

**PLATELET-RICH PLASMA (PRP) FOR
THE TREATMENT OF MUSCLE INJURY**

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ABSTRACT

Muscle injury particularly the hamstring group is the most common type of sports related injury among athletes. Despite its frequent occurrence, the best treatment for hamstring injury is not known. Athletes affected by muscle injury often need considerable amount of time to recover. Recently autologous biological products are being used for treatment of soft tissue injury including that of muscles. Off recent, an autologous platelet-rich plasma (PRP) therapy is one of the 'hot topics' discussed in literatures. A systematic review of the available literatures on PRP therapy showed evidences to support PRP use for rotator cuff injury, lateral epicondylitis (tennis elbow), patellar tendinopathies and Achilles' tendinopathies. As clinical evidence to support its usefulness is limited, a randomised controlled trial is needed to examine the effect of PRP on muscle injury. Besides, current evidences were based on few laboratory animal studies and case reports.

Since information on injury prevalence and management of muscle injury in the local settings is limited, a cross-sectional study was conducted to investigate the pattern of muscle injury among Malaysian athletes. Medical records of athletes diagnosed with muscle injury were examined and injury mechanisms, types, treatment and duration to return-to-play (DRP) recorded. The pattern of muscle injury among Malaysian athletes was comparable to those reported by earlier researchers. Surprisingly, the DRP of local athletes were considerably longer compared with other studies (7.4 versus 3.8 weeks). Further, duration before first consultation, recurrent hamstring injury and female athletes were significant predictors of DRP.

The decision to allow return-to-play was based on athlete's symptoms of pain and objective clinical assessments including muscle flexibility test. An active knee extension (AKE) test was designed for assessment of hamstring flexibility in the RCT. A preliminary study on 14 healthy individuals showed excellent interater and test-retest reliabilities with intraclass correlation coefficient ranges from 0.78 to 0.92. This findings support the inclusion of the AKE test in the RCT.

Twenty-eight athletes diagnosed with grade-2 acute hamstring injury were recruited in an RCT to explore effect of PRP on DRP. Both intervention and control groups were prescribed with a standard rehabilitation program. Additionally patients in the PRP group received a single 3 ml injection of autologous PRP (approximately 5-fold increase in platelets and white blood cells) into the injured muscle. Significantly earlier DRP ($p = 0.013$) was noted among participants in the PRP (median $21.0 \pm$ IQR 13.0 days) compared with control (median $34.0 \pm$ IQR 37.3 days). In addition the PRP group has significantly ($p \leq 0.001$) lower pain severity score ($1.14 \pm$ SE 0.19) than control at all time points ($2.31 \pm$ SE 0.23). Furthermore participants reported no severe adverse effect of PRP therapy. In conclusion the findings of this research suggest PRP therapy is a safe and effective treatment for muscle injury.

ABSTRAK

Kecederaan otot terutamanya kumpulan otot *hamstring* merupakan kecederaan sukan yang kerap dialami oleh atlet. Meskipun kecederaan ini kerap berlaku, rawatan terbaik bagi kecederaan otot masih belum dikenalpasti. Kebanyakan atlet mengambil masa yang panjang untuk pulih dari kecederaan otot. Kebelakangan ini produk *autologous* biologikal mula digunakan bagi merawat kecederaan tisu lembut termasuk kecederaan otot. Kebelakangan, penggunaan *autologous platelet-rich plasma (PRP)* bagi rawatan kecederaan tisu lembut merupakan topik hangat dibincangkan dalam literatur. Memandangkan bukti menyokong penggunaan PRP masih tidak mencukupi kajian berbentuk ujian kawalan rawak diperlukan bagi menentukan kesan PRP untuk kecederaan otot.

Penganalisan ulasan sistematik literatur menunjukkan bukti menyokong penggunaan PRP bagi kecederaan *rotator cuff, lateral epicondylitis, patellar tendinopathies dan Achilles' tendinopathies*. Namun begitu pengetahuan berkenaan penggunaan PRP bagi kecederaan otot masih terbatas. Di samping itu bukti sedia adalah berdasarkan kajian makmal haiwan dan beberapa laporan kes. Sehubungan itu suatu kajian ujian kawalan rawak (*RCT*) dirancang bagi mengkaji kesan PRP terhadap kecederaan otot. Memandangkan maklumat berkenaan prevalen kecederaan dan kaedah rawatan di kalangan atlet tempatan masih terhad. Suatu kajian *cross-sectional* bagi mengenal pasti corak kecederaan otot di kalangan atlet Malaysia dijalankan. Rekod perubatan atlet yang mengalami kecederaan otot diteliti. Maklumat berkaitan dengan kecederaan otot yang dialami termasuk mekanisme kecederaan, jenis kecederaan, jenis rawatan dan masa yang diambil untuk pulih (*DRP*) direkod. Corak kecederaan otot di kalangan atlet Malaysia adalah setara dengan kajian yang dilaporkan oleh pengkaji lain.

