Bull. Egypt. Soc. Physiol. Sci. Vol. (42), issue (2), 144-163

Bull. of Egyp. Soc. Physiol. Sci.

(Official Journal of Egyptian Society for Physiological Sciences)

(pISSN: 1110-0842; eISSN: 2356-9514)

Protective Role of Platelet Rich Plasma in Cardiovascular Dysfunction and Autonomic Dysreflexia Induced by Spinal Cord Injury in Rats

Mohamed H ElSayed[1], and Sherif Khedr[2]

[1] Assistant Professor in the Physiology Department, Faculty of Medicine, Ain Shams University, Cairo, Egypt.

[2] Lecturer in the Physiology Department, Faculty of Medicine, Ain Shams University, Cairo, Egypt.

Submit Date: 18 August 2021
Revise Date: 09 Oct. 2021
Accept Date: 17 Oct. 2021

Keywords

- Spinal cord injury
- platelet rich plasma
- cardiovascular dysfunction
- autonomic dysreflexia

Abstract

Background: Patients with spinal cord injury (SCI) have a high risk of cardiovascular complications during the acute phase following the trauma, affecting their prognosis and quality of life. These cardiovascular complications require prompt medical attention to avoid neurological compromisation, morbidity, and mortality. This work aims to provide an overview of the impact of platelet-rich plasma (PRP) treatment on SCI and its cardiovascular hazardous sequelae. Methods: 26 adult female Wister rats were randomly allocated into three groups; sham-operated control group, a group that underwent compression of the spinal cord at the T4 level, with no further intervention (SCI group), and a treated group with PRP following spinal cord injury at T4 level on the site of injury (SCI-PRP group). Mean arterial pressure (MAP), heart rate (HR), as well as core temperature, were recorded under basal conditions and in response to colorectal distension (CRD). Results: Under the basal condition, hypotension and hypothermia were observed during the initial 4 weeks post-injury while tachycardia was prominent all through the study starting from the 2nd week onwards in the SCI rats compared to sham controls. Meanwhile, the study of cardiovascular sequelae of SCI in response to CRD revealed a marked elevation in the MAP, hyperthermia as well as bradycardia associated with ventricular/supraventricular ectopic rhythm in the SCI group which may be accounted for by autonomic dysreflexia (AD). PRP treatment ameliorated partially the cardiovascular complications under basal conditions and in response to CRD as well. Moreover, rats with SCI showed a significant increase in atherogenic index, body weight gain in addition to hypercholesterolemia and hypertriglyceridemia. This effect was blunted in the SCI-PRP group compared to the SCI group, though not normalized. Histopathological and electron microscopic (EM) examination revealed that the SCI-PRP group had more myelinated regenerating axons of the spinal cord (SC) than the injured group but fewer than the sham group. Conclusion: The application of PRP immediately to the site of SCI facilitated its regeneration, had a potential repairing effect, and prevented, at least partially, cardiovascular complications.

Corresponding author: Mohamed H ElSayed, **Email:** doctorpioneer@yahoo.com **. Tel.** 01006625071,
.

INTRODUCTION

Spinal cord injury (SCI) is one of the most devastating and health debilitating conditions an individual can sustain. A complete injury causes permanent loss of sensation and movement in the affected limbs and trunk, in addition to the loss of bowel, bladder, and sexual functions, thus causing extreme psychological stress (1), not only causing disability but also has a heavy burden on the family and society (2,3).

Cardiovascular dysfunction is one of the major secondary complications of SCI and a source of suffering for the patients and their caregivers. SCI disrupts autonomic pathways (4) and consequently perturbs cardiovascular homeostasis and may be life-threatening in the early stages of SCI (5). Cardiovascular complications include profound hypotension, bradyarrhythmia, and cardiac arrest (6). Although some of these complications can improve in the weeks following SCI, cardiovascular function and regulation rarely return to the pre-injurious baseline. Moreover, bouts of hypertension were reported in response to colorectal distension following SCI, a condition referred to as autonomic dysreflexia (AD) (7). Although AD is a common condition associated with high SCI, the time course of its development has not been thoroughly investigated. Symptoms of AD can present in patients 2–3 months following the injury (8). However, Krassioukov et al. (2003) (9) have reported their occurrence within the first month following injury in 5.7% of patients. Animal studies have reported that full AD develops between 2 and 4 weeks following spinal cord clip compression induced injury at T4 level (10,11).

The treatment of SCI patients has been extensively studied in the field of neuroscience; however, limited progress has been achieved (12) and currently, there is no established therapeutic intervention capable of restoring significant neurological function after SCI. However, there has been tremendous hope for the development of new treatment for many SCI complications with platelet-rich plasma (PRP)_ in recent advances (13).

Treating some clinical conditions using the innovative PRP therapeutic modality was a very tempting approach with promising results and no side effects, moreover, it is affordable, simple, easily performed, noninvasive, and effective (14). PRP has been used clinically in humans since the 1970s for its healing properties on a cellular level and in the fields of surgical sciences (15). It consists of a supra-physiological concentration of platelets that on activation secretes a high concentration of growth factors. While the average concentration of platelets in the whole blood is about 200,000/µl, PRP preparations contain between 4 to 7 times the average value, depending on the PRP preparation system used (16). However, to be clinically effective, it is generally recommended that PRP should have a minimum of 5 times the normal average number of platelets found in whole blood (17,18). Platelets are rich sources of cytokines and growth factors stored within α-granules. These growth factors include platelet-derived growth factor, insulin-like growth factor, vascular endothelial growth factor, platelet-derived angiogenic factor, transforming growth factor-beta, fibroblast growth factor, epidermal growth factor, connective tissue growth factor, and interleukin-8. In addition to growth factors, platelets contain other substances, such as fibronectin, vitronectin, and sphingosine 1-phosphate, that initiate wound healing (19,20).

These growth factors proved beneficial healing responses across multiple specialties e.g., dermatology, urology, and gynecology (21,22). Moreover, platelet growth factors and cytokines play a vital role in initiating and supporting the three stages of healing i.e., inflammation, proliferation, and remodeling (23). Based on this, PRP has been extensively used for topical therapy of various clinical conditions, including wounds and soft tissue injuries (24.25), chronic diabetic foot ulceration (26), maxillofacial surgery, urology as well

as plastic surgery (27). Additionally, PRP is widely used in orthopedic, and sports medicine to relieve pain through the natural promotion of healing in musculoskeletal diseases such as tendonitis, arthritis, ligament sprains, and tears resulting in exceptional healing, and rapid return to regular activities, with complete pain relief (28).

Moreover, because the person's own blood is being used, there is no risk of a transmitting infection and minimal risk of an allergic reaction (29). To our knowledge, we have not come across profound pre-clinical evidence of PRP use in SCI. Therefore, we propose an assumption that the application of PRP could provide a novel and tempting therapeutic approach for SCI in terms of cardiovascular functional measurements and histopathological features in rats. In the following passages, we attempt for the first time, to cover the evidence base for regeneration and repair after SCI by PRP and to hamper its cardiovascular complications.

Materials and Methods:

Experimental animals

All experimental procedures were approved by the Animal Care and Ethics Committee at Ain Shams University and per the guidelines of the National Health and Medical Research Council of Australia for animal research. All animals were killed using an overdose of sodium pento-barbitone (200 mg /kg i.p.) following completion of the study (6 weeks following induction of injury). In our experimental design, rats have been chosen to be our model to study the traumatic SCI as they mimic the morphological, biochemical, and functional changes that occur after SCI in humans (30-33). This work was performed on adult female Wistar albino rats initially weighing 200 to 260 g, purchased from the Research Institute of Ophthalmology (Giza). Female rats were preferred because of the relative ease of manual bladder emptying after SCI, resulting in less frequent urinary tract infections. Rats were maintained in Ain Sham Research Institute under standard conditions of boarding and feeding.

Experimental protocols

Rats (n=26) were randomly allocated into 3 groups:

Group I (Sham group); rats in this group were exposed to the same surgical manipulation as the other groups except for spinal cord crushing.

Group II (SCI group); rats in this group were exposed to spinal cord injury (SCI) at T4 level according to the technique described by Krishna and his colleagues (2013) (34) with some modifications.

Group III (SCI-PRP); these rats were exposed to the same surgical intervention of SCI followed by immediate direct application of freshly prepared and activated PRP to the site of spinal cord injury.

PRP Preparation

Five rats were anesthetized with 50 mg/kg ketamine i.p. followed by exsanguination via cannulation of the abdominal aorta. PRP can only be made from anticoagulated blood. Preparation of PRP begins by adding citrate to whole blood to inhibit the clotting cascade (35). This is followed by two centrifugation steps. The first centrifugation step (5,600 rpm/10 minutes) separates the red and white blood cells from plasma and platelets. The second centrifugation step (2,400 rpm/15 minutes) further concentrates the platelets, producing the PRP separate from platelet-poor plasma (15, 36, 37)**.** Activation of PRP preparation was done by $CaCl_2$ (38) which results in polymerization of fibrin from fibrinogen, creating a gelatinous or putty-like structure that can be placed directly into the region of the spinal cord injury during the operation (39).

