



EFFECT OF PLATELET RICH PLASMA ON REGENERATION OF ACUTE MEDIAN NERVE INJURY

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ABSTRACT

Currently there is a low success rate of function restoration with aid of surgical techniques. Therefore Peripheral nerve repair and healing have gained an increased attention in recent decades; many studies have investigated this subject. There is a clear need for biomedical engineering research to develop novel strategies to improve outcomes following nerve damage. Double-blinded randomized control trial was designed with 10 patients with acute median nerve injuries at wrist who underwent epiperineurorrhaphy with microsurgical technique. 5 cases received outologus platelet rich plasma (PRP) at repair site. Patients were followed up for evaluation of recovery for 1 year. The average recovery percentage of all variants in case group was 70.33% and 67.38% in control group. The average speed of recovery of all variants in case group was 8.16% and 10.21% in control group in aforementioned time interval. Remarkable recovery was found in pinch power, amplitude and distal latency-motor in both group. Majority of variants had better results after PRP injection at repair site but these changes was not remarkable.

Keywords: *Peripheral nerve repair, PRP, nerve recovery.*

INTRODUCTION

The peripheral nervous system is damaged primarily by traumatic injury, surgery, or repetitive compression. Traumatic injuries can occur due to stretch, crush, laceration and ischemia, and are more frequent in wartime, i.e., blast exposure. Peripheral nerve injuries occur surprisingly frequent and are reported in up to 3% of all trauma patients^{1,2,3}. Peripheral nerve repair and healing have gained an increased attention in recent decades; many studies have investigated this subject^{4,5,6}. The aim of surgical nerve repair is to influence regenerating sensory, motor, and autonomic axons to the distal, degenerating nerve segment, to maximize the chance of target reinnervation^{8,9}. Despite the best efforts and modern surgical techniques, functional restoration is often incomplete, with approximately only 50% of surgical cases achieving normal to good function restoration^{7,8,9,10}. Accordingly, there is a clear need for biomedical engineering research to develop novel strategies and grafting options to improve outcomes following nerve damage⁷. Recent studies

reveal that Schwann cells promote peripheral nerve regeneration by releasing neurotrophic factors and forming the bands of Bungner to direct regenerating axons across the lesion^{11,12}. However, the limited supply of cultured Schwann cells limits clinical application of these cells. Nerve recovery is also promoted by the use of neurotrophic factors, including brain-derived neurotrophic factor (BDNF), platelet- rich plasma (PRP), and Matrigel (a complex of extracellular matrix components, such as laminin, collagen IV, entactin, and heparin sulfate proteoglycan)^{13,14,15,16}. PRP is prepared by centrifugation of the patient's own blood¹⁷. PRP consists of various neurotrophic factors, such as neurotrophin-3 (NT-3), angiopoietin-1, glial cell linederived neurotrophic factor (GDNF), and BDNF¹⁸. The use of these neurotrophic factors has been investigated, and facilitated nerve regeneration has been reported^{15,16,18,19}. Low median nerve injury can cause palsy dysfunction in thenar muscles and sensitive alteration of thumb, 2nd and 3rd fingers and radial portion of anular finger. This can affect the muscles such as abductor pollicis brevis, superficial portion of brevis flexor

of the thumb, opponents and 1st and 2nd lumbricals which can result in claw fingers. When tension-free suturing is possible, a simple repair is the preferred procedure; however, patients with loss of nerve tissue, resulting in a nerve gap, are considered for nerve grafting procedure²⁰. In present study patients with acute low median nerve injury were selected. Tension free simple repair by trained microsurgeon was performed and to improve the outcome, PRP was used in the repair site. The outcome was evaluated and illustrated.

MATERIALS AND METHOD

The study was performed between April 2010 and April 2013 at Chamran hospital (Shiraz University of medical sciences, orthopaedic surgery department). The study's goal was to evaluate the Platelet rich plasma effectiveness in the outcome of nerve repair, leading to potential enhancement of nerve regeneration. All treatment protocols were approved by the Ethical Committee of Shiraz University Medical Sciences and a written informed consent was obtained from each patient. Twelve patients who were referred to Chamran hospital emergency room with acute median nerve injury were selected. The patients were examined by senior resident of orthopaedic surgery and divided into 2 control and case groups. This research was double-blinded, where both the surgeon and the examiner were blind. Two patients refused to resume the research and were omitted from sample.

