patients is 16-30. [3,4]. Motor vehicle accidents, falls, gunshot wounds, penetrating injuries from sharp objects, and sports-related injuries are the most common causes of SCI [5]. In light of the significant costs incurred by these patients for their treatment and care, as well as the loss of their labor force and income, as well as their social and psychological problems, this is a serious health issue that impacts not only the patient but also his or her family and the national economy [6]. It has been well-documented in experimental and clinical studies that primary and secondary damage occurs after SCI. Primary damage is the injury at the time of trauma. Meanwhile, secondary damage is a form of damage that develops after the primary damage and occurs with different physiopathological mechanisms including hemorrhage, edema, axonal or neuronal necrosis, demyelination, cyst formation, and ischemia in a longer process [7]. In the literature, some biochemical markers such as IL-6, MDA, and TNF-alpha, are used as indicators of spinal cord injury. However, none of these have yet become routine diagnostic and prognostic markers.

Endothelial cell-specific molecule-1 (ESM-1), or endocan, is a soluble proteoglycan that can be measured freely in law and is usually released from vascular endothelium in inflammatory cells. Endocanis known to be a part of the chain of angiogenesis and endothelial cell cells required for inflammatory process cells [9]. Serum endocan levels are elevated alone or in combination with other biomarkers for different conditions, including some cancers (eg, brain, brain, growth, electrical and ventilation), systemic inflammation, and birth defects [9]. The operation performed shows tumor progression in cancer patients in serum endocan overgrowth and their spread in cases of endothelial cell structures or dysfunction in inflammatory diseases [10, 11]. Recent epidemiological study suggests that endocan is an inflammatory marker of endothelial dysfunction [12]. There is no data on SCI and endocan levels in the literature. The aim of this study was to determine the correlation between SCI, which is known to initiate an inflammatory process, and endocan levels and whether endocan levels have prognostic value in trauma severity.

2. Material and Methods

This experimental study was conducted in the laboratory after obtaining the approval of the ethiccommittee (No: 401). 16 male Sprague-Dawleyrats (weighing between 250-300 g) were used for this study and randomlydivided into 2 groups as; Group 1: Laminectomy (L) group (n: 8) (Control group) and Group 2: Laminectomy+ Trauma (LT) group (n: 8). The rats were kept at a constant room temperature of 23ºC, in the conditions of 12 hours of light, and in conditions that would allow free access to water/food. Rats that developed infection or could not be traumatized during follow-up were excluded from the study.

3. Surgical Procedure

After general anesthesia (60 mg/kg ketamine hydrochloride IP-Ketalar, Pfizer Istanbul, Turkey and 5 mg/kg xylazine -Rompun, Bayer, Istanbul, Turkey), a single dose of 50 mg/kg cefazolin sodium was administered intraperitoneally for infection prophylaxis and the rats were fixed on the operating table in the prone position. After cleaning the surgical area with povidone iodine, paravertebral muscles were dissected after a 3 cm skin incision in the midline in the lumbar region. Total laminectomy was performed at the L4-5 distance and the dura mater was exposed.

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4. Traumaprocedure

A cylindrical glass tube with a diameter of 5mm was placed on the dura mater at an angle of 90 degrees in accordance with Modified Allen's weight reduction technique [13]. A cylindrical fixed weight of 4 g was then dropped onto the spinal cord from a height of 10 cm through the tube.

5. Biochemical analysis

Tail blood was collected from both groups, a total of 4 times starting from the moment of trauma, at 0 minutes, 6, 24, and 48 hours. After centrifugation at 360 rpm for 10 minutes, the separated sera were stored at -80°C. Serum endocan concentration was measured by ELISA method via a manual endocan kit (Boster Biological Technology, USA).

6. Histopathological Evaluation:

After rouitinet issue follow-up in pathology laboratory all tissuesampleswere evaluated in HE stained slides by lightmicro scope. The extent of tissuedamage was evaluated on thebasis of neuronal degeneration and necrosis. The neuronal degenaration was scored semi-quantitatively according to the nuclearshirank age, pyknosis and the hyperchromasia. Score 1 represented normal-almost normal histology where asmild, moderate and severe degeneration was scored as 2, 3 and 4 respectively.

7. Statistical analysis

Statistical analysis was conducted on a computer using the SPSS version 22.0 program. Normally distributed numerical data were expressed as mean ± standard deviation, while non-normally distributed data were expressed as median (minimum-maximum). Categorical variables were expressed as frequency (percentage). Group comparisons for variables with normal distribution were conducted by one sample t-test and ANOVA, and group comparisons for variables without normal distribution were conducted by the Shapiro-Wilk test. Correlation analysis between numerical variables was conducted using Paired sample t-test. P<0.05 was considered statistically significant.

8. Results

8.1. Biochemical results:

Our study consisted of 16 subjects dividedin to two groups: Group 1: L (n:8) and Group 2: LT (n:8). According to results there is an increase in endocan level seach groups and this in crease creates a significant difference between groups (p<0.05) (Table 1).