Spinal cord injury technique

The technique of compression injury was done as described before by clamping of the spinal cord at the fourth thoracic spinal level under anesthesia (90 mg/kg of Ketamine i.p) (34, 40-41). After incision of the skin centered on the T4 spine, fascia and muscles were dissected away from the T3-T5 spinous processes and

laminae. Then the interspinous ligament was sharply divided between T3 and T4 and between T4-T5 using fine scissors. The lamina and spinous process of T4 was completely removed. Following the dissection of the superficial and deep muscles of the back, a laminectomy of the T3 vertebra was performed to reveal the T4 spinal segment. In our model using a specific aneurysm clip (35 g force) was used for clamping and contusion. This clip was then rapidly released from the applicator to produce acute compression injury. The clip was left compressing the spinal cord for 1 minute before removal (42).

Following spinal cord compression injury, PRP gel was inserted directly on the dorsal surface, especially, the lacerated area of the spinal cord in the SCI +PRP group. In all groups, the muscle tissue and skin were then sutured in layers and baneocin powder (bacitracin and neomycin, Bayer, Co.) was applied to the wound soon after the operation and on every morning till healing occurred.

Postoperative care

After anesthesia and SCI surgical procedure rats require specialized veterinary care (43). Rats received an antibiotic (Cephotaxime, 10 mg /kg s.c.) twice daily until no sign of urinary infection (noted by cloudiness of expressed urine) was seen for three consecutive days. Animals also received analgesic (Ibuprofen, 50mg kg/day s.c.) for 3 days and saline solution (5ml of 0.9% saline s.c.) to provide hydration until the rat could drink following the surgery. Manual emptying of the urinary bladder was carried out 3 times a day until the rats developed automatic bladder emptying (about 2 weeks post-transection in our study). In the sham-operated group, rats were exposed to the same surgical and postsurgical manipulation without spinal cord compression. The room temperature was maintained at 25–28°C and rat core temperature was measured regularly until a normal temperature was maintained and then the room temperature was reduced to approximately 22°C. Rats were weighed daily and monitored for skin lesions or swelling.

Colorectal distension

The colorectal distension (CRD) balloon was made from a latex balloon tied to Tygon tubing extended into the balloon approximately 6 cm. Rats were deprived of food but had free access to water 24 h before the experiment. The prepared colorectal distension balloon covered with lubricant was inserted into the descending colon and rectum via the anus under ether sedation. With the end of the balloon positioned 1 cm inside the rectum (the tube was previously marked before anal insertion), the flexible catheter was taped to the base of the tail to prevent displacement (44-45). The tube of the balloon was connected via a Y connector to an air pump and a sphygmomanometer (pressure control device that regulated inflation of the balloon and provided a measure of intracolonic pressure). The balloon that was inserted into the rat rectum was inflated with air and the pressure inside the balloon was continuously monitored and incremented at an approximate speed of 1 mmHg/s to a final pressure of 25 mmHg. The responses to CRD were recorded in restrained manners. The technique involved a 1 min inflation of a colorectal balloon to trigger autonomic dysreflexia (40).

Weekly, mean arterial pressure (MAP) and heart rate (HR) were recorded in response to CRD following 2 hours period of prerecording acclimatization to the restrain. During this period, animals were put into a glass box. After this period, the animals were anesthetized with diethyl ether (40 mg/kg). Then, the rat tail was placed inside the tail-cuff and the cuff was inflated and released a few times to allow the animal to be conditioned to the procedure. MAP values and HR were recorded by a tail sphygmomanometer (NIBP200A, Biopac systems Inc; USA). When rats were more relaxed in their environment, as indicated by a stable HR and MAP, the average response was calculated from three distensions made during the

recording period. The entire cardiovascular response to 1, and 10-min periods following the distension, was recorded and the average peak changes in MAP and HR were calculated (40). The procedure of MAP and HR recording started from the day of surgery (prior to the operative procedure as baseline values) and then, recordings were weekly monitored for the successive 6 weeks.

Temperature recording

A rectal thermometer was used for the recording of core temperature in intact animals (before surgery), then the recording of core temperature was performed twice weekly, one for the recording of basal temperature and the other in response to colorectal distension.

ECG Recording

ECG was recorded in intact rats prior to surgery, and weekly post-surgery. Final ECG recording was done at the end of the study with and without CRD. Needle electrodes were placed under the skin of the 4 limbs of the anesthetized animal, near the paws, and connected to an ECG recorder (Cardimax Fx-2111, Fukuda Denshi Co., Ltd., Japan). Measurements were made for heart rate, R-wave voltage, duration of P-R interval, QRS complex, and the observed Q-T interval (Q-TO). Also, the observed Q-T interval (Q-TO) was corrected for the effect of heart rate (Q-Tc) as follows:

$$\text{Corrected } Q-T\left(Q-T_C\right)=\frac{\text{Observed } Q-T\left(Q-T_O\right)}{\sqrt{R-R \text{ in seconds}}}$$

Blood Sampling

After ECG recording, blood samples were collected from retro-orbital plexus with the help of a capillary tube into a heparinized plastic tube which was then centrifuged at 4000 rpm for 10 minutes to separate plasma. The plasma was then pipetted into clean storage tubes and stored at -20○C for later determination of triglycerides, total plasma cholesterol, and HDL-cholesterol.

Biochemical analysis

1) Plasma lipid profile

Plasma triglycerides (TGs) were assayed using a TG reagent kit (Bayer). Plasma cholesterol was determined in individual rats using a cholesterol reagent kit (Bayer), a direct HDL-cholesterol kit (Bayer), and an LDL-cholesterol kit (Bayer) according to Richmond (1973) (46). All parameters were measured spectrophotometrically with a UNICO 7200 Spectrophotometer (UV-1601PC, Shimadzu and Kyoto, Japan. The atherogenic index was calculated by using the following formula: log10 (TG/HDL-C) (47).

2) Measurement of spinal cord tissue TGF-β and MDA levels

Spinal cord tissue homogenate was prepared with 0.1 M phosphate buffer solution and centrifuged at 12,000 g for 10 min. The supernatant was used to determine TGF-β levels using East Bio-Pharm (China) ELISA kits according to Flanders et al (2016) (48) and MDA levels in tissue were measured by the colorimetric method according to Ohkawa (49) using kits supplied by Bio-diagnostic, Egypt.

Statistical Analysis

All values are expressed as mean ± SEM. Statistical analysis was conducted using the Graphpad Prism 6 software. The comparison of all data was performed by one-way analysis of variance (ANOVA) except data for the mean arterial pressure, heart rate, and temperature which were analyzed using two-way ANOVA, all statistical tests were followed by Tukey's post-hoc test. p-value <0.05 was considered statistically significant.

Results

By the end of our experiments, two rats in the SCI group and two rats in the group SCI-PRP were excluded from the study because they died. Mortalities were related to persistent urinary tract infection.

Bodyweight gain and biochemical analysis (Table 1)

Measurements of the rats' body weight revealed a significant increase in the SCI group compared to the sham-operated, and SCI-PRP treated groups. The increase in the body weight observed in SCI was

blunted in the SCI-PRP treated group leading to a nonsignificant difference compared to the sham-operated.

Analysis of serum lipid profile 6 weeks post-surgery in SCI group, underscored marked elevation in the plasma levels of triglycerides, total cholesterol, LDL as well as atherogenic index in addition to a significant reduction in HDL level compared to the sham control group. Treating SCI with PRP reverted significantly the aforementioned lipid profile compared to the SCI group. While PRP treatment normalized HDL-cholesterol in the SCI- PRP group to a comparable value compared to the sham controls. On the contrary, TGs, total and LDL cholesterol plasma levels, and atherogenic index of plasma in the SCI- PRP group showed a persistent significant elevation compared to the sham control group.

Moreover, there were no significant differences between SCI and sham control groups regarding tissue levels of TGF-β1 and MDA at the end of the study (6 weeks post-surgery). On the other hand, tissue levels turned out to be significantly higher regarding TGF-β1 and lower regarding MDA in the SCI- PRP group compared to the SCI group and sham control group.

