Serotonin, Cyclooxygenase-2, and Some Biochemical Parameters in Patients with Spinal Cord Injury

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Abstract
Spinal cord injury is still a serious disease that affects people throughout the world and the purpose of this study was aimed to investigate the influence of Cyclooxygenase-2 (COX-2) and Serotonin (SERT) in sera of acute spinal cord injury (SCI) patients. The study also included cholesterol, calcium, and sodium as biochemical tests for risk development. SERT was determined using (ELISA) kit, COX-2 enzyme was determined using an assay for the peroxidase activity in serum of spinal cord injury patients based on a colorimetric procedure and the other biochemical parameters, Cholesterol was determined using spectrophotometric assays, Ca and Na were determined using Atomic number. The study included sixty patients with acute SCI and thirty healthy people as control. The differences in age were non-significant between the two groups ($P>0.05$). The results have shown significant ($P<0.05$) levels of SERT between SCI patients and controls. COX-2 level was significantly higher ($P<0.05$) in the serum of SCI patients compared to control. Cholesterol Parameter was significantly different ($P<0.05$) between the two groups. Furthermore, SCI patients exhibited significantly ($P<0.05$) higher calcium and sodium levels than control patients. COX-2 has shown a significant positive moderate correlation with SERT and a negative weak correlation with age in SCI patients. The receiver operating characteristic (ROC) test has shown that SERT, COX-2, Ca, and Na were good markers for diagnosing SCI in serum. In conclusion, patients with acute SCI have elevated COX-2 levels, which may result from the inflammatory induction due to tissue damage and taking part in pain following the injury.
Keywords: Spinal cord injury, Cyclooxygenase-2, Serotonin, Neurotransmitters regulators.

1. Introduction

Spinal cord injury may result from a direct impact of a fast-moving object striking the spine or an indirect force induced by motions of the spine that are outside of the physiological range [1]. Injury to the spinal cord leads to a chain of biological reactions that can take within seconds and that proceed for months or even years [2], The pathophysiology of SCI is expressed in two distinct phases: the primary and secondary SCIs [3], and the search for pharmacological treatment protocols are generally aimed at reducing and minimizing neural injury and neurological sequelae [4]. Secondary spinal cord injury usually develops following a primary lesion induced by spinal cord contusion and the emergence of apoptotic cells has been found to play an important role in the development of secondary injury [5].

Serotonin, also termed 5-hydroxytryptamine (5-HT), affects almost every aspect of human behaviour. this conclusion may appear odd given that only around one in a million CNS neurons make serotonin and that the great majority of total body serotonin is located outside the CNS [6]. During the acute phase of a spinal cord injury recently showed that both the 5-HT1A and 5-HT2A receptor subtypes found on spinal dorsal column axons, and have conflicting effects on axonal excitability [7], Serotonin plays a prominent role in locomotion and increased 5-HT neurotransmission promotes functional recovery after SCI [8].

The cyclooxygenase-2 enzyme plays a key role in the inflammatory and oxidative stress stages (lipid peroxidation, protein, and DNA degradation) in traumatic neuronal tissue [9][10]. This enzyme creates the superoxide anions leukotrienes and thromboxanes Parecoxib, a selective COX-2 inhibitor gent, reduces the production of pro-inflammatory prostaglandins in damaged tissue and provides strong analgesic and anti-inflammatory properties [11]. COX-2 provides a possible link between excitotoxicity and lipid peroxidation in spinal cord injury. COX-2 is induced by neuronal activation, but induction of COX-2 in the brain by synaptic activity is blocked by the NMDA antagonist MK801 and the non-NMDA antagonist NBQX in a seizure model. These data suggest that activation of excitatory amino acid receptors is required for COX-2 gene expression in the brain. Each molecule of arachidonic acid metabolized by cyclooxygenase creates a hydroxy radical, a source for lipid peroxidation [12].

The present study is designed to investigate the levels of SERT and COX-2 in the serum of SCI patients in order to clarify some of the pathways crossed by these two important parameters in the modulation the neurological events systemically (as the levels are determined in serum) after traumatic injury in the spinal cord. Also, to investigate the correlation of SERT and COX-2 with serum calcium,

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Sodium, and Cholesterol parameter, as well as the sensitivity and specificity of SERT and COX-2 as prognostic markers in serum of SCI patients.