Menariknya DRP atlet tempatan lebih panjang berbanding kajian lain (7.4 berbanding 3.8 minggu). Masa sebelum konsultasi pertama, kecederaan hamstring berulang dan atlet wanita adalah faktor signifikan bagi meramalkan DRP.

Keputusan bagi membenarkan atlet untuk kembali bersukan adalah berdasarkan gejala kesakitan dan ujian objektif klinikal termasuk ujian fleksibiliti otot. Ujian *active knee extension (AKE)* dipilih bagi menentukan fleksibiliti hamstring dalam kajian RCT. Kajian awalan melibatkan 14 individu yang sihat menunjukkan kesahan intra-penguji dan uji-ujisemula yang sangat baik dengan koefisi korelasi intra-klas antara 0.78 hingga 0.92. Kajian ini menyokong penggunaan ujian AKE dalam kajian RCT.

Dua puluh lapan atlet yang mengalami kecederaan gred-2 hamstring mengambil bahagian dalam RCT ini. Kedua-dua kumpulan menerima program rehabilitasi yang piawai. Tambahan itu peserta dari kumpulan PRP juga menerima satu suntikan 3 ml *autologous PRP* (mengandungi 5 kali ganda jumlah platelet) pada otot yang cedera. Masa penyembuhan (DRP) bagi peserta kumpulan PRP secara signifikan lebih singkat ($p = 0.013$) (median $21.0 \pm$ IQR 13.0 hari) berbanding kawalan (median $34.0 \pm$ IQR 37.3 hari). Tambahan, peserta kumpulan PRP juga menunjukkan skor tahap kesakitan signifikan ($p \leq 0.001$) lebih rendah berbanding kawalan ($1.14 \pm$ SE 0.19) pada setiap poin masa ($2.31 \pm$ SE 0.23). Di samping itu tiada seorang peserta pun melaporkan kesan sampingan serius terhadap rawatan PRP. Kesimpulannya kajian ini mendapati rawatan PRP adalah selamat dan berkesan bagi kecederaan akut gred-2 hamstring.

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LIST OF ABBREVIATIONS

AB	Autologous Blood
ABI	Autologous Blood Injection
ACD-A	Anticoagulant Citrate Dextrose – Solution A
ACS	Autologous Conditioned Serum
AKE	Active Knee Extension
AOR	Adjusted Odds Ratio
ASIS	Anterior Superior Iliac Spine
BF	Biceps Femoris
bFGF	Beta Fibroblasts Growth Factor
BF _{lh}	Biceps Femoris Long Head
BF _{sh}	Biceps Femoris Short Head
BMI	Body Mass Index
BPI-SF	Brief Pain Inventory – Short Form
CC	Case Control
CG	Control Group
CI	Confidence Interval
CN	Central Nucleated
CNF	Central Nucleated Fibres
CONSORT	Consolidated Standards of Reporting Trials
CRF	Clinical Research Form
CT	Computed Tomography
DRP	Duration to Return to Play
RICE	Rest Ice Compression Elevation
EGF	Epidermal Growth Factor

ELISA	Enzyme Linked Immunosorbent Assay
FGF	Fibroblasts Growth Factor
HGF	Hepatocyte Growth Factor
ICC	Intraclass Correlation Coefficient
IGF-1	Insulin-like Growth Factor-1
IGF-2	Insulin-like Growth Factor-2
IQR	Interquartile Range
KEA	Knee Extension Angle
L-PRP	Leucocyte Platelet-Rich Plasma
LA	Local Anaesthetic
LIF	Leukemia Inhibitory Factor
LMM	Linear Mixed Model
MDC	Minimum Detectable Change
MGF	Melanocyte Growth Factor
MRI	Magnetic Resonance Imaging
NFL	National Football League
NOS	Newcastle-Ottawa Scale
NSAIDS	Nonsteroidal Anti-Inflammatories Drugs
NSI	National Sports Institute
OR	Odds Ratio
P-PRP	Pure Platelet-Rich Plasma
PATS	Progressive Agility and Trunk Stabilization
PDGF	Platelet Derived Growth Factor
PEdro	Physiotherapy Evidence Database
PEP	Platelet-Enriched Plasma
PPP	Platelet-Poor Plasma