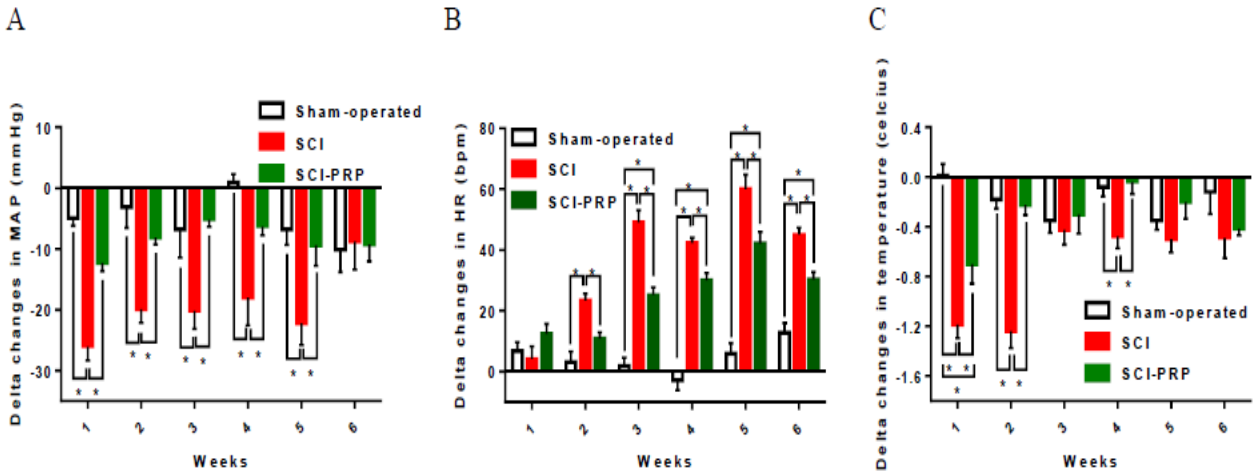

Figure 1: Impact of SCI and PRP treatment on the delta changes from intact pre-surgical value under basal condition in the (A) MAP, (B) heart rate, and (C) core temperature. Data represent mean ± SEM. Significance * $p < 0.05$ between different group by two-way ANOVA using Tukey's multiple comparison post-test. MAP: mean arterial pressure, HR: heart rate, SCI: spinal cord injury, SCI-PRP: spinal cord injury treated with platelet rich plasma.

Table (1): Mean ± SEM values of body weight gain (BWG), spinal cord tissue levels of TGF-β, MDA, and plasma levels of TGs, total cholesterol, LDL-cholesterol, and HDL- cholesterol as well as atherogenic index in different studied groups.

	Sham group (n=10)	**SCI group (n=8)**	**SCI-PRP group (n=8)**
BWG (g)	23.6 ±4.2	31.8 ± 3.4[a]	27.5± 4.6
TGF-ß1 (ng/mL)	92.2±18.9	95.7±17.5	134.7±24. 3[ab]
MDA (µmol/L)	5.3±1.6	5.7±1.1	3.3±0.8 [ab]
TGs (mg/dL)	36.44±6.7	79.5±4.1 [a]	48.2±7.5 [ab]
Total cholesterol (mg/dL)	51.4±5.7	73.4±8.4 [a]	65.3±4.8 [ab]
HDL-cholesterol (mg/dL)	28.6±5.4	21.3±2.3 [a]	26.6±2.1[b]
LDL-cholesterol (mg/dL)	20.3±4.4	46.5±7.2 [a]	35.6±4.2 [ab]
Atherogenic index	0.104±0.01	0.57 ± 0.11 [a]	0.25±0.07 [ab]

a P<0.05 compared to the sham control group. b P<0.05 compared to spinal cord injury (SCI) group

Basal ECG changes: (Table 2)

ECG recordings revealed a significant increase of the HR in the SCI group compared to the sham control group, meanwhile, PRP treatment tended to reduce the HR, however, it was nonsignificant compared to the SCI group. Despite the reduction in the HR in the SCI-PRP, it remained significantly higher compared to the sham control group. Meanwhile, no significant changes were observed in QRS voltage among the studied groups. Calculation of Q-To duration showed a significant increase in the SCI group compared to the sham control group. PRP treatment notably reduced Q-To duration compared to the SCI group and made its value nonsignificant compared to the sham control group.

Table (2): Mean ± SEM values of basal ECG changes in different studied groups

	Sham group (n=10)	**SCI group (n=8)**	**SCI-PRP group (n=8)**
Heart rate (bpm)	315 ±23.13	373 ±14.27 [a]	350 ±12.33 [a]
QRS voltage (µv)	290 ±27	277 ±33	284 ±18
Q-To (msec)	83.38 ±7.21	117.27 ±8.20 [a]	90.72 ±9.63 [b]

a P<0.05 compared to the sham control group. b P<0.05 compared to spinal cord injury (SCI) group

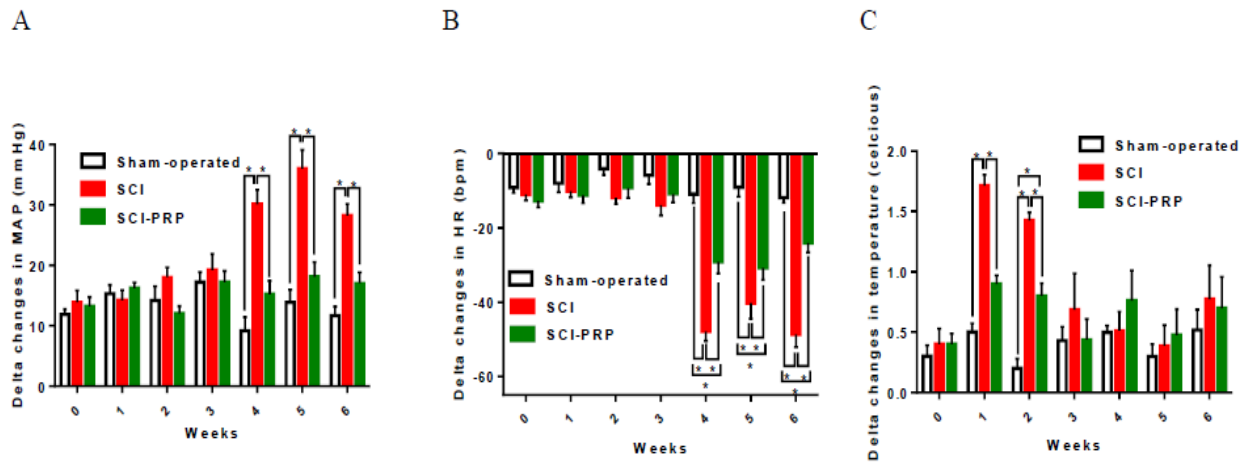

Figure 2: Impact of SCI and PRP treatment on the delta changes in the (A) MAP, (B) heart rate, and (C) core temperature following CRD. Data represent mean ± SEM. Significance * p < 0.05 between different group by two-way ANOVA using Tukey's multiple comparison post-test. MAP: mean arterial pressure, HR: heart rate, SCI: spinal cord injury, SCI-PRP: spinal cord injury treated with platelet rich plasma.

ECG changes in response to CRD

ECG recordings conducted before surgery in response to CRD revealed a normal sinus rhythm in intact rats of all groups. Indeed, there was a high incidence of supraventricular, ventricular tachycardic paroxysms, and ectopic beats in response to CRD in the SCI group. Percentage incidence of rats affected by supra-ventricular arrhythmias in the SCI group was (25%, 25%, 12.5%, 12.5%, 25%, 12.5% respectively from the 1st week to the 6th week post-surgery) and (37.5%, 12.5%, 25%, 37.5%, 25%, 12.5%) of rats were affected by ventricular arrhythmias. Meanwhile, the SCI-PRP group showed a marked reduction of the supra-ventricular arrhythmias in the 1st (12.5%), 3rd (0%), 5th (12.5%) weeks and disappeared in the 6th week, and a less remarkable incidence of ventricular arrhythmias in the 1st (12.5%), 4th (12.5%) weeks and no longer exist in the final two weeks post-surgery. Whilst, the incidence of arrhythmias was similar in SCI and SCI-PRP groups in the 2nd and 4th weeks post-

surgery regarding supra-ventricular arrhythmia and the 2nd and 3rd weeks regarding ventricular arrhythmia. Interestingly, the sham control group didn't show any signs of arrhythmia in all recordings starting from the 1st-week to the 6th-week post-injury.