Inclusion criteria

The patients selected were between 18-45 years old. They all presented acute median nerve injury due to sharp laceration up to 7 cm proximal to distal volar wrist crease, within 7 days prior to admission. The nerve injury classified as Seddon type III and Sunderland type V, the neurotemesis were approved in operating room. If nerve injury was not classified correctly, the case was omitted from research. Another criterion was for patients to present sign of injury of recurrent branch of median nerve (no contraction of thenar muscle).

Exclusion criteria

Patients who refused to participate in research were not included in this study. Patients with contaminated or crushed wounds or with tissue defect were also excluded from the study. Similarly patients with signs and symptoms of neuropathy

before injury such as carpal tunnel syndrome (CTS), Diabetes mellitus, Rheumatoid Arthritis, leprosy and induced neuropathy, or sign of previous surgery in affected hand were not considered.

PRP Preparation

Arthrex double syringe ACP (Autologous Conditioned Plasma) system was used for PRP preparation. Some previous researches approved a system for PRP preparation and product included concentrated growth factor^{21,22,23,24}. According to guidelines, when ACP is used within thirty minutes of blood withdrawal, the use of anticoagulant is not required. After nerve repair, the surgeon left the operation room to blind the research while patient was still under general anesthesia. Approximately 12 cc of venous blood at a rate of 1 cc every two seconds, slowly, was taken from patient's hand which prepared in the operative field with designed syringe. The sample was centrifuged with speed of 1500 rpm for 5 minutes in operation room under aseptic condition. Around 2-3 cc ACP was extracted from primary sample. Then ACP was injected at the nerve repair site. All injections at repair site were performed in less than 20 minutes after sampling.

Surgical Technique

The delay in performing surgery was at most 7 days after the injury. The whole patient's upper extremity was in operation field for later aseptic sampling. One gram cephazolin was injected intravenously as prophylactic antibiotic. In cases that had associated tendon injury, nerve repair was done after meticulous tendon repair. All of the nerves were repaired by the same surgeon and the same technique. Distal and proximal stumps of the nerve were refreshed by sharp knife with?????? technique. Then Epiperineurorrhaphy was done by 8-0 Nylon (Ethilon,...) under 5 to 10 times microscopic magnification. One microscope (Carl Zeiss OPMI Vario/S 88 System) was used for all patients. There was no tension at repair site in neutral wrist position in any of the cases. First suture was applied in the back, middle part of the nerve and second one applied 180 degree from it (in mirror image anterior portion). At this stage if there were protruding fascicles, the repair was considered to be unacceptable because of poor rotational coaptation and the sutures were revised. Otherwise the neurorrhaphy proceeded with application of more Nylon sutures between the previous ones. If the median volar artery of the

median nerve was prominent it was also used as a good guideline for proper rotational coaptation.

The surgeon left the operating room and the senior resident prepared and injected PRP for the patients belong to "case group". One milliliter of PRP was withdrawn with TB syringe and injected under epineurium at the points just proximal and distal to the repair site both volar and dorsally. The amount of injection was 0.2 ml at each site. The needle tip was toward elbow in proximal and toward wrist in distal injection. The last 0.2 cc was injected to repair site. Wound closure was done as routine and long arm slab applied in mild wrist flexion for 6 weeks. Post operation antibiotic were continued for 5 days.

Follow up

The 1st visit took place 2 weeks post surgery. Sutures were removed and slab was changed. The patients were then seen monthly for physical examination to evaluate the nerve regeneration process by presence or absence and progression of Tinel sign. Ten months after operation first EMG and NCV were requested and details of physical examinations such as tinnel sign, two point discrimination, grip and pinch power measurements (which measured by Jammar Dynamometer set), light touch test (which measured by Touch Test Sensory Evaluators set) were noted. Next session

was 12th months after operation when patients underwent complete examination. Obtained data were re-evaluated and carefully compared to describe results.