PRES	Progressive Running and Eccentric Strengthening
PRFM	Platelet-Rich Fibrin Matrix
PRGF	Platelet-Rich Growth Factor
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PRP	Platelet-Rich Plasma
PRTEE	Patient-Rated Tennis Elbow Evaluation
PVC	Poly Vinyl Chloride
RCT	Randomised Controlled Trial
RICE	Rest Ice Compression Elevation
ROM	Range of Movement
RTP	Return-to-Play
SD	Standard Deviation
SE	Standard Error
SEM	Standard Error of Measurement
SER	Strength in External Rotation
SLR	Straight Leg Raise
SM	Semi Membranosus
SPSS	Statistical Package for Social Sciences
SR	Systematic Review
SST	Simple Shoulder Test
ST	Semi Tendinosus
TENS	Transcutaneous Electrical Nerve Stimulation
TGF- β	Transforming Growth Factor - β
UMMC	University Malaya Medical Centre
US	Ultrasound
VAS	Visual Analogue Scale

VEGF	Vascular Endothelial Growth Factor
EGF	Epidermal Growth Factor
WBC	White Blood Cell

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Chapter 1 Introduction

1.1 Background

Muscle injury is one of the commonest injury seen in athletes. This injury commonly affects athletes engaging in sports that involved in high-speed sprinting and kicking. Muscle injuries were reported in Australian rule football, rugby union, soccer and American football (Brooks *et al.*, 2005; Fuller *et al.*, 2005; Brooks, 2006; Elliott *et al.*, 2011; Ekstrand, 2012; Murphy *et al.*, 2012). Despite of its frequency the best management of muscle injury is still unclear (Reurink *et al.*, 2012). Sports medicine practitioners used various treatment methods in attempt to hasten recovery and allow early return-to-play (RTP). In the early phase following an injury the treatment objective is to limit the extent of injury. This can be achieved by applying the RICE principles which involve rest, intermittent application of ice, compression and elevation of the affected region (Järvinen *et al.*, 2000).

In the later stage, treatment focusses on control of symptoms and enhancing recovery. Several approaches are often combine including use of rehabilitation programmes, electrotherapeutic modalities, hyperbaric oxygen therapy and injection of substances to the injured area (Worrell, 1994; Drezner, 2003; Bennett *et al.*, 2005; Järvinen *et al.*, 2007; Heiderscheit *et al.*, 2010; Stevens *et al.*, 2010; Franklyn-Miller *et al.*, 2011). Clinical evidence to support these modalities for muscle injury is limited (Orchard *et al.*, 2008; Reurink *et al.*, 2012). Despite various treatment modalities, the duration of RTP following muscle injury is lengthy (Wright-Carpenter *et al.*, 2004; Askling, 2006). Currently, there is no consensus on the optimal treatment for muscle injury. More recently, injection of autologous platelet-rich plasma (PRP) have gain

much attention for muscle injury. Theoretically, PRP contains many growth factors and cytokines that is essential for regenerating muscle fibres. These substances are stored within the α - and dense granules of platelets (Mejia *et al.*, 2011). Preliminary animal study showed significant acceleration of muscle healing in injured mice (Wright-Carpenter *et al.*, 2004). Human study to demonstrate the potential capacity of PRP for muscle injury is limited. Controversies exist as two case control studies found contrasting effects of PRP for muscle injury (Wright-Carpenter *et al.*, 2004; Rettig *et al.*, 2013). More well design study to examine the effect of autologous PRP on muscle injury are needed (Engebretsen *et al.*, 2010).

A short review of the skeletal muscle structure and function is presented in this chapter. This is followed by a discussion on the pathobiology of muscle injury and muscle healing. The epidemiology of muscle injury specifically the hamstring muscle (most frequent muscle injured) including its incidence, mechanisms of injuries and injury severity are explored. Finally, the current management of muscle injury is discussed.

