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Effect of Platelet-Rich Plasma Treatment on Antioxidant Enzymes' Activity following Hamstring Injury among Malaysian Athletes

(Kesan Rawatan Plasma Kaya-Platelet ke Atas Aktiviti Enzim Antioksidan Selepas Kecederaan Hamstring dalam Kalangan Atlet Malaysia)

LINGLING LAI*, MOHD NAHAR AZMI MOHAMED, MOHAMED RAZIF MOHAMED ALI, KHONG TENG-KEEN & ASHRIL YUSOF

ABSTRACT

The objective of the present preliminary study was to investigate the effect of platelet-rich plasma (PRP) treatment alongside rehabilitation programme compared with rehabilitation programme alone on antioxidant enzymes' (superoxide dismutase, SOD; catalase, CAT) activity and time to return to play (RTP) following hamstring injury among Malaysian athletes. Participants diagnosed with grade-2 acute hamstring injury (n=10) were randomised into 2 groups of PRP treatment and rehabilitation programme (PRP-T) and rehabilitation programme alone (CON). Blood samples were collected at baseline and 2 fortnightly (week-2, W2; week-4, W4) for the biochemical assessments. Participants were certified to have recovered upon fulfilling RTP criteria. PRP-T group showed a significantly lower CAT activity compared to CON group (47.55% lower at W2, 37% lower at W4, p<0.05), while there was no significant difference in SOD activity between the groups. Although PRP-T group benefited from an earlier (~15 days) time to RTP compared to the CON group, no statistically significant difference was noted; however, the effect size was large (Cohen's d=0.9). These findings suggested that PRP treatment alongside rehabilitation potentially reduces the degree of secondary tissue damage and facilitates muscle recovery through regulation of antioxidant enzymes, which subsequently hastens time to RTP. Athletes and coaches could consider PRP as viable treatment for hamstring injury.

Keywords: Autologous; blood injection; muscle injury; oxidative; rehabilitation

ABSTRAK

Objektif kajian awal ini adalah untuk mengkaji kesan rawatan PRP bersama program pemulihan berbanding dengan program pemulihan sahaja ke atas aktiviti enzim antioksidan (superoksid dismutase, SOD; catalase, CAT), serta masa untuk kembali beraksi selepas kecederaan hamstring dalam kalangan atlet Malaysia. Peserta yang didiagnosis dengan kecederaan hamstring akut gred-2 (n=10) dibahagikan secara rawak kepada kumpulan rawatan PRP bersama program pemulihan (PRP-T) dan program pemulihan sahaja (CON). Sampel darah dikumpulkan pada awalan dan setiap 2 mingggu (minggu-2, W2; minggu-4, W4) untuk penilaian biokimia. Peserta disahkan pulih setelah memenuhi kriteria RTP. Kumpulan PRP-T menunjukkan aktiviti CAT jauh lebih rendah berbanding dengan kumpulan CON (47.55% lebih rendah pada W2, 37% lebih rendah pada W4, p<0.05), manakala tiada perbezaan signifikan bagi aktiviti SOD antara dua kumpulan. Walaupun kumpulan PRP-T mendapat manfaat daripada masa lebih awal (~15 hari) untuk kembali beraksi berbanding kumpulan CON, tiada perbezaan statistik yang signifikan didapati; walau bagaimanapun, saiz kesannya adalah besar (Cohen's d=0.9). Penemuan ini menunjukkan bahawa rawatan PRP bersama program rehabilitasi berpotensi mengurangkan tahap kerosakan tisu sekunder dan menggalakkan perkembangan pemulihan otot melalui pengawalan enzim antioksidan, justeru mempercepatkan masa untuk kembali beraksi. Atlet dan jurulatih boleh mempertimbangkan PRP sebagai rawatan berkesan untuk kecederaan hamstring.

Kata kunci: Autologus; kecederaan otot; oksidatif; pemulihan; suntikan darah

INTRODUCTION

Hamstring injuries are the most common type of sport injury that occur in sports that involve sprinting, jumping and rapid change in directions such as in football, rugby, and athletics (Agre 1985; Ekstrand et al. 2011). In Malaysia, surveys found that grade-2 muscle strain is prevalent, with hamstring as one of the muscle groups most frequently injured in various sports (Mohamad Shariff et al. 2011; Shariff et al. 2009). These injuries are of major concern to athletes and coaches as it would lead to a significant time-off, as well as treatment costs.