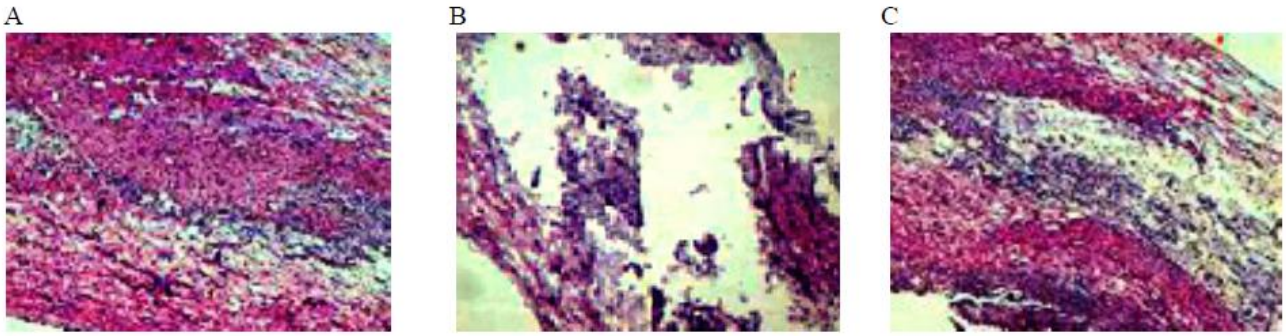

Figure 3: Sections of (A) sham control. Spinal cord was intact with no injury, cavities or inflammation. (B) SCI group showed a clear and wide vacuolization of the injured spinal cord in addition to the appearance of some inflammatory cells in the field together with some cell debris within the cavities. (C) In rats of the SCI-PRP group, the gaps in the SCI region became narrower than those of the SCI group with less inflammatory response and cell debris. SCI: spinal cord injury, SCI-PRP: spinal cord injury treated with platelet rich plasma.

Study of mean arterial pressure (MAP) delta changes from intact pre-surgical value under basal condition (Figure 1A)

During the first 5 weeks of the recovery period, the drop in the MAP was marked compared to the pre-surgical level in the SCI group. The maximal reduction of MAP in SCI and SCI-PRP groups was at the 1st-week post-surgery compared to the initial presurgical values. In the SCI-PRP group, delta change of the lowered MAP was significantly reduced compared to SCI and became near to normal values during the same period (from the 1st to 5th-week post-injury). On the 6th week of the recovery period, the drop in the MAP was corrected to the presurgical level and the delta change of MAP was nonsignificant among all groups.

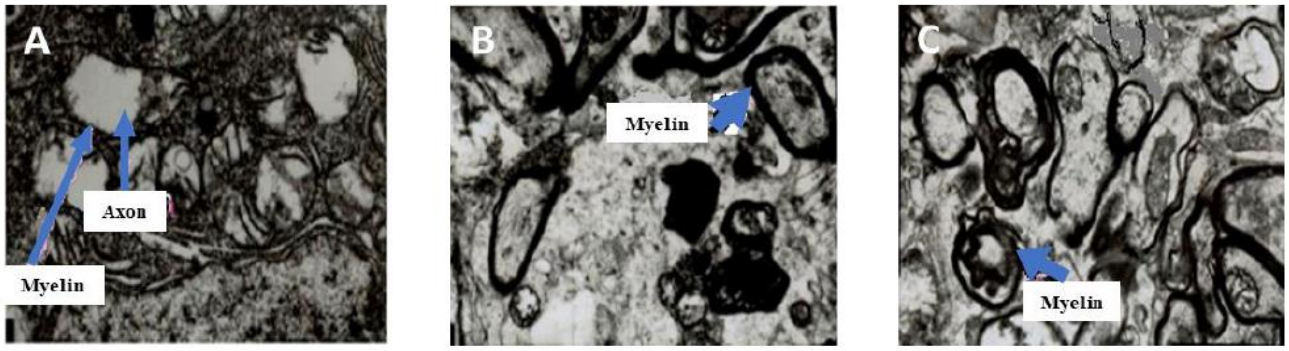

Figure 4: Electron microscopic (EM) micrographs showing axons, myelin and extra-axonal space of spinal cord sections in (A) sham-operated, (B) SCI, and (C) SCI-PRP groups. Section of rat SC subjected to SCI and followed by platelet rich plasma (SCI-PRP) showed dense and complete myelination, less intra-axonal cavities and vacuolization and regenerating more numerous axons compared with those in the SCI group. Mitochondria are commonly observed in the axoplasm of regenerating axons in the PRP group unlike those of SCI group. SCI: spinal cord injury, SCI-PRP: spinal cord injury treated with platelet rich plasma.

Study of HR delta changes from intact pre-surgical value under the basal condition: (Figure 1B)

HR values were increased and delta changes of HR from intact presurgical values were significant starting from the 2nd-week post-surgery in the SCI

group and the 3rd week in the SCI-PRP group to the end of the study compared to sham control values. Such tachycardic changes in the SCI-PRP group were significantly reduced compared to the SCI group from the 2nd week to the end of the recovery period i.e., 6th week, however, remained significantly higher than the sham group.

The core temperature of the sham control group was relatively stable all through the 6 weeks duration of the study. Meanwhile, SCI group core temperature dropped significantly, and delta changes were significant in the first, second, and 4th weeks post-surgery compared to the sham control group. On the other hand, statistical analysis revealed that the delta changes decreased significantly in the SCI-PRP group compared to the SCI group in such weeks. While SCI-PRP rats' core temperature markedly dropped during the first week following injury (from 36.9 to 36.2°C) and delta change was significant compared to the sham control group, normal core temperature was restored by the 2nd-week post-surgery.

Changes in rats' MAP in response to CRD: (Figure 2A)

Delta changes of the MAP in all groups of rats in response to CRD were nonsignificant compared to each other in intact animals. Interestingly, our data revealed an enhanced response in the form of elevated blood pressure in the SCI group, especially during the chronic phase of injury which started from the 4th week and extended to the 6th week of the study. During the first 3 weeks following SCI, rats exhibited MAP responses similar to that of intact animals and delta changes of MAP in response to CRD were nonsignificant compared to those of sham controls. In contrast, during the 4th to the 6th week following injury, the MAP delta changes to CRD were significantly higher than those of the sham control rats.

Regarding the SCI-PRP group, our results showed also nonsignificant differences in the delta changes of the MAP in response to CRD in the initial 3 weeks post-injury compared to SCI and sham control groups. Interestingly, the delta changes in the MAP during the last 3 weeks (4th – 6th) of the recovery period were significantly lower in the SCI-PRP group compared to those observed in the SCI group and divulged comparable values to their corresponding sham control group.

Changes in rats' HR in response to CRD: (Figure 2B)

Statistical analysis revealed nonsignificant delta changes of HR in response to CRD in the intact and during the initial 3 weeks of the study among different studied groups. However, marked bradycardic responses to CRD were observed in SCI and SCI-PRP groups in response to CRD compared to corresponding sham control results during the last 3 weeks post-surgical. The post-injury peak changes of HR in response to CRD were more evidenced in the SCI group. On the other hand, the SCI-PRP group showed significantly lower HR delta changes compared to the SCI group during the last 3 weeks although it remained significantly higher compared to the sham control group.

Changes in rats' core temperature in response to CRD: (Figure 1C)

In intact rats, as well as in the last 3 weeks post-surgery (3rd – 6th), animals exhibited mild increases in core temperature in response to CRD with nonsignificant differences in delta changes in all studied groups. The only significant hyperthermic responses to CRD in the SCI group were those reported during 1st and 2nd weeks post-compression injury. Such hyperthermic responses to CRD observed in the initial two weeks post-surgery were significantly reduced in the SCI-PRP group compared to the SCI group, however, not normalized, and remained significantly higher compared to the sham control group during the 2nd week.

Discussion

It is well established that SCI disturbs the physiological regulation of the cardiovascular system. However, little is known about the temporal progression of such disturbances from the acute to the chronic stages of the SCI. In our study, different cardiovascular parameters were recorded as intact values prior to SCI followed by weekly recordings for 6 successive weeks after injury. Each recording was taken as a basal value and in response to CRD as well.

Results revealed a significant lowering in the MAP following the induction of SCI compared to the intact pre-surgical value. The fall in the MAP was maximum one week following SCI (about 26 mmHg) and remained to the 5th-week post-surgery. This observation was in agreement with the literature that suggested a lowered sympathetic activity in rats experiencing SCI during the acute phase as a part of generalized depression of the spinal reflexes, a well-established phenomenon known as spinal shock, which occurs during this period (50). Moreover, the observed hypotension might be due to the loss of tonic vasoconstriction below the level of the lesion which was usually maintained by supra-spinal structures (51) in addition to the inability of the nerves to regulate arterial blood pressure (52). Besides, a lack of skeletal muscle pumping action (53) cardiovascular deconditioning (54), lowered noradrenaline (55), and/or altered salt and water balance (56) had also been hypothesized to contribute to the hypotension.

This opinion was supported by the previous clinical experiment conducted by Gao and his coworkers (57) which showed that cardiovascular complications were believed to be common in quadriplegia because injuries in the cervical or upper thoracic regions disrupted the control of sympathetic outflow to major vascular beds and thus impair blood pressure regulation. Earlier reports also observed that following a high SCI, patients usually develop long-lasting hypotension due to decreased level of sympathetic activity (55).

Our data underscored the recovery of the MAP in the SCI group to the pre-surgical value during the 6th-week, and the loss of significant difference compared to the sham control group. The recovery of the normal basal MAP levels at the end of the study may be due to some descending pathways that were left intact after spinal cord (SC) clip compression as demonstrated by the EM picture together with the partial subside of SC edema and inflammation that followed the injury (Figure 4).

