Experimental Use of Blood PRP for Regeneration of Manually Sciatic Nerve Damaged in Dogs

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Authors’ contributions

This work was carried out in collaboration between both authors. Author BJH designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author GM managed the analyses of the study. Authors BJH and GM managed the literature searches.

ABSTRACT

The study has done on (10) adult healthy male dogs separated as two group (control group and group treated with PRP) each group had (5 dogs), with body weight ranged between (15-20 kg). Blood was collected from peripheral cephalic vein (10 ml in anti-coagulant tube) in five dogs and double centrifugation protocol was followed at 800 rpm and 1600 rpm for 10 minutes to separate the PRP. The PRP then injected directly in the manually damaged sciatic nerve in both side of legs. The result show that the left legs showed moderate knelling, pain and swelling was also showed. The histo-pathological examination of proximal longitudinal section of sciatic nerve at 3 months after treatment showed Increase number of Schwann cell and vacuolated degenerated nerve fibers with distorted arrainment. The histo-pathological examination of middle transverse part of nerve fawned increase number of Schwann cell and vacuolated degenerated nerve fibers with distorted arrainment. The histo-pathological examination of longitudinal section of the distal part of sciatic nerve showed Irregular arrangement of nerve fibers irregular spaces in between and moderate regeneration.

Keywords: Sciatic nerve; dogs; PRP.
1. INTRODUCTION

Platelet-rich plasma (PRP) is a biological therapy the blood to obtain products with a higher platelet concentration than in blood. It provides a transient fibrin scaffold as a controlled drug delivery system of growth factors suitable. For regenerative. Medicine [1]. PRP has been used as a medical strategy to treat diverse types of injuries in the field of orthopedics, including peripheral nerve lesions, new treatments administering growth factors, and cells for promoting nerve regeneration. exist [2]. PRP an auto logos product with proven therapeutic effects for musculoskeletal disorders, is a new treatment option for peripheral nerve injury [3]. PRP has been used for cellular growth as an auto logos scaffold, and it has been proved that in association with mesenchymal stem cells, it potentiates tissue regeneration [4]. Platelet rich plasma (PRP) has been observed to enhance healing of the donor and recipient sites of soft tissue flaps and grafts including skin, mucosa, muscles, and dermal fat [5]. The addition of PRP to the regenerating nerve fibers immediately after their reanastomosis has improved the process of early regeneration of the nerve fibers [6]. Some of many attempts for regeneration of nerve damage is (PRP); [3]; [7] reported that there are positive effects of platelet-rich plasma (PRP) on tissue regeneration. PRP is used as an auto logos cell-free therapy and contains many bioactive factors of plasma and \( \alpha \)-granules of platelets, which are involved in wound healing and tissue repair [8].

2. MATERIALS AND METHODS

2.1 Animals of Study

The study has done on [9] adult healthy male dogs separated as two group (control group and group treated with PRP) each group had (5 dogs), with body weight ranged between (15-20 kg). The animals of study were kept in animals house college. of veterinary medicine – University of Basra in a cages along the period of study, good nutrition and management had been provided. to the animals. The study were started in February 2020. extended about 5 months. Based on manually damaged the sciatic nerve of both side of leg using surgical opinion and made damage by artery forceps.

2.2 Blood Collection and Separation of P.R.P.

Blood was collected from peripheral cephalic vein (10 ml in anti-coagulant tube) in five dogs and double centrifugation protocol was followed at 800 rpm and 1600 rpm for 10 minutes at room temperature respectively. The platelet count was evaluated through hematology analyzer. The mean concentration of platelets in PRP was (1247.2 ± 30.74) x 10³/mm³, involving more than four times increase in platelet count. This method appears to produce an adequate concentration of platelets and may be appropriate for clinical use. The PRP then injected directly in the manually damaged sciatic nerve in both side of legs. The PRP separated then injected of 4 ml of it in the site of and around the damaged nerve, of both side, the operation repeated weekly for 2 weeks.

2.3 Post Operative. Histo-Pathology

Taken them, microscopic examination of nerve sections to determine the number of Schwann cells, arrangement of nerve fibers, vacuolated degenerative nerve fibers, intramural and extraneural scarring, the axonal alignment and density of nerve fibers. The Neuro. histo pathological examinations were done with stain (eosin stain and hematoxylin ). Separated the left sciatic nerves from each animal and nerve samples fixed. the nerve sample on to plastic plate. using stay sutures to keep the nerve tissues straight [10], then saved in special container containing 10% formalin. Three samples of 1-cm length each. were harvested from the proximal, middle .site of injury) and .distal portions of the sciatic Nerve [9].

3. RESULTS

3.1 Clinical Finding

3.1.1 Control group

In this group of animals showed sever pain, swelling and knelling on the left leg after treatment then become moderate (pine and swelling) after 21 days, at the 28 day (A.T) was become normal. The animals show sever knelling after. Treatment, Then become moderate after 42 day and disappear in 60 day (A.T). At the first day (A.T) was showed flaccid of the muscle flexion and the become less gradually then become normally at 90 day.
3.1.2 Platelet Rich Plasma (PRP) group

The left legs of this animals was showed moderate knelling, pain and swelling was as showed. Sever knuckling also to the 28 day after treatment. Then the knuckling was become gradually to moderate and then become normal at 48 day after treatment, We also showed also flaccid of muscle flexion at the first day and then retune to normal progressively at 72 day after treatment (Table. 1).