Common recommendations for injury management would involve rest, ice, compressive dressing and elevation (R.I.C.E) of the affected extremity (Mohamad Shariff et al. 2011). Lately, combination of rehabilitation programme and platelet-rich plasma (PRP) treatment has been employed with the view to hasten time to return to play (RTP) (Hamid et al. 2012). PRP has recently gained much attention among

Malaysian clinicians and is currently, widely used in the field of sports medicine, orthopaedics, fasciomaxillary, urology and aesthetic medicine.

Biologically, PRP is the platelet's concentrate that contains various secretory proteins which potentially initiate and enhance healing process (El-Sharkawy et al. 2007). Most of the studies investigating effects of PRP were carried out on non-Asian populations (Bernuzzi et al. 2014; Bubnov et al. 2013), interestingly, one study reported its usage among Malaysian athletes (Hamid et al. 2014). Its high efficacy and safety (Wang-Saegusa et al. 2011), coupled with a positive patients' satisfaction feedback (Lai et al. 2014), indicate that PRP treatment is a promising treatment that warrants further investigation on its biological effects which contribute to the muscle recovery.

Generally, muscle healing follows the stages of inflammation, proliferation, and remodelling coordinated by innate cellular interactions (Mishra et al. 2009). Each stage produces large quantity of reactive oxygen species (ROS) such as superoxide ($O_2^{\bullet-}$), hydroxyl radical ($^{\bullet}OH$) and hydrogen peroxide (H_2O_2) which play integral roles in injury prognosis (Gutteridge & Halliwell 1989). These free radicals initiate cellular damage through lipid and protein peroxidation, which in turn propagates more free radicals which subsequently lead to secondary tissue damage (Jackson 1999). In response to these elevated ROS, redox-sensitive regulatory mediators would trigger action of antioxidant enzymes, such as superoxide dismutase (SOD) and catalase (CAT) to control the secondary tissue damage (Michiels et al. 1994). SOD is the primary antioxidant enzyme that actively neutralises $O_2^{\bullet-}$ radicals into oxygen and H_2O_2 (Hassan 1988), subsequently CAT catalyses the breakdown of H_2O_2 into water and oxygen (Gutteridge & Halliwell 1989). In order to provide an optimal protection from oxidative damage, SOD and CAT act in a synergistic way to maintain an appropriate balance of anti-oxidative activity (Michiels et al. 1994).

The levels of antioxidant enzymes' presence could be an indicator of recovery process taking place, attenuation of these mediators is reflective of progression in healing. Previous studies found that regulated antioxidant enzymes' activity through antioxidants supplementation potentially attenuated oxidative stress and facilitate regeneration (Lee et al. 2014; Li et al. 2005). It is hypothesised that the levels of antioxidant enzymes will increase during the healing period and PRP treatment may limit this rise. Hence, the objective of present preliminary study was to investigate the effect of PRP treatment on antioxidant enzymes' activity and time to RTP following hamstring injuries among Malaysian athletes.

Materials and Methods

The present single-blinded randomised controlled study was approved by the University of Malaya Medical Centre Medical Ethics Board (No. 907.25). All participants were recruited from the Sport Medicine Clinic of University of Malaya Medical Centre. Patients suspected to have symptoms of injured lower limb were screened for eligibility. Patients who fulfilled the inclusion criteria of: Age above 18 years, and (2) acute grade-2 hamstring injury (within 7 days from date of injury) were eligible for present study. However, patients who had received other forms of treatment during that time and/or unable to comply with scheduled follow-up assessments were excluded.

Initially, suspected patients were clinically assessed by a sport physician. The degree of injury was further confirmed through a diagnostic ultrasonography (US) (Philips IU 22 ultra-sound with 17-5 MHz Probe), and clinically classified based on grading system recommended by Peetron et al. (2002) and Takebayashi et al. (1995). Patients diagnosed with acute grade-2 hamstring injury were invited to participate in present study. The nature and objective of present study was explained to all the participants and written consent was obtained upon participation.

Participants were randomised into PRP treatment (PRP-T) or control (CON) groups using a computer-generated block randomisation list with 5 participants in each group. The PRP-T group received a PRP injection along with a routine rehabilitation programme, while the CON group prescribed rehabilitation programme only. Participants in both groups underwent the same rehabilitation programme (Table 1), which focused on progressive agility and trunk stabilisation (PATS) exercises, as these exercises were found to be effective in promoting earlier RTP (Sherry et al. 2004).