1.2 Skeletal muscle

Skeletal muscle cells are unique as they have the capacities of contracting in response to activation by an action potential (Marieb, 2009; Tate, 2011). Each muscle in the human body comprised of multiple bundles of muscle fascicles. These muscle fascicles itself are collection of muscle fibres grouped. Each muscle fibre is an elongated cells extending throughout the length of the muscle (Figure 1.1).

Muscle bundles usually comprised of various muscle fibre subtypes. Depending on the muscle main role, the proportion of fibre subtypes distribution differs. Fast contracting muscles have a higher percentage of Type II fibres, whereas slower contracting muscle comprised mainly of Type I fibres. Therefore, sprinters, have a higher percentage of muscle Type II fibres while long-distance runners have a lower percentage of Type II fibres in the vastus lateralis muscle (Komi *et al.*, 1977).

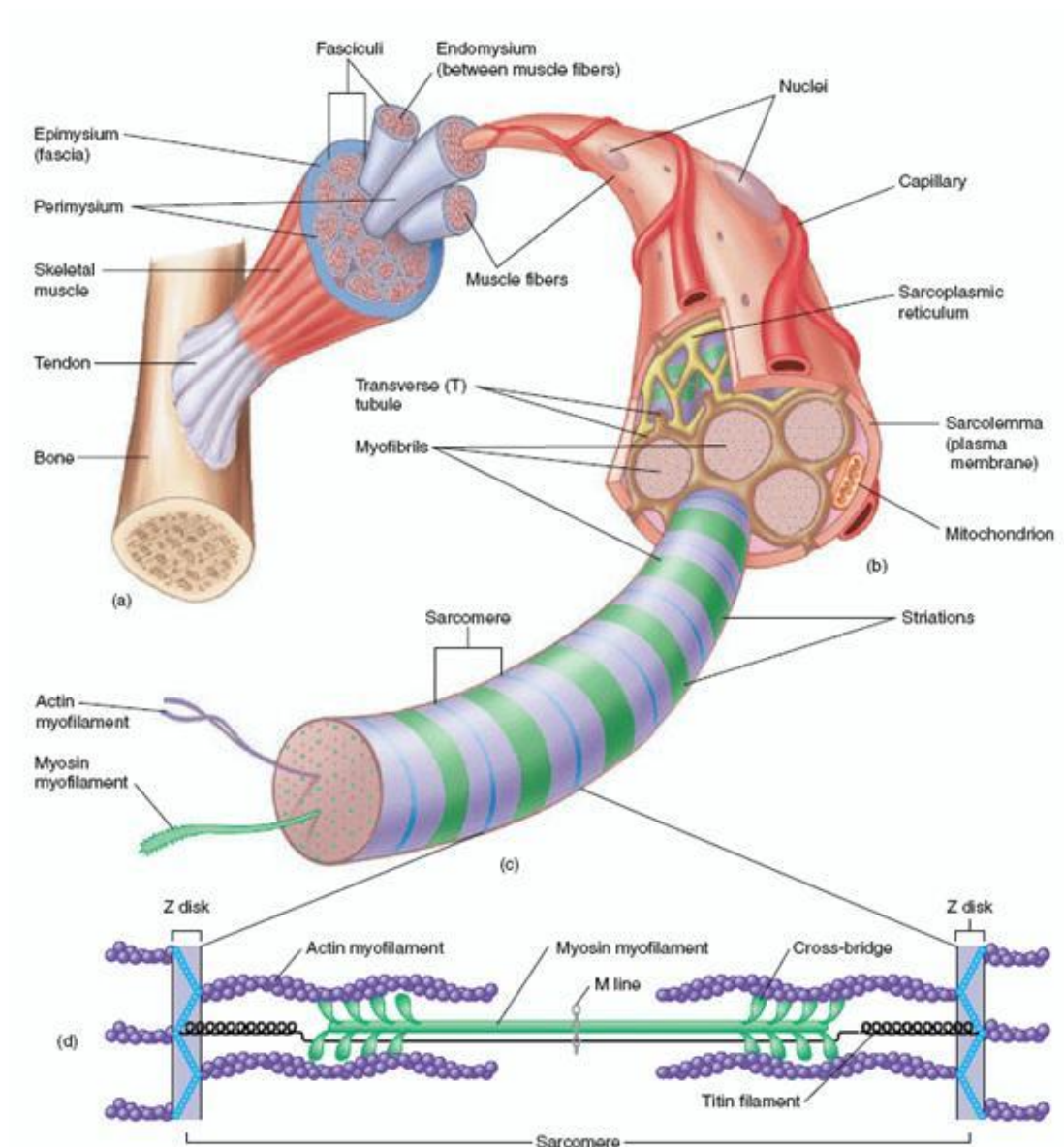


Figure 1.1. Skeletal muscle structure (Source: Seeley's Principle of Anatomy & Physiology, 2nd Edition 2012, p. 201).