3.2 Histopathological Examination

3.2.1 Control group

The histo-pathological. examination of longitudinal section of the proximal. part of left sciatic nerve at 3 month (A.T) necrotic-degenerated nerve.fibers (Fig. 2). Was showed changes at middle. Transaction part of sciatic nerve, irregular and degenerated myelin sheath, and presence of vacuolated degenerated nerve fibers. associated with Schwann cells. The distal longitudinal section of distal part of nerve. sections showed Irregular arrangement of nerve fibers. and presence of few vacuolated degenerated nerve fibers (Fig. 1).

3.2.2 Platelet Rich Plasma (PRP) group

The histo-pathological examination of proximal longitudinal section of sciatic nerve at 3 months after treatment showed increase number of Schwann cell and vacuolated degenerated nerve fibers with distorted arrainment (Fig. 3). The histo-pathological examination of middle transverse part of nerve fawned increase number of Schwann cell and vacuolated degenerated nerve fibers with distorted arrangement (H&E40X) (Fig. 4). The histo-pathological examination of longitudinal section of the distal part of sciatic nerve showed Irregular arrangement of nerve fibers irregular spaces in between and moderate regeneration. (Fig. 5).

Table 1. Clinical finding of PRP group

<table>
<thead>
<tr>
<th>PRP group</th>
<th>Knelling</th>
<th>Pain</th>
<th>Swelling</th>
<th>Muscle flexion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weak 1</td>
<td>Moderate</td>
<td>Moderate</td>
<td>Moderate</td>
<td>Flaccid</td>
</tr>
<tr>
<td>Weak 2</td>
<td>sever</td>
<td>moderate</td>
<td>moderate</td>
<td>Flaccid</td>
</tr>
<tr>
<td>Weak 3</td>
<td>sever</td>
<td>less</td>
<td>normal</td>
<td>Flaccid</td>
</tr>
<tr>
<td>Weak 4</td>
<td>sever</td>
<td>absent</td>
<td>normal</td>
<td>Flaccid</td>
</tr>
<tr>
<td>Weak 5</td>
<td>moderate</td>
<td>absent</td>
<td>normal</td>
<td>Flaccid</td>
</tr>
<tr>
<td>Weak 6</td>
<td>moderate</td>
<td>absent</td>
<td>normal</td>
<td>Flaccid</td>
</tr>
<tr>
<td>Weak 7</td>
<td>moderate</td>
<td>absent</td>
<td>normal</td>
<td>Flaccid</td>
</tr>
<tr>
<td>Weak 8</td>
<td>normal</td>
<td>absent</td>
<td>normal</td>
<td>Moderate</td>
</tr>
<tr>
<td>Weak 9</td>
<td>normal</td>
<td>absent</td>
<td>normal</td>
<td>Moderate</td>
</tr>
<tr>
<td>Weak 10</td>
<td>normal</td>
<td>absent</td>
<td>normal</td>
<td>Moderate</td>
</tr>
<tr>
<td>Weak 11</td>
<td>normal</td>
<td>absent</td>
<td>normal</td>
<td>Moderate</td>
</tr>
<tr>
<td>Weak 12</td>
<td>normal</td>
<td>absent</td>
<td>normal</td>
<td>Normal</td>
</tr>
<tr>
<td>Weak 13</td>
<td>normal</td>
<td>absent</td>
<td>normal</td>
<td>Normal</td>
</tr>
</tbody>
</table>

Fig. 1. The proximal longitudinal section of sciatic nerve at 3 months (A.T) of control group showed irregular Schwann cells (necrotic-degenerated nerve fibers) (40X)
Fig. 2. The distal longitudinal section of sciatic nerve of control group. Irregular arrangement of nerve fibers and presence of few vacuolated degenerated nerve fibers (40X).

Fig. 3. Proximal longitudinal section of sciatic nerve of PRP group. Increase number of Schwann cell vacuolated degenerated nerve fibers with distorted arrangement (H&E 40X).

Fig. 4. The transverse section of middle part of sciatic nerve of PRP group. Increase number of Schwann cell vacuolated degenerated nerve fibers with distorted arrangement (H&E 40X).
Fig. 5. The distal longitudinal section of sciatic nerve of PRP group Irregular arrangement of nerve fibers ( ) irregular spaces in between and moderate regeneration ( ) (40X)

Table 2. Statistical analysis of conductive velocity of control, and PRP, group at 3 months after treatment

<table>
<thead>
<tr>
<th>Groups</th>
<th>Conductive velocity at 3 months (A.T)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Right nerve</td>
</tr>
<tr>
<td>Control</td>
<td>25m/s</td>
</tr>
<tr>
<td>Platelet rich plasma (PRP)</td>
<td>25 m/s</td>
</tr>
</tbody>
</table>

3.3 Conductive Velocity at 3 Months after Treatment

The sciatic nerves were isolated from left legs to electrophysiological examination at 3 months after treatment. They accrue differences between control and PRP groups. We observed the PRP groups gave best comparative with control group Table (2).

4. DISCUSSION

In control group the animals show sever knelling after treatment. Then become moderate after 42 day and disappear in 60 day, the result was agreed with [11]. While the animals that treated with PRP showed sever pain and swelling at 7 day (A.T) then become moderate at 14 day after that was became normally the result was agreed with [12]. When they reported that the sciatic nerve return to the normal after 2 weeks of treatment with PRP. At first day (A.T) showed sever knelling to the 35 day (A.T) and then become normally at 63 day progressively, the result was not agreed with [13] when they reported that the period of recovery take about 12 week after treatment of damaged sciatic nerve with PRP.

5. CONCLUSIONS

The study conclude that blood PRP can be use in treatment of nerve damage. The PRP is the most important easy and cheap method for regeneration the nerve damage with no noticed clinical complications to the damaged tissue.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Animal ethic Committee approval has been collected and preserved by the author.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/61369