The PRP injection was given immediately after randomisation. The Biomet Gravitational Platelet Separation System GPS™ III (Biomet Biologics, Inc., Warsaw, Indiana) was used to prepare the PRP. Using an aseptic technique, 54 mL of blood was drawn from the participant's antecubital vein into a 60 mL syringe primed with 6 mL of citrate anticoagulant (ACD-A), then centrifuged for 15 min at 3200 rpm. Approximately, 6 mL of platelet concentrate was produced and 3 mL of sodium bicarbonate (8.4%) was added into the platelet concentrate to neutralise its acidotic environment. A 3 mL of PRP was injected into the injured region under US guidance and subsequently, the injected region was iced for 15 min.

A standard clinical examination to evaluate the participant's readiness for RTP was conducted weekly until recovery. Blood samples were collected at 3 time points, i.e. baseline (W0), week-2 (W2) and week-4 (W4) for the assessment of antioxidant enzymes' activity. Blood was drawn from participant's antecubital vein into serum separator tubes (SST), allowed to clot and then centrifuged (Heraeus™ Multifuge™ X1R Centrifuge, USA) at 3000 rpm for 15 min. The serum was separated and kept at -80°C until assayed. Whole blood was collected in K2 EDTA vacutainer tubes and stored at 4°C for biochemical analysis.

Primary outcome assessment focused on the changes of antioxidant enzymes' activity between groups throughout the reference time frame. Stored serum was thawed and assayed using commercially available SOD enzyme-

TABLE 1. The progressive agility and trunk stabilisation (PATS) exercises

	Stage 1	Sets	Duration
1.	Low to moderate intensity side-stepping	3	1 min
2.	Low to moderate intensity grapevine stepping, both directions	3	1 min
3.	Low to moderate intensity forward and backward stepping over a tape line while moving sideways	2	1 min
4.	Single-leg stand progressing from eyes open to eyes closed	4	20 s
5.	Prone abdominal body bridge	4	20 s
6.	Supine extension body bridge	4	20 s
7.	Side bridges, left and right side	4	20 s
8.	Icing in sitting position	-	20 min
	*Stage 2		
9.	Moderate to high intensity side-stepping	3	1 min
10.	Moderate to high intensity grapevine stepping, both directions	3	1 min
11.	Moderate to high intensity forward and backward stepping while moving sideways	2	1 min
12.	Single-leg stand windmill touches	4	20 s
13.	Push-up stabilisation with trunk rotation	2	15 reps
14.	Fast feet in ground	4	20 s
15.	Proprioceptive neuromuscular facilitation trunk-pull downs (along with Thera-Band), right and left	2	15 reps
16.	Symptoms free practice	-	-
17.	Icing if any symptoms of pain	-	20 min

Key: Low intensity, a velocity of movement that is less than or near that of normal walking; moderate intensity, a velocity of movement greater than normal walking but not as great as sport; high intensity, a velocity of movement similar to sport activity.
*Participants were allowed to progress to next stage of PATS exercises after the sport physiotherapist has assessed their progression without symptom of pain

linked immunosorbent assays (ELISA) kit (Northwest Life Science Specialties, LLC, Vancouver, WA) according to the manufacturer's guidelines. Serum was analysed in duplicate and spectrophotometrically measured using a microplate reader (Multiskan FC, Thermo Fisher Scientific, Finland). Stored whole blood was assayed for the erythrocyte CAT activity using an UV-VIS scanning spectrophotometer (Shimadzu UV-1800, Japan), according to protocol by Aebi (1984). The erythrocyte CAT activity was analysed through the decomposition rate of hydrogen peroxide (H_2O_2) at 240 nm. The rate constant was determined based on the exponential decay of H_2O_2 and corrected using sodium azide (NaN_3) as CAT inhibitor. The homogenate CAT activity was then normalised against the haemoglobin (Hb) concentration (mM/g) read at 630 nm.

The secondary outcome of the present study was time to RTP, which was defined as time (days) from the day of injury until the day participant fulfilled criteria for RTP. Participants were required to fulfil the RTP criteria recommended by Hamid et al. (2014) (Table 2), before they were allowed to recommence their pre-injury state of activity.

Statistical analyses were performed using the Statistical Package for the Social Science version 19.0 (SPSS Inc, Chicago, IL, USA). Data was checked for normality using the Shapiro-Wilk test and $p>0.05$ was established. Differences at baseline were analysed with an independent *t*-test for continuous variables and Chi-square test for binary variables. Effect size was calculated based on Cohen (1988). In order to analyse the changes over time, data was analysed using two-way repeated measures ANOVA and Bonferroni's *post hoc* test. Data were presented as mean±SD. A p value of <0.05 was considered to be statistically significant.