Type II muscle fibres are more susceptible to eccentric contraction-induced injury than Type I fibres. This was demonstrated in both human and animal studies (Friden *et al.*, 1983; Jones *et al.*, 1986). It is hypothesized that depletion of high-energy phosphates and resulting formation of actin-myosin cross-bridges in the rigor state is responsible for the increased injury susceptibility of type II fibres. Breaking the rigor cross-bridges through mechanical loading induced by eccentric contractions could lead to muscle injury (Patel & Cuizon, 1998).

Differences in the cytoskeletal proteins between Type I and II muscle fibres might also play a role in injury susceptibility. Type I fibres have higher levels of certain cytoskeletal proteins that provide structural support to sarcomeres and the cell membranes. These structures provide protection from eccentric contraction-induced injury (Koh, 2002). Other protective substances, including a family of “stress proteins” may also affect the risk of injury. These stress proteins, also known as “heat shock proteins” protects cells from mechanical stress during eccentric contraction. The levels of “heat shock proteins” are higher in Type I than II fibres (Koh, 2002).

1.3 Skeletal muscle injury

Muscle injury is one of the commonest sports related injuries affecting elite or recreational athletes (Järvinen *et al.*, 2000; Brooks *et al.*, 2005; Ekstrand, 2008). These injuries occur through various mechanisms, including direct trauma (e.g., lacerations, contusions and strains) and indirect causes (e.g. ischemia and neurological dysfunction) (Schiaffino *et al.*, 2008; Tiidus, 2008; Tate, 2011). More than 90 % of sports related muscle injuries are either contusions or strains (Järvinen *et al.*, 1993; William, 1999),

while muscle laceration occurs less frequent. A muscle contusion occurs when a muscle is subjected to sudden heavy compressive force, such as a direct hit to the muscle often seen in contact sports (Junge *et al.*, 2004; Järvinen *et al.*, 2005; Junge *et al.*, 2010). Muscle strains on the other hand are often reported during explosive activities such as jumping and sprinting, when the muscle develops tension while lengthening (eccentric contraction) (Crisco *et al.*, 1994; Gabbe *et al.*, 2004; Järvinen, 2005). Muscle strain occurs especially when the contractions are performed by muscle not previously conditioned with eccentric contractions (Jones *et al.*, 1986; Garrett, 1996; Schultz, 1989; Lieber & Friden, 2007; Tiidus, 2008; Flann *et al.*, 2011). Patel *et al.* (1998) hypothesised the low oxidative capacity of fast glycolytic fibres predisposes to injury during repetitive eccentric contractions through depletion of high-energy phosphates. Depletion of high-energy phosphates subsequently leads to formation of actin-myosin cross-linkages in the rigor state. Added mechanical loading induced by eccentric contractions will cause breaking of this rigor cross bridges causing muscle injury (Patel & Cuizon, 1998; Scott *et al.*, 2001).

Garret (1996), reported that certain muscle are more susceptible to strain injury. Muscle that crosses multiple joints or has complex architecture such as the hamstring; quadriceps and gastrocnemius muscles have higher risk of injury (Komi *et al.*, 1977; Garrett, 1996; Ekstrand, 2012; Ropiak & Bosco, 2012). Hamstring muscle injuries are the most common muscle injury reported in athletes (Garrett, 1996; Patel & Cuizon, 1998; Junge *et al.*, 2004; Ekstrand, 2006; Eirale *et al.*, 2013).

1.3.1 Pathobiology of muscle injuries and healing

Injury to muscle, whether through direct trauma or indirect strain, follows a fairly constant healing pattern. Unlike bone, muscle has limited ability to regenerate new fibres; following injury muscle heals through repair process. Three phases of muscle healing have been identified; (1) destruction phase, (2) repair phase and (3) remodelling phase (Figure 1.2) (Kalimo *et al.*, 1997; Hurme *et al.*, 2006; Koh, 2008). The latter 2 phases are usually closely associated and chronologically overlapped each other. In each phases, complex interaction between various cells including inflammatory cells, platelets, fibroblasts, satellite cells, and substances such as cytokines and growth factors are involved (Järvinen *et al.*, 2000; Koh, 2002; Järvinen, 2005; Tiidus, 2008).