Table 1: Meanvalues of serum Endocan levels for each group

		Std. Error	Sig. (p)
Control	0.36	0.05877	0.001
0th min	0.53125	0.03336	0.001
6th hour	0.38125	0.0326	0.003
24h hour	0.82875	0.04605	0.002
48th hour	0.74375	0.05364	0.001

The differences in the mean values among the groupsare greater than would be expected by chance; there is a statistically significant difference (p=<0,005).

When the control group and trauma groups is compared, there is a significant difference between control and trauma groups after 24h and 48h time periods (p<0.05). On the other hand, there is no significant difference after 6 hours, and there is a relatively small differene between the group 1 (Table 2).

Table 2: Meanvalues of serum Endocan levels for control- each group and theirp values

	Mean	Std. Deviation	Sig (p)
Pair 1 Control- 0th min	-0.17125	0.22408	0.067
Pair 2 Control- 6th hour	-0.02125	0.21663	0.789
Pair 3 Control- 24h hour	-0.46875	0.18795	0
Pair 4 Control- 48th hour	-0.38375	0.17254	0

8.2. Histopathological results

In the light microscopic examinations of the L group, the gray and white matter neuroglial structuring of the spinal cord was normal/almost normal (Score 1) (Figure 2). The neuronal degeneration scores of the LT group were score 2 (n:2), score 3 (n:4), and score 4 (n:2) (Figure 3). Mean neuronal degeneration scores (NDS) were 0 and 1.125 (p=0.001) for Groups L and LT, respectively. Differences between neuronal degeneration scores were statistically significantamongthetwogroups (p<0.05). Serum endocan levels of rats were correlated with neuronal degeneration scores determined after the histopathologic examination.

Figure 1: Dura materexposedafterlaminectomy

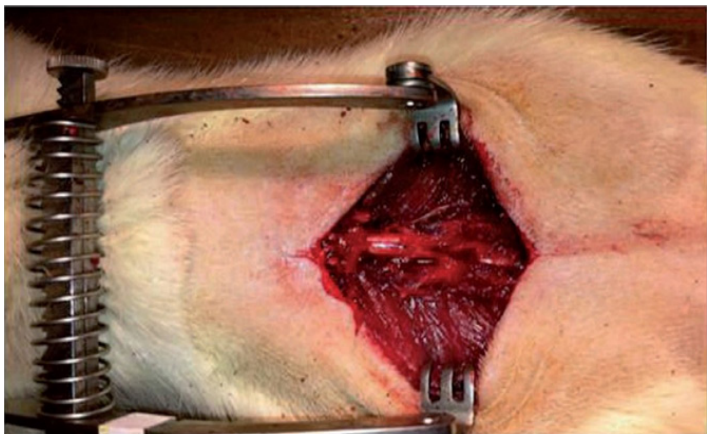

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Figure 2: Non-degenerated normal neurons in normal spinal cordsections, score1 (H&EX100)

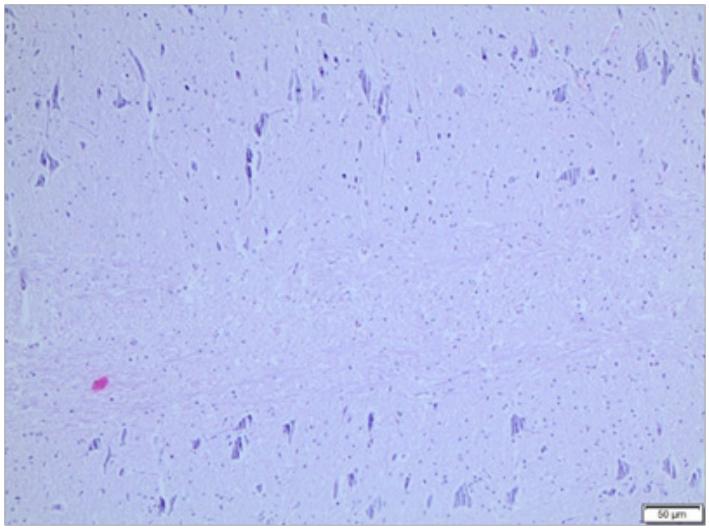
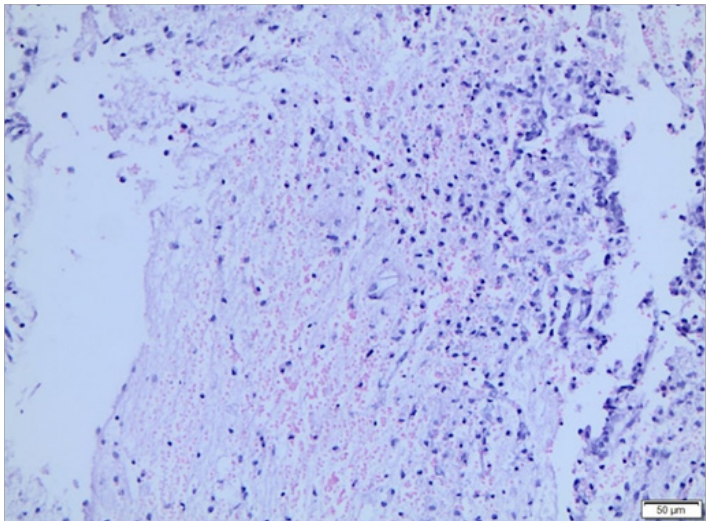