TABLE 2. Criteria for return to play (RTP)

Symptom	Assessment	Condition
Pain	Hamstring palpation; isometric hamstring contraction	Pain free
Range of movement	Active knee extension test	Symmetrical (≤10°) compared to healthy side
Hamstring strength	Isokinetic dynamometer (60, 180 and 300 °/s)	Concentric strength (≤10%) compared to healthy side

Results

The mean age of participants was 22.8±4.03 years in the PRP-T group, and 24.8±6.42 years in the CON group (p=0.57). Majority of the participants were male (70%), in which majority were Malay ethnicity (40%). Half of the participants were national athletes, whereas the other half was state/club/school athletes, who actively engaged in several popular sports in Malaysia. Details of the hamstring injury in both groups were documented and compared (p=0.29). The mean time to RTP was 27.6±14.99 and 42.2±17.3 days for PRP-T and CON group, respectively (p=0.19). However, Cohen's d analysis showed large effect size for RTP between the groups (d=0.9). No significant difference in anthropometric description, injury characteristic and RTP between groups was noted (Table 3).

Due to hamstring injury, antioxidant enzymes' activity was actively present during the healing process. Our analysis showed that there was a significant interaction between groups and time in CAT activity, where the CAT activity in CON group significantly rose above baseline and became comparably higher than PRP-T group at W2 (difference of 47.55% between groups, p=0.04) (Figure 1). However, the difference between groups became closer with a trend of reduced activity from W2, though nothing significant was noted at W4. In contrast, although there was a reduction in SOD activity (from W0 to W4) in both groups, there was no statistically significant difference between groups throughout the reference time frame (Figure 2).

Discussion

Despite reported clinical studies demonstrating the efficacy of PRP in treating muscle injuries, the biological interactions of muscle repair and regeneration capacity within the initial weeks of PRP treatment is still unclear. To the best of our knowledge, this is the first study that investigated the clinical effect of PRP treatment on antioxidant enzymes' activity and time to RTP following grade-2 hamstring injury among Malaysian athletes. The main finding of the present study showed that CAT activity was suppressed in the PRP-T group, compared to CON group which remained elevated throughout the study period. On the other hand, SOD did not show any difference between groups. Although the time to RTP between groups was not significant, effect size calculation showed a large interaction, which could be a determining factor in treatment choice.

Being one of the major antioxidant enzyme *in-vivo*, SOD is an important cellular antioxidant defence against oxidative activity resulting from inflammation and redox-active compounds from muscle damage and muscle

TABLE 3. Anthropometric description, injury characteristic and RTP of participants

Description	PRP-T group	CON group	p
Age, y, mean±SD	22.8±4.03	24.8±6.42	0.57
Weight, kg, mean±SD	79.95±26.9	64.97±10.32	0.29
Height, m, mean±SD	1.72±0.08	1.69±0.08	0.53
Gender, n (%)			0.49
Male	4 (80)	3 (60)	
Female	1 (20)	2 (40)	
Sports, n (%)			0.56
Athletics	1 (20)	0 (0)	
Soccer	1 (20)	1 (20)	
Others (basketball, hockey, netball, rugby, tennis)	3 (60)	4 (80)	
Participation level, n (%)			0.06
National	4 (80)	1 (20)	
State/Club/School	1 (20)	4 (80)	
Circumstance of injury, n (%)			0.99
Training	4 (80)	4 (80)	
Competition	1 (20)	1 (20)	
Injury mechanism, n (%)			0.37
Sprinting	4 (80)	4 (80)	
Jumping	0 (0)	1 (20)	
Slip	1 (20)	0 (0)	
Hamstring injured, n (%)			0.29
Biceps femoris	5 (100)	3 (60)	
Semimembranosus	0 (0)	1 (20)	
Semitendinosus	0 (0)	1 (20)	
RTP, days, mean±SD	27.6±14.99	42.2±17.3	0.19