Data analysis

All data were analyzed by SPSS program. Two groups of variants including electrodiagnostic variants (EMG, NCV, Volitional activity) and functional score variants which were measured by mentioned set were used. All electrodiagnostic study was performed by one physiatrist and all functional scoring was done by one senior resident. Both of them were blinded about case or control patients. Electrodiagnostic variants include motor and sensory distal latency, amplitude and volitional activity (Table 1). The functional variants include grip and pinch power and light touch score. All variants corresponding to 10th and 12th month were analyzed and speed of regeneration and functional recovery were compared. Full recovery was considered as distal latency of motor, sensory and amplitude to be 3.8ms, 3.4ms, >5mv respectively. In addition it should be without any fibrillation, positive short wave and fasciculation with adequate MUAP (motor unit action potential) according to EMG and NCV study. The functional full recovery was defined as having functional score of contralateral hand which was used as reference.

Table1
Volitional Activity classification and quantitative number we gave for analysis.

EMG Variants	Bonous
No MUAP	0
Single MUAP	1
Discrete Activity	2
Decreased MUAP and Polyphasic Surface	3
Adequate MUAP	4

RESULT

Case group

The statistical analysis was performed on case group and progression of all variants observed one by one. Initially, evaluation of the progression of distal latency (motor and sensory) of injured median showed descending mean's vector which is more prominent in distal motor latency that means better improvement in distal motor latency. The amplitude had remarkable ascending mean's vector

which revealed satisfactory healing trend. Volitional activity had no significant advancement in this interval though satisfactory recovery in comparison with previous months was observed. The change of grip power in aforementioned period was variable. This was expected due to slight effect of low median nerve injury and difference of grip power among patients. The pinch power had satisfactory progression and it displayed acceptable healing at end of research. The monofilament touch test in this interval revealed descending progression with acceptable inclination. Totally, according to

considered base as ideal recovery, recovery percentage can be calculated which is shown below (Figure 1). As shown in this figure the average recovery percentage was 70.33% and were more prominent in pinch power and volitional activity and distal motor latency which revealed acceptable motor, at first ,and sensory recovery in case group

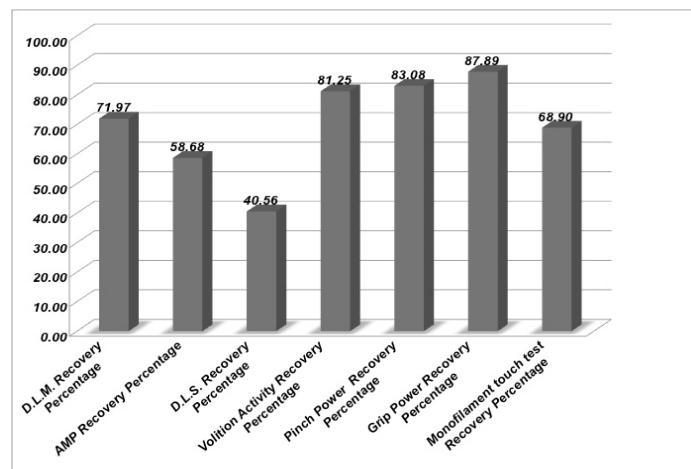


Figure 1
Recovery Percentage of case group.

Control group

On the other hand, recovery process of control patients was evaluated. The progression of distal latency (Motor and Sensory) at follow up of 10th to 12th month had satisfactory descending inclination almost similar to case group. Amplitude had increasing trend with sharp angle. Volitional activity similar to case group, had no significant progression. The changes of pinch power and monofilament touch test were promising with acceptable advancement. The progression of grip

after 12 month. Speed of recovery of variants between months 10th to 12th were calculated. The mean speed of recovery of all variants was 8.16% (per 2 month), the fastest recovery was observed in amplitude, distal latency-motor and slowest recovery in volitional activity and grip power.