Additionally, another factor that could potentially assist in normalizing the hypotension during the last week of recovery was the HR, since the autonomic control of the heart was still intact (both the vagal control and the sympathetic component that exits the spinal cord from (T1–3) (55). Interestingly, the HR was elevated following SCI starting from the 2nd-week post-surgery and reached its maximal level during the last two weeks of recording (the fifth and sixth weeks). Such tachycardia served to compensate and counter the hypotension that developed in these animals. Tachycardia may be mediated, in part, by a decrease in the vagal drive as well as an increase in sympathetic excitation to the heart which remained intact despite SCI (40).

Another consequence of the SCI was the significant fall in core temperature, particularly during the first 2 weeks following the compression injury. The initial marked fall in core temperature in the SCI group compared to the sham control group was probably induced by the decrease in the sympathetic activity due to the spinal shock condition. The decreased sympathetic activity would have led to a loss of vasoconstrictor tone in cutaneous blood vessels and thus increased skin blood flow, causing heat loss through dissipation to the colder ambient air (40). Moreover, the dip in the body temperature was previously reported to be due to a loss of the adaptive control of the blood

vessel's diameter in response to the environmental changes. Thus, permanently expanded blood vessels lose heat more quickly rendering the rats more liable for hypothermia (58).

High-level SCI was found to impair thermoregulation because it disconnects the thoracic spinal cord from the central thermoregulatory centers thus preventing central control of cutaneous blood flow, sweating, piloerection, and shivering (59). Therefore, spinal patients have a greater susceptibility to changes in core temperature resulting from environmental temperature fluctuations, exercise, or infection (60-61).

In our study and under basal condition, hypothermia was particularly marked in the SCI group on the first 2 weeks after compression injury but improved to some degree during the last weeks of the experiment although it showed a mild significant decrease compared to the sham control group during the 4th-week recording. In the SCI group, delta changes of core temperatures from intact values were insignificant in the last weeks of injury compared to the sham control group.

The partial recovery of core temperature may have been due to the resolution of spinal shock. Another explanation for the partial recovery of core temperature is a compensatory increase in thermogenesis, perhaps mediated by the interscapular brown adipose tissue. Like the heart, the interscapular brown adipose tissue is controlled by sympathetic preganglionic neurons located in the thoracic spinal cord above the level of injury (62). Thus, interscapular brown adipose tissue sympathetic discharge up-regulation might have played an important role in compensating for the heat lost from cutaneous territories located below the lesion (40).

Regarding body weight changes, our finding of increased body weight gain might be caused by immobilization of rats exposed to the SCI compared to the sham control group. Increased body weight and subsequent expected subcutaneous fat deposition (thermal insulator) might add a further explanation for the partial recovery of core temperature at the last weeks of recovery.

In our experimental setup, further to the persistent hypotension observed in the SCI group, bouts of hypertension were found upon exposing rats of this group to colorectal distension (CRD), a condition known as autonomic dysreflexia (AD) (63). The analysis of the time course of development of AD revealed that it took 4 weeks for full hypertension to occur in SCI rats. MAP, indicative of AD, was progressively increased in the SCI group of rats compared to the pre-surgical value. The full picture of hypertension was observed in the last 3 weeks of the experiment (4^{th} - 6^{th} week) and recording of MAP during this period showed that values of MAP became significantly high compared to the sham control group. Thereby, responses to CRD started from the 4^{th}-week post-injury onward regarding hypertensive response and the 3^{rd} week post-injury onward regarding bradycardic response. In agreement with our results, AD was reported in patients with SCI above T6. They regularly experience life-threatening bouts of extreme hypertension that were accompanied by pronounced bradycardia (64).

During AD, noxious stimuli below the lesion, such as bowel or bladder distension, could cause exaggerated activation of the spinal circuits caudal to the injury that project to the sympathetic pre-ganglionic neurons. In turn, this usually triggers a sympathetically mediated peripheral vasoconstriction in the gut, muscle, and skin vascular beds causing extreme systemic hypertension (65-66). Finally, parasympathetic-induced bradycardia could be present during episodes of AD as a baroreflex-mediated response to hypertension (64)

Interestingly, in our experimental setup, although there was spontaneous hypotension recorded in basal conditions, especially in acute phases of SCI which became recovered after 6 weeks post-injury, MAP was found to increase in the last weeks post-surgery in response to CRD which is characteristic to AD. It is

generally accepted that loss of supraspinal input to the spinal sympathetic circuits, reduced overall sympathetic activity, disruption of spinal reflexes, and plastic changes in the spinal cord and peripheral autonomic circuits, all contribute to hypotension and the development of AD as well (67). Sympathetic hypoactivity following high thoracic or cervical SCI causes a reduction in circulating levels of epinephrine and norepinephrine (NE) (68) which might be, later, compensated for by hypersensitivity to vasoconstrictor substances. Indeed, tetraplegics have an enhanced pressor response to intravenous NE infusions and phenylephrine (69). The pressor response was not associated with increased plasma NE, renin, aldosterone, vasopressin, or arginine, suggesting such a response was due to adrenoceptor hypersensitivity (70). Indeed, the delay in the development of AD may be related to the time course of formation of increased afferent arbors in the lower spinal cord, under the influence of increased intraspinal nerve growth factor levels following the trauma (71).

ECG study data of this work exhibited long Q-To in the SCI group under basal condition and 6 weeks post-injury. Such observation indicated higher excitability of hearts of this group. Herein, increased incidence of ventricular and supra-ventricular ectopic beats and /or tachycardia in response to CRD may support the suggestion of increased cardiac excitability. Altered cardiac sympathetic activity in basal conditions in addition to increased adrenoceptor sensitivity after the appearance of AD might contribute to the appearance of such complication.

Moreover, the lipid profile of the SCI group of rats revealed significant elevation of plasma levels of TGs, total and LDL- cholesterol as well as lowered HDL- cholesterol level and increased atherogenic index compared to the sham control rats. Such lipid profile was probably accounted for by immobilization of rats for 6 weeks after SCI and might share in deteriorating the cardiac condition. Additionally, the high atherogenic index might contribute to the increased blood pressure to the normal value at the end of the study, after being hypotensive all through the period of basal recordings.

Hyperthermia was also an early manifestation of AD in response to CRD in SCI rats. The significantly increased rectal temperature in the first 2 weeks in this group of rats in response to CRD had preceded hypertension which started significant elevation from the 4th week following SCI onward (Figure 2A and C). Such observation would aid the care of spinal patients just after the appearance of hyperthermia as an early sign of AD, by allowing earlier elimination of the triggering stimuli and avoiding the use of vasoactive drugs. Whilst rats of the SCI group showed normothermic response to CRD like the sham control group during the late weeks post-injury although AD became more prominent. Interestingly, in a clinical consideration, during this period, increased sympathetic cholinergic activity can cause excessive sweating which can contribute to a lowering of body temperature. Consistent with our results, it was reported that episodes of AD were known to challenge thermoregulation and lead to severe hyperthermia (72) and early elevation of core temperature in response to CRD (33).

On the other hand, in this study, the SCI-PRP group showed that cardiovascular complications of SCI as well as disorders of AD were improved considerably due to partial healing of SC demonstrated by histopathological and transmission EM examinations (Figure 3 and 4). This study demonstrated that PRP was able to accelerate the healing of the injured spinal cord. The number of axons in the SCI-PRP group was more pronounced than that in the SCI group, together with nearly complete myelination of regenerating axons. In addition, in rats of the SCI-PRP group, the cavities in the SCI region became smaller than those of the SCI group. In accordance with our results, in tissue culture, PRP was shown to promote axon growth in spinal cord

tissue (73-74). Moreover, other reports also precluded that the application of PRP promotes axon regeneration in animal models and assists to promote remyelination of peripheral nerves (73,75-77) followed by all the subsequent steps of tissue remodeling, wound repair, and axon regeneration. (78).

Data of this work revealed that hypotension (delta MAP) observed in SCI rats was significantly reduced in SCI- PRP group starting from 1st-week post-injury to the 5th-week. Delta changes of MAP were reduced in SCI- PRP group to nonsignificant values compared to the sham control group during this period.

Regarding HR changes in the SCI-PRP group under basal conditions, there was also a significant reduction of tachycardia (delta change from intact pre-injury values) compared to SCI rat values that became normalized at the 2nd-week post-injury only and remained significantly higher compared to the sham control values afterward (Figure 1B). Indeed, reduced tachycardia in the SCI-PRP group could be secondary to the correction of MAP, as tachycardia was essentially a baro-response to hypotension observed in the SCI group.