1.3.1 (a) Destruction phase

Excessive mechanical force can lead to tearing of the sarcoplasm in muscle injury. As myofibres are long and string-like cells there is a possibility that necrosis can extend along the entire muscle length. This is prevented by a condensation of cytoskeletal material known as contraction band (Järvinen, 2005).

Cellular contents released from injured cells serve as chemo attractants (wound hormones) further heightening the inflammatory reaction (Järvinen, 2005).

Inflammatory cells (mainly macrophages) infiltrate injured muscle to remove necrotic cells via phagocytosis while leaving the basal lamina intact. The intact basal lamina serves as scaffolds for satellite cells to form new myofibres (Hurme *et al.*, 1992; Hurme *et al.*, 1993).

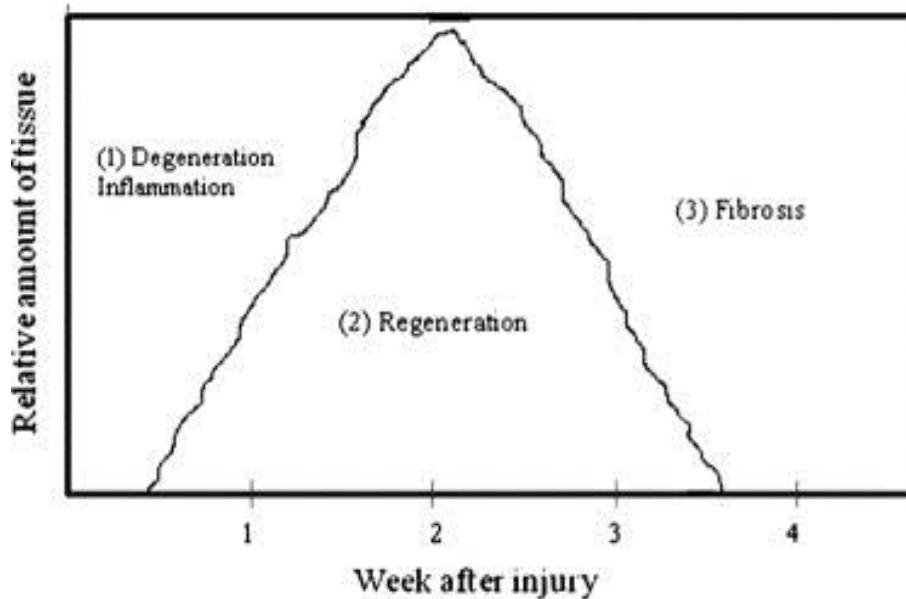


Figure 1.2. Different stages of muscle healing after injury (Source: Huard *et al.*, 2002, p. 826).

1.3.1 (b) Repair and remodelling phase

The repair phase starts once the destruction phase has subsided. This phase begins with two concomitant processes that are supportive but at the same time competitive; the regeneration of disrupted myofibres (including nerves) and the formation of a connective tissue scar (Kalimo *et al.*, 1997; Hurme *et al.*, 2006). Optimal recovery of muscle contractile function depends on a balance progression of both processes.

Immediately after injury, the gap that develops between ruptured muscle fibres is filled with blood clot (haematoma) (Figure 1.3). Within the next few hours, inflammatory cells including phagocytes arrived to the injured site to remove necrotic cells and haematoma. This is followed by invasion of fibroblasts, forming early granulation tissue. Later, fibroblasts start to synthesise proteins and proteoglycans to restore the integrity of the connective tissue framework (Lehto *et al.*, 1986; Hurme *et*

al., 1991; Hurme *et al.*, 2006). In the early stage fibroblasts synthesise fibronectin, tenascin-C and type III collagen. After few days Type I collagen is replaced with the considerably stronger scar tissue (mainly Type II collagen) (Hurme *et al.*, 1991).

