# Use of platelet – rich plasma (PRP) therapy of muscular contusions in

### Iraqi Arabian horses

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### Abstract

Platelet-rich plasma (PRP) is regarded as a modern therapeutic technique in horses, so there are few researches .

The study is conducted on (8) Iraqi Arabian horses, which are divided equally and randomly into (2) groups, treatment and control group. Autologous PRP is prepared for treatment group. All experimental horses are exposed to hard trauma causing muscular contusions. Treatment group are treated two times a week for (6) weeks.

Due to the effect of many growth factors, the results shows benefit effect of PRP, perfuse granulation tissue which is characterized by excellent proliferation of fibroblasts and highly proliferation of endothelial cells to form new blood vessels.

This study according to our knowledge is the first research in muscular contusions of Arabian horses, which is focusing on the histological effect of autologous PRP on the muscular contusions which is very common acute injuries in Iraqi Arabian horses.

Muscular contusions were a major common surgical affect in horses, especially sport horses. According to our knowledge there was no any study of platelets rich plasma (PRP) use to treat muscular injuries in horses.

## Introduction

Despite the high prevalence of muscle strains, there was a limited evidence base for the majority of management techniques and treatment, in particular minimizing the risk for recurrent muscle injuries, had progressed little in the past (30) years. Moreover, although numerous risk factors for muscle injury had been identified (1), evidence suggested that the greatest risk factor for a recurrence remains a previous injury to that muscle, perhaps a result of scar tissue formation at or near the injury site (2).

(3) were studied the effects of autologous (PRP) on recovery in the tibialis anterior muscle of rats. PRP was isolated by a technique involving centrifugation of whole blood, allowing extraction of the specific part of the plasma containing a high concentration of platelets. These platelets were rich in growth factors that could stimulate myogenesis (4), (5), (6), and mitigate inflammation (7), (8).

Healing had been shown to take place in response to local application of growth factors (9), (10).

Platelets were an important component involved in haemostasis and stored growth factors in alpha granules, which were activated to release these factors at the site of injury (11).

There were currently several methods of concentrating growth factors. Platelets rich plasma (PRP) involved extracting the protein of plasma that contains higher concentration of platelets after centrifugation of autologous whole blood. PRP had been found to contain between (4-8) times the normal platelet concentrations compared with whole blood (4).

PRP preparations had certain steps in common. The first step was the withdrawal of the peripheral blood, followed by centrifugation to yield (3) layers – the red layer (containing erythrocytes), white layer (leukocytes and inflammatory cytokines) and the yellow layer (containing plasma, platelets and growth factors) (12).

Besides yielding greater concentrations of growth factors, PRP preparations also presented some advantages over other methods of obtaining growth factors. Platelets had an important role in coagulation and hemostasis (12).

The alpha granules of platelets also secreted other cytokines / proteins (endostatins, platelet factor 4) that were involved in the healing process (13).

Platelets had also been found to have analgesic properties, releasing protease- activated receptor 4 peptides (14).

Muscle healing took place in a series of steps, which overlapped – inflammation, proliferation and remodeling. The steps were regulated by the presence of growth factors and cell interactions. There was a large concentration of cytokines found in healing muscle, evidence for the role of growth factors in muscle healing. Growth factors not only enhanced muscle regeneration, but also enhanced muscle force (15).

The aim of this study was to demonstrate the beneficial use of (PRP) in the treatment of muscular contusion of the local Arabian horses.

### Materials and methods

Eight local Arabian horses are used (2 stallions and 6 mares), their ages are (6-6.5) years, which divide randomly and equally into (2) groups, treatment group and control group.

Whole blood (100) ml are aspirated from the peripheral blood circulation (jugular vein) of each horse of treatment group (autologous blood), twice centrifugation at (4000) rpm for (10) minutes. The yellow layer (containing plasma, platelets and growth factors) is aspirated gently, adding 10% calcium chloride then keeping the solution in incubator at  $37^{\circ}$ c for 24 hours to activate platelets, to release high concentration of growth factors and preserve in (-80 ° c).

All the experimental horses have sedation by acepromazine maleate (2-4 mg/lb.B.W.).Their muscles (superficial gluteal muscle) are exposed to hard trauma by a metal object causing muscular contusion.

The treatment group are injected locally 4ml.of plasma rich platelets (PRP) preparations, (2) times a week for (6) weeks, directly after contusion (Fig- 1).

Biopsies are taken from both groups (7, 14, and 21) weeks for histo-pathological examination. The histo- pathological slides are stained with eosin & hematoxiline stains and exam under light microscope (Olympus Incorporation - Japan).



Fig.-1: injection of PRP in the superficial gluteal muscle

#### Results

On day (7) of the treatment group with ( PRP ), the histological assessment shows significant changes, particularly regarding wound healing. There are marked irregular muscle

fibers and scattered infiltration of inflammatory cells, with presence of few hemorrhage between skeletal muscle fibers (Fig. 2).

After (14) days of treatment with ( PRP ), shows higher infiltration of inflammatory cells and abundant irregular arrangement of skeletal muscle fiber, also presence of granulation tissue which characterized the wound with formation of new blood vessels (Fig. 3).