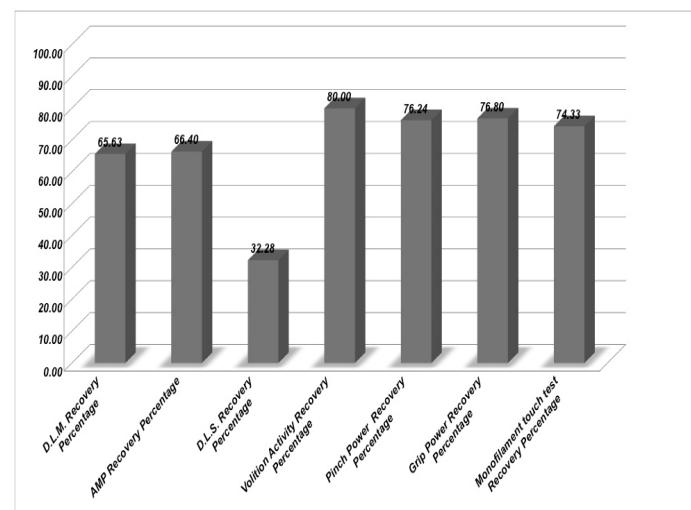


Figure 2
Recovery Percentage of control group.

DISCUSSION

Despite more than 100 years of intense laboratory and clinical investigations, results of nerve repairs are somewhat discouraging, with only 50% of patients regaining useful function. At this time, there is many researches under way, and pharmacologic agents, immune system modulators, enhancing factors and entubulation chambers offer promising future improvement in nerve repair outcomes. Following biomedical engineers' increased attention to growth factors which theoretically enhances nerve regeneration; many procedures were created to concentrate these factors. Thereafter many researches were undertaken to clarify these effect. In 2007, Dr Farrag and Dr lehar carried out an animal study which showed an improved functional outcome with the use of PRP on regeneration of peripheral nerve²⁵. Later in 2008, Sariguney did other animal study. He revealed PRP did not obviously enhance peripheral nerve regeneration, but it may be involved in the process of remyelination of regenerating axons²⁶. After that Ding et al. in 2009, performed another animal study and concluded positive effect of PRP on regeneration of peripheral nerve injury²⁷. Piskin et al. concluded that platelet gel does not have any effect on axonal regeneration according to the animal study they performed in 2009²⁸. In 2010, Dr Cho HH, performed a research which revealed the use of PRP promotes facial nerve regeneration in an animal model of facial nerve axotomy²⁹. According to literature and most of aforementioned researches the PRP theoretically and experimentally had positive influences on peripheral nerve regeneration. No study based on humans was found in literature to approve the effect of PRP on peripheral nerve regeneration. Therefore the author believes that this study is the first experiment to evaluate the effect of PRP on regeneration of peripheral nerve. Due to frequency of referred median nerve injury to the clinic where the study was performed, this injury was selected for this study. With consideration of previous animal researches, 10 patients were chosen as case

and control group and effect of PRP was evaluated in patients. The patients closely followed up every two months, The Tinel sign was analyzed in each visit. It revealed the progression or cessation of nerve regeneration in follow up visits. In all visits progression was observed in both groups. No cessation was found. The speed was slight but it was progressive. This variant was subjective and it could not be measured correctly and precisely, therefore was omitted from analysis. To evaluate the effectiveness of PRP on peripheral nerve regeneration, in addition to comparison of each variant which were compared, in previous chapter, the authors believed that the final recovery at end of follow up is proper indicator to show this purpose. The final recovery was calculated for both groups and was compared. The Figure (3) shows the comparison of recovery percentage. As can be seen, the variants such as pinch power, volitional activity, distal sensory latency and distal motor latency had more recovery percentage in case group at end of research. While the variants such as amplitude and monofilament touch test had more recovery in control group but these changes were not statistically significant. On the other hand, the speed of recovery was considered as cross-sectional indicator (between month 10th to 12th) to show effectiveness of PRP on peripheral nerve regeneration, but, it would be better that more sectional measurements (more than two sections) were considered to calculate this index to survey more accurate results but due to many technical problem it was computed only at two stages. The speed of recovery for amplitude and distal motor latency in control group was very remarkable .On contrary the speed of recovery for distal sensory latency in case group was more satisfactory too in time interval (Figure 4). However, these differences were not significant statistically. Totally, the mean speed of recovery was 10.21% and 8.16% in control and case group respectively. Therefore the proper result to fully support higher speed of recovery in case group compared to control group was not found.