Muscle tissue limited regeneration capacity occurs by means of a pool of undifferentiated reserve cells called the satellite cells. These cells are located underneath the basal lamina of individual myofibre (Kalimo *et al.*, 1997; Tiidus, 2008). Satellite cells proliferate and later differentiate into multinucleated myoblast in response to injury. In a laboratory studies on single muscle fibres culture, Bischoff *et al.*, (2006) showed satellite cells activation and proliferation occurred only when exposed to crushed muscle extract. These cells remain quiescent when exposed to normal muscle extract or crushed extract from non-muscle tissues. They concluded that myogenic satellite cells, and muscle regeneration are regulated by multiple growth factors released by injured muscle (Figure 1.4). Their conclusion was supported by *in vitro* and *in vivo* studies by other researches (Table 1.1).

The newly formed multinucleated myotubes will then fused with the injured myofibres that have survived the initial injury (Järvinen *et al.*, 2007). On both ends of connective tissue scar, survived muscle fibres form multiple branches in attempt to pierce through the scar separating them. But, after extending for a short distance, most regenerated myofibres only managed to adhere to connective tissue forming minimyotendinous junction with scar (Vaittinen *et al.*, 2002). As scar tissue contracts with time, the stumps adhere closer to one another (Hurme *et al.*, 1993). Also, the regenerative capacity of skeletal muscle is significantly reduced with age. This diminished capacity is attributed to the deterioration and slowing down of each phases of the repair (Järvinen *et al.*, 1983).

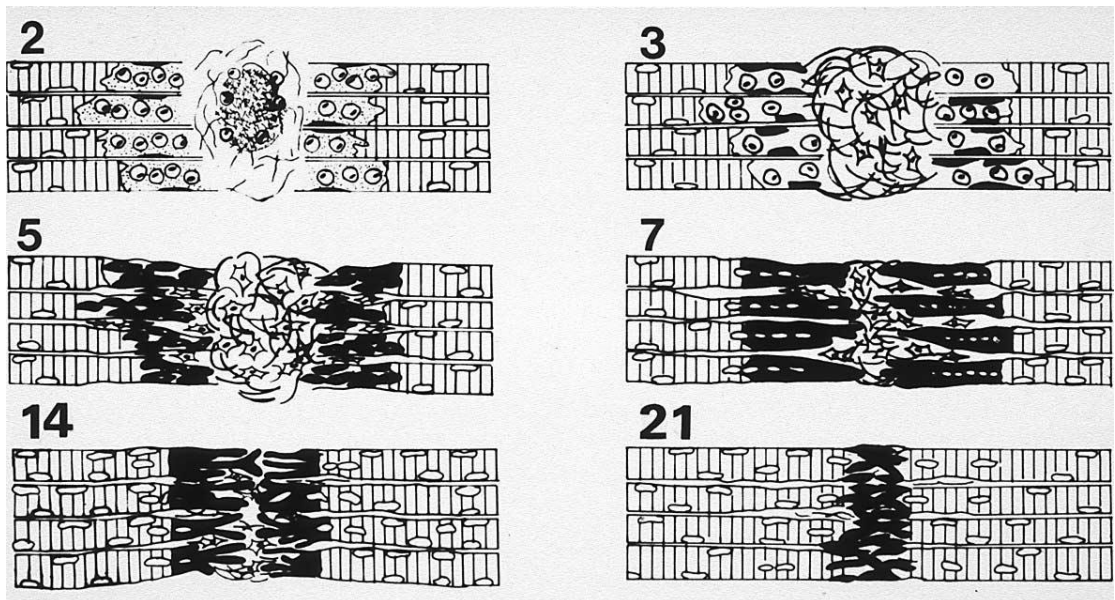


Figure 1.3. A schematic illustration of the healing skeletal muscle.

Day 2: necrotized parts are being removed by macrophages while, concomitantly, the formation of connective tissue scar by fibroblasts has begun. Day 3: satellite cells have become activated within the basal lamina cylinders. Day 5: myoblasts have fused into myotubes with the connective tissues become denser. Day 7: the regenerating muscle fibres extend out of the old basal lamina cylinders and begin to pierce through the scar. Day 14: the scar further condensed and reduced in size, and the regenerating myofibers closing the gap. Day 21: the interlacing myofibers are virtually fused with little intervening connective tissue (scar) in between (Source: Järvinen *et al.*, 2007, p. 319).