Meanwhile, the study of core temperature under basal condition revealed that hypothermia pronounced in SCI rats in 1st and 2nd weeks post-surgery was significantly decreased in the SCI-PRP group. Starting from the 2nd week till the end of the experiment, hypothermia was completely abolished, and insignificant changes were present between the SCI-PRP group and the sham control group. Partial recovery of body temperature in the PRP enriched group was ascribed to the incomplete regeneration of the spinal cord and the subsequent repair of sympathetic activity.

Concerning the study of MAP, HR, and rectal temperature changes in response to CRD, MAP was markedly increased in the SCI group compared to the sham control group. However, rats of the SCI-PRP group showed significantly decreased delta changes compared to the SCI group. Thereby, hypertensive response to CRD was normalized in the group treated by PRP in the last 3 weeks of the study. Exaggerated hypertensive response to CRD is characteristic of AD that was pronounced in the SCI group. Sympathetic hypersensitivity due to dropped sympathetic activity was a direct promoting factor for such dysreflexia observed maximally in the chronic phase of SCI. Owing to accelerated healing of SC by the use of PRP gel, AD parameters started to fade and blood pressure gain in response to CRD in the SCI-PRP group tended to be similar to the sham control group. Likewise, bradycardic responses to CRD (delta HR) showed a significant decline in the SCI-PRP group compared to the SCI group in the final 3 weeks records, however, such values of delta HR were not normalized and remained significantly high compared to the sham control group. We speculated that the partially ameliorated HR response to CRD in the SCI-PRP group was secondary to the recovered hypertensive response. Indeed, both parameters gave a piece of evidence for the tendency of AD recovery in SCI rats subjected to PRP.

A study of changes of core temperature in response to CRD revealed that SCI-PRP significantly lowered delta changes from intact pre-surgical values in the first 2 weeks following SC injury compared to the SCI group. This finding provided further support to AD partial recovery in the SCI-PRP group. Nevertheless, values of core temperature delta changes remained significantly elevated compared to the sham control group. Interestingly, Hyperthermic response to CRD in the initial weeks post-injury is considered an early sign of AD and appeared in the acute phase of SCI although the complete picture of AD became more pronounced in the chronic phase of injury in the form of hypertension associated with bradycardia as responses to CRD.

Protection of PRP against cardiovascular complications extended to shorten Q-To interval duration under basal condition and to abolish ventricular/supra-ventricular ectopic beats and/or

tachycardia in response to CRD. In addition, it significantly reduced plasma TGs, total and LDL-cholesterol compared to the SCI group. Normalization of these values, as well as increasing HDL- cholesterol, reducing atherogenic index compared to the SCI group, renovated a notion that the application of PRP partially protected against cardiovascular sequelae and AD disruptions of SCI. Such protective effects might be attributed to their healing effects on SC after its compression injury, in addition to the antioxidant effect of PRP which was proved in this work by the significantly reduced oxidative stress marker, MDA compared to other groups. Improved lipid profile and body weight gain in the SCI-PRP group in our study may be due to better mobilization of the animal as a consequence of partial SC repair.

Our results of the SCI-PRP group revealed increased transforming growth factor ß1 (TGF ß1) the known growth factor that might mediate the SC tissue healing. Although many GFs are associated with wound healing, TGF-β1 appears to be one of the more integral modulators (79). TGF-β increases the production of collagen from fibroblasts (80), causes chemotactic attraction and activation of monocytes, macrophages, and fibroblasts. The activated fibroblasts enhance the formation of extracellular matrix and collagen and stimulate the cells' ability to contract the provisional wound matrix (81-82).

Consistently, it has been demonstrated that PRP provided a mean for growth factors delivery that exceeds those released by the body, thereby, accelerating the healing process (83). The potent restorative function of PRP is mainly based on the neurotrophic capacity of preparation rich in growth factors (GFs) which could be certified to ameliorate the pathological process of central nervous system diseases (84). Effects of PRP on acute SCI presented in our study were accounted for by the rapid release of GFs as approximately 70% of the stored GFs are released from active platelets within 10 minutes, and nearly 100% of the GFs are released within 1 hour (85). In addition, the sustained release of large quantities of growth factors, cytokines, and other mediators found in PRP may help to stimulate wound healing and resolve chronic inflammation (3) which explains the protective effects of PRP on chronic SCI and limited the cardiovascular and AD complications.

Additionally, other growth factors were reported to enhance local tissue healing upon using PRP such as platelet-derived growth factor, vascular endothelial growth factor, fibroblast growth factor, epidermal growth factor, hepatocyte growth factor, insulin-like growth factor 1, angiopoietin-2, and interleukin-1beta (86). The basic cytokines identified in platelets play important roles in cell proliferation, chemotaxis, cell differentiation, regeneration, and angiogenesis (86). Moreover, following trauma, platelets are the primary driving force behind the development of inflammation. Active platelets aggregate and release factors that promote vasodilation, increase blood flow, and increase capillary permeability (87-88). This increased permeability allows plasma proteins to move from the blood into the wound site, causing the initial phase of inflammation. Although platelets initially act to initiate and enhance trauma-triggered inflammation, subsequently, as the inflammatory process and necrotic tissue elimination are resolved, platelets take on an anti-inflammatory role in releasing enzyme inhibitors within wounds, as well as suppressing cytokine release and promoting tissue proliferation and regeneration (89).

Conclusion:

The progression of cardiovascular impairments due to SCI is still far from being completely managed. However, this study is an important step towards clinical and preventative treatment following SCI, particularly for those with cardiovascular problems. Our work revealed that biologically active factors released by platelets within platelet-rich plasma which had neurotrophic and antioxidant properties conferred, at least partially, spinal injury healing process and

promoted axon and myelin regeneration. Reestablishment of nearly normal spinal structural and functional properties was the basis for the partial elimination of cardiovascular hazards of SCI as well as AD-related disruptions.

Conflict of Interest: The authors declare that they have no conflict of interest.

Ethical approval: All applicable national and institutional guidelines for the care and use of animals were followed. All animal studies have been approved by the Research Ethical Committee, Faculty of Medicine, Ain Shams University. All animal studies have been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

References

1- **Devivo MJ**: Epidemiology of traumatic spinal cord injury: trends and future implications. Spinal Cord; 50: 365–372, 2012.

2- **Krassioukov A**: Autonomic function following cervical spinal cord injury. Respir Physiol Neurobiol; 169: 157–164, 2009.

3- **Sell SA, Ericksen JJ, Reis TW, Droste LR, Bhuiyan MB, Gater DR**: A case report on the use of sustained-release platelet-rich plasma for the treatment for chronic pressure ulcers. J Spinal Cord Med; 34: 122–127, 2011.

4- **Krassioukov A**: Autonomic function following cervical spinal cord injury. Respir Physiol Neurobiol; 169: 157–164, 2009.

5- **Wu JC, Chen YC, Liu L, Chen TJ, Huang WC, Cheng H, Tung-Ping S**: Increased risk of stroke after spinal cord injury: a nationwide 4-year follow-up cohort study. Neurology; 78: 1051–1057, 2012.

6- **Bilello JF, Davis JW, Cunningham MA, Groom TF, Lemaster D, Sue LP**: Cervical spinal cord injury and the need for cardiovascular intervention. Arch Surg; 138: 1127–1129, 2003.

7- **Krassioukov A, Warburton DE, Teasell R, Eng JJ**: A systematic review of the management of autonomic dysreflexia after spinal cord injury. Arch Phys Med Rehabil; 90: 682–695, 2009.

8- **Karlsson AK**: Autonomic dysfunction in spinal cord injury: clinical presentation of symptoms and signs. Prog Brain Res; 152, 1–8, 2006.

9- **Krassioukov AV, Furlan JC, Fehlings MG**: Autonomic dysreflexia in acute spinal cord injury: an under-recognized clinical entity. J Neurotrauma; 20, 707–718, 2003.

10- **Marsh DR, Wong ST, Meakin SO, MacDonald JI, Hamilton EF, Weaver LC**: Neutralizing intraspinal nerve growth factor with a trkA-IgG fusion protein blocks the development of autonomic dysreflexia in a clip-compression model of spinal cord injury. J Neurotrauma; 19, 1531–1541, 2002.

11- **Marsh DR, Weaver LC**: Autonomic dysreflexia, induced by noxious or innocuous stimulation, does not depend on changes in dorsal horn substance P. J Neurotrauma; 21, 817–828, 2004.

12- **Mazwi NL, Adeletti K, Hirschberg RE**: Traumatic Spinal Cord Injury: Recovery, Rehabilitation, and Prognosis. Curr Trauma Rep; 1, 182–192, 2015.

13- **Salarinia R, Sadeghnia HR, Alamdari DH, Hoseini SJ, Mafinezhad A, Hosseini M**: Platelet rich plasma: Effective treatment for repairing of spinal cord injury in rat. *Acta Orthop Traumatol Turc*; 51(3):254-257, 2017

14- **Dawood AS, Salem HA**: Current clinical applications of platelet-rich plasma in various gynecological disorders: An appraisal of

theory and practice. Clin Exp Reprod Med.;45(2):67-74, 2018.