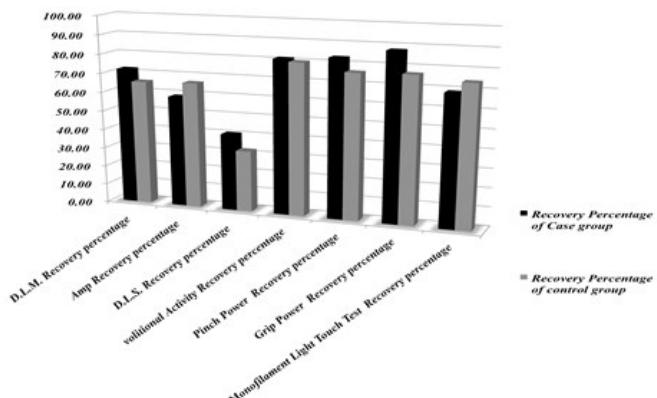


Figure 3
Comparison of recovery percentage in both groups.

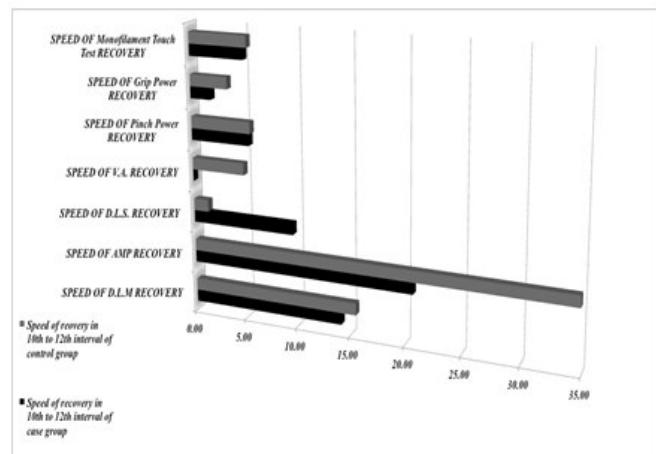


Figure 4
Comparison of recovery speed in 10th to 12th interval.

In summary, based on the results which were obtained and analysis with Mann-Whitney Test and the considered P.Value of .05, conclusions were drawn. Higher improvement level was observed in distal sensory and motor latency, volitional activity and pinch power in case group compared to control group. At the same time the amount of improvement of monofilament touch test and amplitude in control group was higher compared to case group at the end of the research. However, these differences were not statistically significant. On the other hand, the speed of recovery of amplitude and distal motor latency were higher in control group in mentioned time interval. In the same period, distal sensory latency in case group showed higher results compared to the other group. Nevertheless like recovery percentage, these differences were not statistically significant. It seems, despite previous studies and theoretical ideas about the positive effect of PRP on peripheral nerve regeneration after nerve injury, no substantial effect was found in present study. In present study the major conclusions were based on two

aforementioned indices. The authors believe that the recovery percentage is more accurate indicator than speed of recovery to survey the final results in this study. Because the data for speed of recovery is based on two measurements were obtained at two times which were only 2 month apart. It would be much better if we were able to do more measurements at more different interval. Unfortunately it was not possible because: Firstly, the electrodiagnostic studies were performed in first 3 patients before the 10th postoperative month didn't show any findings. So we abandoned it for remaining patients. Secondly, the time limitation we had for preparation of the thesis precluded more electrodiagnostic studies to be carried out after 12th month. The following hypothesis may be the possible explanations for effectiveness of PRP in this study.

1. Although PRP is reported to promote &accelerate healing in many tissue, the elevated intraneuronal pressure due to injection to the nerve may have adverse effect which neutralize the positive neurotrophic influence

of PRP.

- Technically, nerve repair is not performed in a water seal fashion, thus there is no way to prevent extravasation of the injected material from the repair site. The injected PRP may be dispersed and leak from the suture line. Considering that the PRP is injected only one time (at operation time), this dispersion of the material may cause inadequate concentration of PRP for long enough duration which is needed for beginning & progression of the nerve regeneration.
- Small sample size may also explain the negative result. Although, this is definitely a

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weak point of this study, but previous animal studies have been performed with more or less similar sample size. Further studies on more patients may change our prospect about PRP influence in nerve repair.

CONCLUSION

This study showed that PRP has no significant effect on peripheral nerve regeneration in human. Considering the weak points of the present study further human base investigation with great sample size and longer follow up period may be required.

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