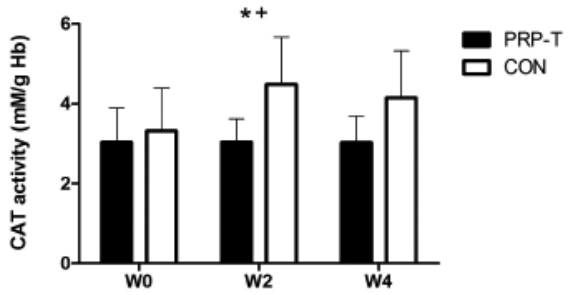

FIGURE 1. CAT activity between PRP-T and CON groups on W2 and W4

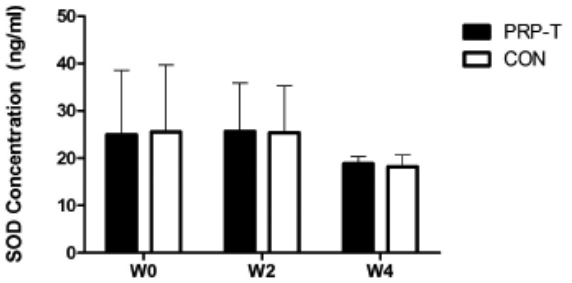

FIGURE 2. SOD activity between PRP-T and CON groups on W2 and W4

metabolism (Hassan 1988). A study by Vaille et al. (1990) has shown that an intravenous injection of SOD attenuated the inflammation by infiltration of inflammatory cells to facilitate the regeneration process. In the present study, it seems that PRP may not influence this redox sensitive enzyme, hence the SOD activity was similar between groups. Although statistical difference was not detected in SOD, both groups showed a reduction trend from W0 to W4. It has been suggested that trained athletes have an enhanced antioxidant defence system that is capable to overcome lipid and protein peroxidation (Gomez-Cabrera et al. 2008), which subsequently restraint secondary tissue damage. Although past study indicates that antioxidant enzymes increase following an oxidative event (i.e. exercise induced muscle injury) (Duthie et al. 1990), the rise may not be physiologically proportionate to the needs of pro-oxidant event; there can be little to no change in SOD activity (Laughlin et al. 1990).

As a consequence of both, presence of phagocytic cells attracted to the injured site and enhanced oxygen consumption (Brancaccio et al. 2010), high amount of metabolic by-products (i.e. H_2O_2) are produced. H_2O_2 is relatively more reactive than superoxide as it could readily be converted to reactive $^{\bullet}OH$ radical (Halliwell et al. 2000). H_2O_2 could damage cell membranes, denature proteins, disrupt cell chromosomes and initiate inflammatory cascades, where these secondary damages could lead to muscle atrophy, possibly delaying the healing process (Tiidus 2008). In the present study, CAT activity in PRP-T group was significantly lower compared to CON group, which implies that the H_2O_2 production from inflammatory responses was suppressed. This phenomenon could be attributed to PRP's capability to activate antioxidant response elements (ARE) through nerve related growth factor 2 (Nrf2) (Tohidnezhad et al. 2011). This reaction is found to be capable of regulating inflammatory responses during muscle healing process that favours muscle regeneration (Ishii et al. 2000). In turn, it is speculated that these elevated antioxidant enzymes and inflammation modulating activities potentially reduced the formation of free radicals. The findings from present study indicate that PRP possibly affects production of H_2O_2.

The present study showed that PRP treatment improved time to RTP, where the PRP-T group (27.6 days) showed a trend of hastened time to RTP compared to CON group (42.2 days). This finding was consistent with most published PRP studies with a range of 17 to 35 days for RTP (Bernuzzi et al. 2014; Hamid et al. 2014; Hamilton et al. 2010). Although statistical analysis revealed no significant difference in RTP between both groups, the difference of around 15 days can be an important treatment choice for athletes to recommence to their sport activity.

CONCLUSION

The use of PRP treatment following grade-2 hamstring injury could potentially reduce the degree of secondary tissue damage and facilitated muscle recovery through regulation of antioxidant enzymes (i.e. CAT). It was suggested that PRP may serve as an auxiliary treatment for injury management in hasten time to RTP.

FUTURE DIRECTION

Although the findings in the present study are promising, further research should attempt to investigate in a broader scale of oxidative stress that involve inflammatory markers, whole spectrum of antioxidant enzymes and other free radical biological markers, such as gluthatione peroxidase (GPx), malondialdehyde (MDA), thiobarbituric acid reactive substances (TBARS), isoprostanes (IsoPs), myeloperoxidase (MPO) and protein carbonyl groups. These data would provide detailed changes of oxidative activity after PRP treatment that contributes to a hastened healing.

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Lingling Lai & Mohd Nahar Azmi Mohamed
Sport Medicine Department
University of Malaya Medical Centre
59100 Kuala Lumpur
Malaysia

Lingling Lai*, Mohd Nahar Azmi Mohamed & Mohamed Razif Mohamed Ali
Sport Medicine Unit, Faculty of Medicine
University of Malaya, 50603 Kuala Lumpur
Malaysia

Khong Teng-Keen & Ashril Yusof
Sports Centre, University of Malaya
50603 Kuala Lumpur
Malaysia

*Corresponding author; email: lling5432@gmail.com

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