The abbreviates: Serotonin (SERT), Cyclooxygenase-2 (COX-2), Calcium (Ca), and Sodium (Na), No.of samples (N), standard deviation (SD), and Standard Error (SE), area under the curve (AUC).

2. Experimental part

Collection of Blood Sample

Serums of 60 patients with spinal cord injury, whose ages ranged (15-80) years, and 30 normal subjects, whose ages ranged (16-80) years were collected from the Neurosurgical Teaching Hospital in Baghdad, Iraq, between September and December 2021. Blood samples were taken from the patients between 1 and 7 days following the accident. Five ml of venous blood was collected from the anterior cubital vessel using a disposable syringe from all participants and moved to a clean simple gel tube. Collecting blood was centrifuged within 15 minutes and the resulting serum was divided into three sections by micropipette into Eppendorf tubes and placed at -20 °C before the biochemical examination.

3. Kits

1.1 Serotonin ELISA Kit

It was supplied by (Shanghai YL Biont /CHINA). It has been worked out according to the methods attached to it.

1.2 COX-2: Estimation of COX-2 Activity in spinal cord injury

Estimation of COX-2 Activity in spinal cord injury patients: The activity of the COX-2 enzyme is estimated by an assay for the peroxidase activity in serum of spinal cord injury patients based on a colorimetric procedure [13]. This method depends on measuring the enzyme-catalysed oxidation of tetramethyl-Phenylenediamine (TMPD) by hydrogen peroxide. The blue reaction was measured at 610 nm. One unit of activity is defined as the amount of enzyme required to convert 1µmol of hydrogen peroxide to the product under assay conditions.

1.3 Cholesterol

It was supplied by (Linear Chemicals / Spain) and it used the spectrophotometric method.

1.4 Calcium (Ca) and Sodium (Na)

Estimation Activity in spinal cord injury patients by Flame Atomic Absorption Spectrophotometer (FAAS)/ Puck scientific / Germany.

4. Statistical Analysis

The statistical results were processed by using SPSS-V22. Descriptive analyses of each parameter were detected, and the results are expressed in form of mean±standarad deviation (SD). Independent-sample t-test method was used for the comparison between the means of control and the patient's group. In addition, Spearman's rank correlation coefficient analysis was carried out to determine the relationships between all study variables in this work.

5. Result and discussion

3.1 The anthropological characteristics

Unfortunately, there's no way to reverse damage to the spinal cord. But researchers are continually working on new treatments, including prostheses and medications, that might promote nerve cell regeneration or improve the function of the nerves that remain after a spinal cord injury. This study is focused on patients with spinal cord injury in order to investigate the potential role of COX-2, Serotonin, Cholesterol, Ca, and Na compared with normal people. The anthropological characteristics of the 60 patients with spinal cord injury are analyzed and summarized in (Table 1). The average age of the patients was 31.167years, and there were significant differences in all characteristics between the patients and the control group. Preoperative serum levels of COX-2, Serotonin, Cholesterol, Ca, and Na were measured in 60 patients. Determination of parameter levels was based on mean values for each serum factor measured and are summarized in (Table 2).

Table 1. The anthropological characteristics of patients with spinal cord injury and control groups

Characteristic		Control	SCI patients	*p*-value
N		30	60	-
Age (year)	Mean	34.875	31.167	0.258
	SD	16.680	14.751	
	SE	2.637	1.904	
Male N (%)		24 (60%)	32 (53.33%)	0.511
Female N (%)		16 (40%)	28 (46.67%)	

*non-significant P > 0.05

3.2 SERT, COX-2, and Some Biochemical parameters and their effect on functional recovery after SCI

Table 2. The Serotonin, COX-2, Cholesterol, Ca, and Na in the sera of spinal cord injury patients compared to control group

Parameter		Control	SCI patients	*p*-value
Serotonin	Mean	36.168	52.557	0.0001
	SD	5.938	15.706	
	SE	0.939	2.028	
COX2	Mean	2.316	3.223	0.0001
	SD	0.791	0.644	
	SE	0.125	0.083	
Cholesterol (mg/dL)	Mean	156.213	186.355	0.0001
	SD	28.678	30.532	
	SE	4.534	3.942	
Ca (mg/dL)	Mean	8.850	9.944	0.0001
	SD	1.364	1.203	
	SE	0.216	0.155	
Na (mEq/L)	Mean	142.625	152.283	0.0001
	SD	4.694	4.770	
	SE	0.742	0.616	