15- **Mei-Dan O, Laver L, Nyska M, Mann G**: Platelet rich plasma--a new biotechnology for treatment of sports injuries. Harefuah;150(5):453-7, 490, 2011

16- **Marx RE**. Platelet-rich plasma (PRP): what is PRP and what is not PRP? Implant Dent; 10: 225–, 2001

17- **Hayenesworth SE, Kadiyala S, Liang L, Bruder SP**: Mitogenic stimulation of human mesenchymal stem cells by platelet releasate suggests a mechanism for enhancement of bone repair by platelet concentrates. Transactions of the 48th Annual Meeting of the Orthopaedic Research Society. Dallas, poster no. 462., February 2002

18- **Liu Y, Kalén A, Risto O, Wahlström O**: Fibroblast proliferation due to exposure to a platelet concentrate in vitro is pH dependent. Wound Repair Regen; 10:336–340, 2002.

19- **Jo CH, Roh YH, Kim JE, Shin S, Yoon KS**: Optimizing platelet-rich plasma gel formation by varying time and gravitational forces during centrifugation. J Oral Implantol; 39:525–532, 2013.

20- **International Cellular Medicine Society**. Platelet Rich plasma (PRP) guidelines [Internet] Las Vegas: International Cellular Medicine Society; Available from: http://www.cellmedicinesociety.org/icms-guidelines/guidelines, 2011.

21- **Everts PA, Hoogbergen MM, Weber TA, Devilee RJ, van Monftort G, de Hingh IH**: Is the use of autologous platelet-rich plasma gels in gynecologic, cardiac, and general, reconstructive surgery beneficial? Curr Pharm Biotechnol; 13:1163–117, 2012.

22- **Lai LP, Stitik TP, Foye PM, Georgy JS, Patibanda V, Chen B**: Use of platelet-rich plasma in intra-articular knee injections for osteoarthritis: a systematic review. PM R.; 7:637–648, 2015

23- **Rendu F, Brohard-Bohn B**: The platelet release reaction: granules' constituents, secretion and functions. Platelets;12(5):261-273, 2001.

24- **Anitua E, Aguirre JJ, Algorta J, Ayerdi E, Cabezas AI, Orive G, Andia I**: Effectiveness of autologous preparation rich in growth factors for the treatment of chronic cutaneous ulcers. J Biomed Mater Res B Appl Biomater; 84: 415–421, 2008.

25- **Frykberg RG, Driver VR, Carman D, Lucero B, Borris-Hale C, Fylling CP, Rappl LM, Clausen PA**: Chronic wounds treated with a physiologically relevant concentration of platelet-rich plasma gel: a prospective case series. Ostomy Wound Manage; 56: 36–44, 2010.

26- **Driver VR, Hanft J, Fylling CP, Beriou JM, Autologel Diabetic Foot Ulcer Study Group**: A prospective, randomized, controlled trial of autologous platelet-rich plasma gel for the treatment of diabetic foot ulcers. Ostomy Wound Manage; 52:68–87, 2006.

27- **Eppley BL, Pietrzak WS, Blanton M:** Platelet-rich plasma: a review of biology and applications in plastic surgery. Plast Reconstr Surg; 118: 147e–159e, 2006.

28- **Dhillon RS, Schwarz EM, Maloney MD**: Platelet-rich plasma therapy: future or trend? Arthritis Res Ther.;14:219, 2012.

29- **Behnia-Willison F, Nguyen TTT, Norbury AJ, Mohamadi B, Salvatore S, Lam A**: Promising impact of platelet rich plasma and carbon dioxide laser for stress urinary incontinence. Eur J Obstet Gynecol Reprod Biol X.; 5:100099, 2019.

30- **Metz GA, Curt A, van de Meent H, Klusman I, Schwab ME, Dietz V**: Validation of the weight-drop contusion model in rats: A comparative study of human spinal cord injury. J Neurotrauma; 17:1–17, 2000.

31- **McTigue DM, Popovich PG, Jakeman LB, Stokes BT:** Strategies for spinal cord injury repair. Prog Brain Res; 128:3–8, 2000.

32- **Norenberg MD, Smith J, Marcillo A**: The pathology of human spinal cord injury: Defining the problems. J Neurotrauma; 21:429–140, 2004.

33- **Fleming JC, Norenberg MD, Ramsay DA, Dekaban GA, Marcillo AE, Saenz AD, Pasquale-Styles M, Dietrich WD, Weaver LC**: The cellular inflammatory response in human spinal cords after injury. Brain; 129:3249–3269, 2006.

34- **Krishna V, Andrews H, Jin X, Yu J, Varma A, Wen X, Kindy M**: A contusion model of severe spinal cord injury in rats. J Vis Exp; (78):50111, 2013.

35- **Anitua E, Andia I, Ardanza B, Nurden P, Nurden AT**: Autologous platelets as a source of proteins for healing and tissue regeneration. Thromb Haemost; 91(1):4-15, 2004.

36- **Simman, RD, Hoffmann A, Bohinc,R J MS, Peterson W C , Russ AJ**: Role of platelet-rich plasma in acceleration of bone fracture healing. Annals of Plastic Surgery; 61 (3): 337-344, 2008

37- **Girgin M, Binnetoglu K, Duman K, Kanat BH, Cetinkaya Z, Ayten R, Ilhan YS, Ilhan N, Seker I, Timurkaan N**: Effects of platelet rich plasma on fascial healing in rats with fecal peritonitis. Acta Cir Bras; 31(5):314-9, 2016.

38- **Cavallo C, Roffi A, Grigolo B, Mariani E, Pratelli L, Merli G, Kon E, Marcacci M, Filardo G**: Platelet-Rich Plasma: The Choice of Activation Method Affects the Release of Bioactive Molecules. BioMed Research International; Article ID 6591717, 7 pages, 2016.

39- Chen Nan-Fu, Sung Chun-Sung, Wen Zhi-Hong, Chen Chun-Hong, Feng Chien-Wei, Hung Han-Chun, Yang San-Nan, Tsui Kuan-Hao, Chen Wu-Fu: Therapeutic Effect of Platelet-Rich Plasma in Rat Spinal Cord Injuries. Frontiers in Neuroscience; 12: 252, 2018

40- **Laird AS, Carrive P, Waite PM**: Cardiovascular and temperature changes in spinal cord injured rats at rest and during autonomic dysreflexia. J Physiol; 577(Pt 2):539-548, 2006.

41- **Poon PC, Gupta D, Shoichet MS, Tator CH**: Clip Compression Model Is Useful for Thoracic Spinal Cord Injuries Histologic and Functional Correlates. Spine; 1;32(25):2853-9, 2013.

42- **Talac R, Friedman JA, Moore MJ, Lu L, Jabbari E, Windebank AJ, Currier BL, Yaszemski MJ**: Animal models of spinal cord injury for evaluation of tissue engineering treatment strategies. Biomaterials; 25:1505–1510, 2004.

43- **Santos-Benito FF, Muñoz-Quiles C, Ramón-Cueto A**: Long-term care of paraplegic laboratory mammals. J Neurotrauma; 23:521–536, 2006.

44- **Burton MB, Gebhart GF**: Effects of Kappa-Opioid Receptor Agonists on Responses to Colorectal Distension in Rats with and without Acute Colonic Inflammation. J Pharmacol Exp Ther; 285(2):707-15, 1998.

45- **Yang JP, Yao M, Jiang XH, Wang LN**: Establishment of model of visceral pain due to colorectal distension and its behavioral

assessment in rats. World J Gastroenterol; 12(17): 2781–2784, 2006.

46- **Richmond W**: Preparation and Properties of a Cholesterol Oxidase from Nocardia sp. and Its Application to the Enzymatic Assay of Total Cholesterol in Serum. Clinical Chemistry; 19 (12): 1350-1356, 1973.

47- **Nwagha UI, Ikekpeazu EJ, Ejezie FE, Neboh EE, Maduka IC**: Atherogenic index of plasma as useful predictor of cardiovascular risk among postmenopausal women in Enugu, Nigeria. Afr Health Sci; 10(3):248-252, 2010.

48- **Flanders KC, Yang YA, Herrmann M, et al**: Quantitation of TGF-β proteins in mouse tissues shows reciprocal changes in TGF-β1 and TGF-β3 in normal vs neoplastic mammary epithelium. Oncotarget;7(25):38164-38179, 2016.

49- **Ohkawa H, Ohishi N, Yagi K**: Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem; 95(2):351-8, 1979.

50- **Popa C, Popa F, Grigorean VT, et al**: Vascular dysfunctions following spinal cord injury. J Med Life.;3(3):275-285, 2010.