(21) days of treatment with (PRP), the histo-pathological exam shows profuse granulation tissue which characterize by proliferation of fibroblast to form fibrosis and highly proliferation of endothelial cells to form new blood vessels (Fig. 4).

While the histological assessment of the control group on day (7) shows severe Hemorrhage between skeletal muscle and slightly infiltration of inflammatory cells also there is irregular muscle fiber (Fig.5).

After (14) days post wound in control group, there are marked irregular arrangement with severe degeneration of striated muscle fiber. Also presence of hemorrhage between muscle fiber (Fig.6).

On day (21) post wound in control group, there are clear higher infiltration of inflammatory cells, also shows weak granulation tissue and characterize by the muscle fiber which visible distraction and are separated with degeneration of myocytes (Fig.7).



Fig, -2: treated group (PRP) histopathological section of the superficial gluteal muscle at (7) days shows there is marked irregular muscle fiber (blue arrow) and *scattered* infiltration of inflammatory cells (thin arrows). Necrosis and degeneration muscle fiber (red arrow). also there is hemorrhage between the skeletal muscle fibers (yellow arrow). 10X H&E



Fig,- 3: treated group (PRP) histopathological section of the superficial gluteal muscle at (14) days. There is higher infiltration of inflammatory cells mainly macrophage irregular arrangement of skeletal muscle fibers (blue arrows). Also there is marked granulation tissue which characterized by proliferation of fibroblast and formation of new blood vessels (green arrows). 40X H&E.



Fig.- 4: treated group (PRP) histopathollogical section of the superficial gluteal muscle at (21) days there is presence of granulation tissue which characterized by proliferation of fibroblast to form fibrosis (green arrows) and proliferation of endothelial cells to form new blood vessels (blue arrows) also there is infiltration of inflammatory cells (red arrows).(10XH&E)



Fig,- 5: control group histopathollogical section of the superficial gluteal muscle at (7 ) days There are severe heamorrhage between skletal muscle ( green arrows ) and shows slitly inflammatory cells ( rea arrow ) also there is visible irrigular muscle fiber( blue arrow) ( 10 XH& E)



Fig-6: control group histopathological section of the superficial gluteal muscle at (14) days there is marked irregular arrangement with severe degeneration of striated muscle fibers (red arrows) also there is hemorrhage between muscle fiber (thin arrows) also infiltration of inflammatory cells (green arrows).



Fig-7: control group histopathological section of the superficial gluteal muscle at (21) days shows high infiltration of inflammatory cells (green arrows) also irregular muscle fiber weak granulation tissue and this characterize by the muscle fibers which shows distracted and separated with degeneration of myocytes.

#### **Discussion:**

There are limited studies of the PRP effect on the muscle injuries because PRP therapy has grown popularity over the few in past years. PRP is a safe therapy given its autologous nature and long- term usage with out any major complications. It is produced from the animals' peripheral vein and is centrifuged to achieve a high concentration of platelets with in a small volume of plasma. It is then re-inject at a site of muscular contusion (acute injury). There are many preparation procedures of PRP and activation of platelets to release mean growth factor like (TGF\u00c61, TGF\u00c62, TGF\u00c63, Vascular Endothelial GF) that responsible of acceleration the proliferation of cells (myogenesis). According to (19) double- centrifugation method is able to achieve higher platelet concentrations than the single- centrifugation.

The histological evaluation of PRP effect is very clear as shows by the results of the present study specially after (21) days, there is perfuse granulation tissue which is characterized by proliferation of fibroblasts to form fibrosis and highly proliferation of endothelial cells to form new blood vessels (Fig.- 4) while the histological assessment of the control group at the same period shows, clear higher infiltration of

inflammatory cells, weak granulation tissue, visible distractions of myocytes. These results are accompanied with (16).

The actual mechanisms of action of PRP are extensive because of the release of a myriad of bioactive factors.

The benefit use of PRP for the healing acceleration of muscular contusion may be due to the injection of concentrated platelets in the injured muscle, once is activated, results in an exponential increase in numerous growth factors. However, the function of many growth factors, chemokines, cytokines, and inflammatory mediators has not been elucidated, nor have the interactions between factors and their influence on neighboring cells, specially the transforming growth factor (TGF $\beta$ 1) (16), (17).

Many questions are related to PRP remain unanswered, such as the maximum concentration of platelets in PRP, ideal procedures of preparation, the correct PRP formulations, the types of cells which are contained in PRP, its frequency of applications, so further researches are needed to explain the quantification of specific growth factors release by PRP which effect on angiogenesis and myogenesis as well as the functional recovery before PRP can be used in a wide clinical application (18).

This technique is still need focusing on many questions, the ideal procedures preparation, frequency of application, the correct PRP formulation, types of cells which are contained in PRP and the quantification of the specific growth factors release by PRP which effect on angiogenesis and myogenesis.

This study according to our knowledge is the first research in muscular contusion of Arabian horses, which is focusing on the histological effect of autologous PRP on the muscular contusion which is very common acute injury in Iraqi Arabian horses.

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