*significant p ≤ 0.05

Results showed a significant increase (P<0.05)in the mean value of Serotonin .5-HT is a monoamine neurotransmitter synthesized from tryptophan, an essential amino acid, by a subset of neurons referred to as serotonergic neurons that are present in the CNS [14]. Several factors point to a direct role of 5-HT modulation in motor activity: its activation in the commissural region; its release variations within the ventral horn related to the locomotor activity [15]. In a study performed on mice, has been a rise in collected levels of serotonin, variability in bleeding may have caused the rises in serotonin levels following injury to the spinal cord [16]. A countering results were obtained by Bilchak al. (2021) decrease in serotonin following SCI, the loss of descending 5-HT supply causes 5-HT receptors to undergo varying degrees of plasticity, depending on the specific subtype [17]. Maybe acute exercise raises the blood-brain transport of tryptophan (the precursor of serotonin), thereby increasing the rate of 5-HT synthesis and metabolism, and it could also lead to an increment in serum levels. Changes in platelets due to exercise could also lead to an augmentation in serum 5-HT since they are the main carrier of 5-HT in the periphery [18].

The level of serum COX-2 was elevated significantly (P<0.05) in SCI Patients with SCI have reported to experience an episodes of pain (including neuropathic pain) and arisen in the inflammatory and improve recovery after an SCI [19]. COX-2 is an immediate-early gene that may be induced in response to the transient increase in extracellular excitatory amino acids evoked by spinal cord trauma, its expression may contribute to oxidative stress and lipid peroxidation following injury, and its expression is inhibited by glucocorticoids [20]. Overexpression of COX-2 is associated with an increase in micro-vessel density. Moreover, COX-2 is closely related to with angiogenesis, which is induced by inflammatory cytokines [21]. In SCI, pro-inflammatory cytokines are overexpressed and released in large amounts to modulate the inflammatory events as a consequence of the trauma [22].

The cholesterol parameter was significantly (P<0.05) between SCI patients and control and the value was within the normal range limits. Serum lipids are

directly associated with the lifestyle of individuals in terms of diet (intake) and motion (expenditure) [23]. Laclaustra et al. (2015). Spiralized people are suffers from a reduced level of activity, mostly because of muscle paralysis, which often leads to a low physical capacity, probably leading to unfavorable lipid profiles [24]. Greater saturated fat intake was associated with higher serum cholesterol after controlling for age [25]. A study showed in his that individuals with chronic SCI had higher levels of total cholesterol [26].

The level of serum calcium was elevated significantly ($P<0.05$) in SCI patients at the present study. Javidan et al. (2014), have reported similar results for calcium but in chronic SCI patients. The workers have recommended the dietary regulation of calcium in SCI patients because of its effects during the injury [27]. Other studies have reported hypercalcemia during the acute phase of SCI [28], [29], [30], [31]. another study showed ten to twenty-three percent of people with SCI suffer from hypercalcemia. Hypercalcemia is characterized by excessive bone resorption as a result of immobility, as well as accelerated bone turnover in developing youngsters and their big and active bone mass, especially in teenage boys. Because hypercalcemia impairs renal function, the increased calcium load is not eliminated effectively by the renal, leading to reduced calcium elimination and renal concentrating capacity. In patients with hypercalcemia, symptoms such as stomach discomfort, nausea, vomiting, malaise, lethargy, polyuria, polydipsia, and dehydration appear gradually. People with the disease may also have changes in behavior or a psychotic episode [32]. The increase in calcium level could be explained by the loss of phosphorus from bones and muscle tissues and results from relative hypoparathyroidism. Secondarily, it can directly inhibit the 1,25(OH)D synthesis [33].