Figure 3. Diffusehemorrage and congestion (H&EX100)



9. Discussion

We found that there was a statistically difference between control and trauma group regarding endocan levels. Further more, there was also positive correlation between the severity of SCI and endocan levels. The primary injury is a direct injury to the spinal cord at the time of trauma, and there is no treatment that can reverse the effects of this type of injury with current treatment options [14]. Secondary spinal cord injury is a process that starts within minutes or hours and continues for weeks following the primary injury. In this period, many intertwined pathologic processes, especially ischemia due to impaired perfusion, are involved in the occurrence of secondary injury. This pathological process includes different sub-reactions such as mitochondrial damage, apoptosis and cell death [7]. The aim of research on secondary spinal cord injuries is to find and use pharmacological agents and measures to protect neurons in the lesion area, which are still alive and connected with distal neurons after primary injury, to increase their resilience or to halt pathological processes that will damage them. Some of the secondary damage mechanisms include free radical theory, lipid peroxidation, and inflammatory changes [15]. A free radical is a chemical compound with an unpaired, free electron in its outer orbit. This electron is transferred to other biological molecules, leading to oxidation. Excessive increase in free radicals causes cell death [16, 17]. A key step in ensuring the survival of cells is to prevent the formation of intense free radicals. Because potential oxygen-toxic metabolites are continuously formed in normal cellular respiratory processes. [15, 18].

Even though neurological examinations, MRI evaluations, and electrophysiological examinations can provide insight into the severity of the disease in patients with spinal cord injury, all of them have limitations. For instance, neurological examinations in patients with multiple extremity fractures or uncooperative patients, and electrophysiological examinations due to loss of reflexes in patients with spinal shock may be inadequate in diagnosing and prognostic follow-up. Middendrop et al. emphasized the importance of the need to find potential biomarkers in patients with spinal cord injury due to these limitations [19]. Endocan is an indicator of angiogenesis and endothelial cell activation [8]. It was first described and reported in 1996 [20]. Endocan may be involved in molecular interactions required for the regulation of a wide range of biologically active modalities, such as cell adhesion, migration, and biological processes related to proliferation and neovascularization. A group of specialized cells known as tip cells are more important for blood vessel growth and development than other cells known as stalk cells and mediate vascular growth by acting as sensors [9]. Furthermore, it is thought to be involved in the pathogenesis of vascular disorders, inflammation, and endothelial dysfunction [8]. Considering that endocanis released especially from inflamed endothelial cells, this suggests that its spread from vascular endothelial cells may also increase in inflammatory functions involved in the spinal cord injury process. The contents show that the serum shows a correlation with the pathology of endocan vessels [11]. In our study, the presence of serum endocan was correlated with the degree of neural degeneration performed histopathologically. This suggests that endocan is an inflammatory marker of endothelial dysfunction and may increase in color with the severity of the pathology [12].

In a study by Balta et al. showing the relationship between endocan in Behçet's Disease, a chronic inflammatory disease, plasma endocan levels in 33 Behçet's patients were found to be higher than in 35 healthy volunteers. The endocan level has shown a significant positive correlation with CRP, erythrocyte sedimentation rate, and disease activity [21]. Köse et al. in a controlled study conducted in 53 patients with acute coronary syndrome, they showed that serum endocan levels increased significantly compared to the control group [22]. Similarly, Balta et al. found an increase in serum endocan levels in 18 hypertensive patients who did not receive treatment compared to the control group [12]. Based

on these findings, the researchers revealed that endocan levels were associated with disease severity and mortality and suggested that endocan may be a marker of endothelial dysfunction. On theotherhand, ourstudy had severallimitations: thenumber of ratswasinsufficient, sampleswere not takenfrom CSF, and blood samples were taken only during sacrifice, ratherthan at different times after the trauma.

10. Conclusion

Serunendocan levels can be used as a prognostic indicator of traumatic spinal cord injury. Routine follow-up of serum endocan levels after trauma may help to understand the severity of secondary spinal cord injury that may develop, to make early diagnosis of risky groups and to reduce the effects of secondary injury that may develop. However, ditional prospective clicical studies are still needed to validate this conclusion.

11. Highlights

- Endocanis released by inflamed vessel endothelial cells and is an indicator of endothelial cell dysfunction.
- Serum endocan levels increase significantly, especially in the first 24-48 hours after trauma.
- Increased serum endocan levels in spinal cord injury, which is one of the inflammatory processes, may be a potential indicator of neuronal ischemia.
- Routine follow-up of serum endocan levels after spinal cord injury may reduce the early detection of secondary damage and the associated morbidity.

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