51- **Krassioukov A & Claydon VE**:The clinical problems in cardiovascular control following spinal cord injury: an overview. Prog Brain Res; 152, 223–229, 2006.

52- **West CR, Popok D, Crawford MA, Krassioukov AV**: Characterizing the temporal development of cardiovascular dysfunction in response to spinal cord injury. J Neurotrauma;32(12):922-930, 2015.

53- **Faghri PD, Yount J**: Electrically induced and voluntary activation of physiologic muscle pump: a comparison between spinal cord-injured and able-bodied individuals. Clin Rehabil; 16: 878–885, 2002.

54- **Vaziri ND**: Nitric oxide in microgravity-induced orthostatic intolerance: relevance to spinal cord injury. J Spinal Cord Med; 26: 5–11, 2003.

55- **Mathias CJ & Frankel HL** : Autonomic disturbances in spinal cord lesions. In Autonomic Failure: A Textbook of Clinical Disorders of the Autonomic Nervous System, ed.Mathias CJ & Bannister R, pp. Oxford University Press, Oxford; 494–513, 1999.

56- **Frisbie JH**: Postural hypotension, hyponatremia, and salt and water intake: case reports. J Spinal Cord Med 200; 27: 133–137, 2004.

57- **Gao SA, Ambring A, Lambert G & Karlsson AK**: Autonomic control of the heart and renal vascular bed during autonomic dysreflexia in high spinal cord injury. Clin Auton Res; 12: 457–464, 2002.

58- **West CR, Popok D, Crawford MA, Krassioukov AV**: Characterizing the temporal development of cardiovascular dysfunction in response to spinal cord injury. J Neurotrauma; 32:922–930, 2015.

59- **Price MJ, Trbovich M**: Thermoregulation following spinal cord injury. Handb Clin Neurol; 157:799-820, 2018.

60- **McLean DE, Kearney J & Cawley MF**: Environmentally responsive temperature instability in pediatric spinal cord injury. Spinal Cord; 37, 705–709, 1999.

61- **Price MJ & Campbell IG**: Thermoregulatory responses of spinal cord injured and able-bodied athletes to prolonged upper body exercise and recovery. Spinal Cord; 37:772–779, 1999.

62- **Morrison SF**: Central pathways controlling brown adipose tissue thermogenesis. News Physiol Sci; 19: 67–74, 2004.

63- **West Christopher R, Squair Jordan W, McCracken Laura, Currie Katharine D, Somvanshi Rishi, Somvanshi Rishi , Yuen Violet , Phillips Aaron A. , Kumar Ujendra , McNeill John H. , and Krassioukov Andrei V:** Cardiac Consequences of Autonomic Dysreflexia in Spinal Cord Injury. Hypertension; 68:1281–1289, 2016.

64- **Krassioukov A, Warburton DE, Teasell R, Eng JJ:** A systematic review of the management of autonomic dysreflexia after spinal cord injury. Arch Phys Med Rehabil; 90: 682–695, 2009.

65- **Teasell RW, Arnold JMO, Krassioukov A, Delaney GA**: Cardiovascular consequences of loss of supraspinal control of the sympathetic nervous system after spinal cord injury. Arch Phys Med Rehabil; 81: 506–516, 2000.

66- **Krassioukov A, Claydon VE**: The clinical problems in cardiovascular control following spinal cord injury: an overview. Prog Brain Res; 152: 223–229, 2006.

67- **Krassioukov A**: Autonomic function following cervical spinal cord injury. Respir Physiol Neurobiol; 169: 157–164, 2009.

68- **Schmid A, Huonker M, Barturen JM, Stahl F, Schmidt-Trucksass A, Konig D et al**.: Catecholamines, heart rate, and oxygen uptake during exercise in persons with spinal cord injury. J Appl Physiol; 85: 635–641, 1998.

69- **Arnold JM, Feng QP, Delaney GA, Teasell RW**: Autonomic dysreflexia in tetraplegic patients: evidence for alpha-adrenoceptor hyper-responsiveness. Clin Auton Res; 5: 267–270, 1995.

70- **Krum H, Louis WJ, Brown DJ, Clarke SJ, Fleming JA, Howes LG**. Cardiovascular and vasoactive hormone responses to bladder distension in spinal and normal man. Paraplegia; 30: 348–354, 1992.

71- **Krenz NR &Weaver LC**: Changes in the morphology of sympathetic preganglionic neurons parallel the development of autonomic dysreflexia after spinal cord injury in rats. Neurosci Lett; 243: 61–64, 1998.

72- **Colachis SC 3rd**: Hypothermia associated with autonomic dysreflexia after traumatic spinal cord injury. Am J Phys Med Rehabil; 81: 232–235, 2002.

73- **Emel E, Ergun SS, Kotan D, Gursoy EB, Parman Y, Zengin A, Nurten A**: Effects of insulin-like growth factor-I and platelet-rich plasma on sciatic nerve crush injury in a rat model. J Neurosurg; 114:522–528, 2011.

74- **Takeuchi M, Kamei N, Shinomiya R, Sunagawa T, Suzuki O, Kamoda H, Ohtori S, Ochi M**: Human platelet-rich plasma promotes axon growth in brain-spinal cord coculture. Neuroreport: 23:712–716, 2012.

75- **Cho HH, Jang S, Lee SC, Jeong HS, Park JS, Han JY, Lee KH, Cho YB**: Effect of neural-induced mesenchymal stem cells and platelet-rich plasma on facial nerve regeneration in an acute nerve injury model. Laryngoscope; 120:907–913, 2010.

76- **Yu W, Wang J, Yin J**: Platelet-rich plasma: a promising product for treatment of peripheral nerve regeneration after nerve injury. Int J Neurosci; 121:176–180, 2011.

77- **Wu CC, Wu YN, Ho HO, Chen KC, Sheu MT, Chiang HS**: The neuroprotective effect of platelet-rich plasma on erectile function in bilateral cavernous nerve injury rat model. J Sex Med; 9:2838, 2012.

78- **Kuffler, D P:** Platelet-Rich Plasma and the Elimination of Neuropathic Pain. Mol Neurobiol; 48:315–332, 2013.

79- **Bir SC, Esaki J, Marui A, et al**: Therapeutic treatment with sustained-release platelet-rich plasma restores blood perfusion by augmenting ischemia-induced angiogenesis and arteriogenesis in diabetic mice. Journal of Vascular Research;48(3):195–205, 2011.

80- **Bir SC, Esaki J, Marui A, et al:** Angiogenic properties of sustained release platelet-rich plasma: characterization in-vitro and in the ischemic hind limb of the mouse. Journal of Vascular Surgery; 50(4):870–879, 2009.

81- **Pierce GF, Mustoe TA, Lingelbach J, Masakowski VR, Gramates P, Deuel TF**: Transforming growth factor β reverses the glucocorticoid-induced wound healing deficit in rats. Possible regulation in macrophages by platelet-derived growth factor. Proceedings of the National Academy of Sciences of the United States of America; 86(7) :2229–2233, 1998.

82- **Pérez-Rojas JM, Cruz C, García-López P, et al**: Renoprotection byα-mangostin is related to the attenuation in renal oxidative/nitrosative stress induced by cisplatin nephrotoxicity. Free Radical Research; 43(11):1122–1132, 2009.

83- **Rozman P, Bolta Z:** Use of platelet growth factors in treating wounds and soft-tissue injuries. Acta Dermatovenerol Alp Panonica Adriat; 16:156–65, 2007.

84- **Shen YX, Fan ZH, Zhao JG, Zhang P**: The application of platelet-rich plasma may be a novel treatment for central nervous system diseases. Med Hypotheses;73(6):1038-1040, 2009.

85- **Marx RE, Carlson ER, Eichstaedt RM, Schimmele SR, Strauss JE, Georgeff KR:** Platelet-rich plasma: growth factor enhancement for bone grafts. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontics;85(6):638–646, 1998.

86- **Werner S, Grose R:** Regulation of wound healing by growth factors and cytokines. Physiological Reviews;83(3):835–870, 2003.

87- **Juchem G, Weiss DR, Knott M, Senftl A, Forch S, Fischlein T, Kreuzer E, Reichart B, Laufer S, Nees S**: Regulation of coronary venular barrier function by blood borne inflammatory mediators and pharmacological tools: insights from novel microvascular wall models. Am J Physiol Heart Circ Physiol; 302: H567–H581, 2012.

88- **Cloutier N, Pare A, Farndale RW, Schumacher HR, Nigrovic PA, Lacroix S, Boilard E**: Platelets can enhance vascular permeability. Blood; 120:1334–1343, 2012.

89- **El-Sharkawy H, Kantarci A, Deady J, Hasturk H, Liu H, Alshahat M, Van Dyke TE**: Platelet-rich plasma: growth factors and pro- and anti-inflammatory properties. J Periodontol; 78:661–669, 2007.