Sodium (Na) functions to regulate neurotransmitter activity by removing it from the synaptic cleft [34][35]. Primary and secondary damage is involved in the pathophysiology of acute spinal cord injury (SCI), which includes both kinetic and sustained compression of the spinal cord by displaced bone fragments[36]. In experimental models of SCI, voltage-sensitive sodium channel blockers provide powerful neuroprotection in experimental models of SCI. It is hypothesized that blocking Na^+ channels may lead to neuroprotection by reducing cellular swelling and energy demands to remove excess Na+ as well as improving membrane integrity[37]. permeability due to sodium is an important feature in the pathogenesis of neuronal degeneration of the pathophysiology of spinal cord injury (SCI) in lowing injury to the central nervous system (CNS) involves a primary mechanical injury and a delayed and secondary injury [38]. The results of sodium agreed with the research of Hyun-Yoon Ko and Sungchul Huhet al.2010, sodium was elevated significantly ($P<0.05$) [39], The results of its opponents have been obtained by (Ohbe et al. 2019), The results include hyponatremia they assessed risk factors for hyponatremia in acute SCI at critical care centers in Japan and found associations between severe hyponatremia and various risk [40].

6. Correlation

The relationship of parameters with each other in the pathological condition of SCI was evaluated and shown in terms of r and p-value. Table 4 displays the Pearson's correlation coefficient of SERT, COX-2, Cholesterol, Ca, and Na with others of the study in SCI patients. There was a weak negative correlation was observed between COX-2 and Na in the sera of SCI patients (r= -0.287*, P=0.026), as shown in Fig 1 and Table 3.

Table 3. Correlation between parameters in SCI patients

Parameter	Serotonin		COX-2		Ca		Na	
	r	*p*	r	*p*	r	*p*	r	*P*
COX2	0.029	0.828	-	-	0.103	0.436	-0.287*	0.026
Ca	0.026	0.843	0.103	0.436	-	-	-0.248	0.056
Na	-0.059	0.652	-0.287*	0.026	-0.248	0.056	-	-
Cholesterol	-0.010	0.942	0.189	0.148	0.008	0.952	-0.073	0.581
Age	0.056	0.673	0.086	0.514	-0.030	0.817	-0.250	0.054
*A correlation was observed between COX-2 and Na.								

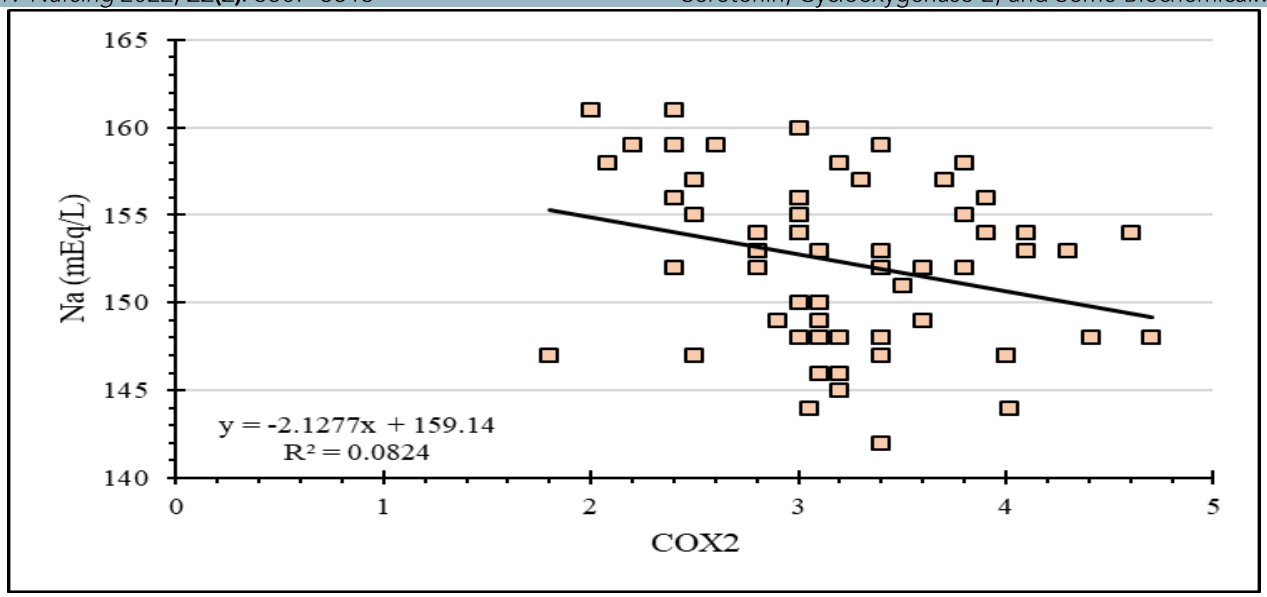

Figure 1: Correlation between COX2 and Na in SCI patients.

3.4 Receiver Operating Characteristic

The receiver operating characteristic (ROC) curve is a calculation tool that uses the given points of the particular test on the sensitivity and specificity axis to examine the usefulness of this test in the prognostic and diagnostic prediction of the disease. This is achieved by measuring the AUC created by the points. Here in this study, the ROC curve was used to examine the possibility of using SERT and COX-2, Ca, and Na in the prediction of SCI. SERT and COX-2 were shown to have good sensitivity as a prognostic marker for SCI within one week from injury. On the other hand, both Ca and Na were shown good sensitivity as a prognostic marker for SCI as shown in Fig 2 and Table 4.

Table 4. ROC parameters and cut-off values.

Parameter	Serotonin	COX2	Ca	Na
AUC	0.828	0.807	0.753	0.919
SE	0.040	0.045	0.053	0.027
p-value	0.0001	0.0001	0.0001	0.0001
Cut-off value	39.80	2.96	9.05	146.5
Sensitivity	71.7%	73.3%	70%	90%
Specificity	72.5%	75%	70%	80%

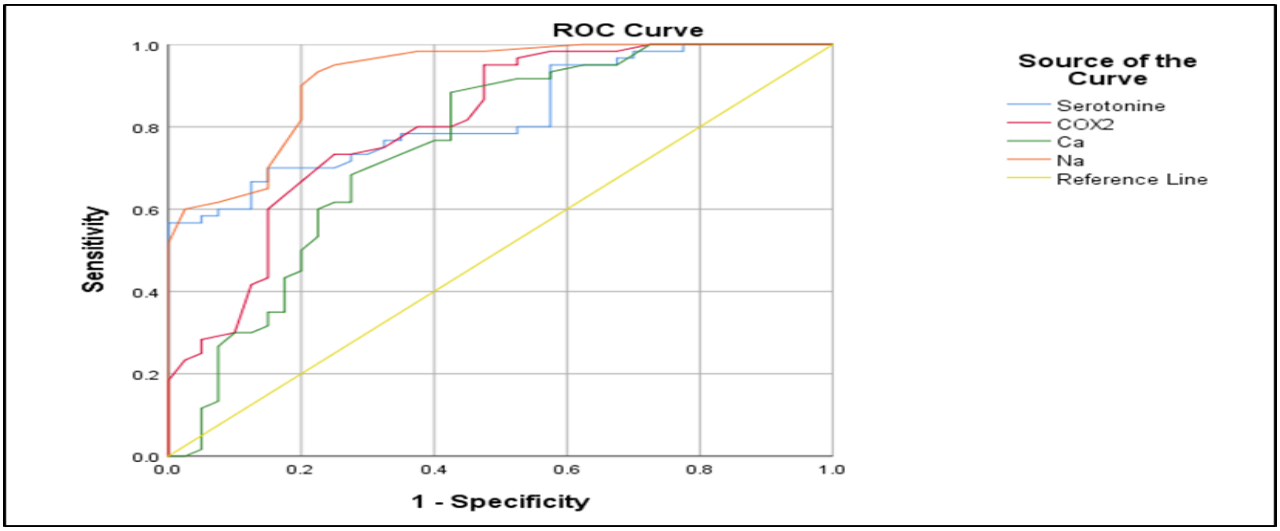

Figure 2. ROC curve of serotonin, COX2, Ca, and Na in the screening of SCI patients concerning healthy control.

7. CONCLUSIONS

The level of serum SERT was significantly increased in SCI patients after one week from an injury that it plays an important role modulation of motor activity by stimulated to control CPG activation as well as motoneuron output, and the level of serum COX-2 was elevated significantly in SCI patients compared to control, which indicates over-release of proteinoids, and thereby, developing inflammatory and pain, moreover, the levels of serum Ca and Na were elevated significantly in SCI patients and Serum COX-2 has a negative weak correlation with age in SCI patients, and this may reflect a minor aging effect in immunomodulation after SCI. However, required in the future Patients with SCI need more follow-up (2, 4, 8, 12, and 16) weeks after injury, we suggested for use and selective use of selective COX-2 inhibitors is highly recommended after an SCI to improve the recovery from injury.

Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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