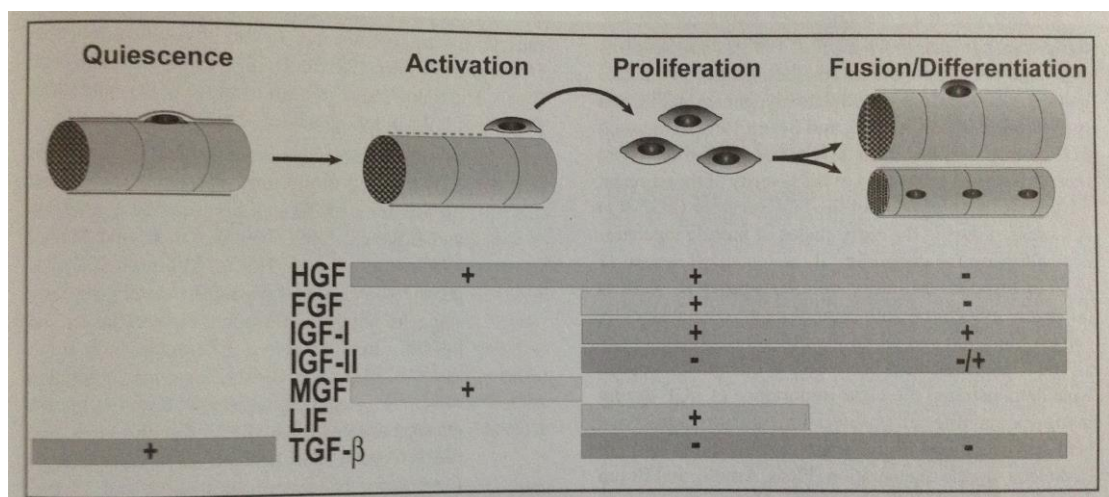


Figure 1.4. Regulators of myogenic satellite cells activation, proliferation and differentiation. FGF = fibroblasts growth factor, IGF = insulin-like growth factor, LIF = leukaemia inhibitory factor, TGF - β = transforming growth factor beta (Source: Tiidus 2008, p. 83).

Table 1.1 : Growth factors affecting myogenic satellite cells activity.

<i>In vitro</i> studies		Myogenic satellite cells activity		
Growth factor	Activation	Proliferation	Differentiation	References
HGF	Increased	Increased	Decreased	(Allen <i>et al.</i> , 1995; Miller <i>et al.</i> , 2000)
FGF		Increased	Decreased	(Thompson <i>et al.</i> , 1989)
IGF-1		Increased	Increased	(Thompson <i>et al.</i> , 1989)
IGF-2		Increased	Increased	(Florini, 1987; Haugk <i>et al.</i> , 1995)
MGF	Increased			(McKoy <i>et al.</i> , 1999)
LIF		Increased		(Austin & Burgess, 1991)
TGF- β		Decreased	Decreased	(Allen & Boxhorn, 1989)
<i>In vivo</i> studies		Myogenic satellite cells activity		
HGF	Increased			(Tatsumi <i>et al.</i> , 1998)
FGF		Increased	Decreased	(Armand <i>et al.</i> , 2005)
IGF-1		Increased	Increased	(Chakravarthy <i>et al.</i> , 2000)
IGF-2		Decreased	Decreased	(Kirk <i>et al.</i> , 2003)
MGF	Increased			(Hill <i>et al.</i> , 2003)
LIF		Increased		(Kurek <i>et al.</i> , 1997)
TGF- β		Decreased		(Thomas <i>et al.</i> , 2000)

1.4 Epidemiology of muscle injury

1.4.1 Incidence of muscle injury

One of the frequently injured muscles is the hamstring's muscle group (biceps femoris, semitendinosus and semimembranosus). Hamstring muscle injury accounts for 12 – 52 % of all injuries suffered in sports events (Askling *et al.*, 2004; Feeley *et al.*, 2008; Shariff *et al.*, 2009; Ekstrand *et al.*, 2011; Orchard & Seward, 2011; Murphy *et*

al., 2012). A two-year prospective study among professional rugby union players reported higher incidence of hamstring injury during rugby match (5.6 per 1000 match hours) than at training (0.27 per 1000 training hours). They also noted majority of hamstring injury often occurred during running. Injuries resulting from kicking however were associated with longer recovery period (36 days) (Brooks *et al.*, 2005; Brooks, 2006; Elliott *et al.*, 2011; Ekstrand, 2012; Murphy *et al.*, 2012). An injury rate of 0.87 per 1000 hours of exposure was reported in competitive sprinters. The incidence of hamstring injuries was higher at the beginning of the season, with 58.3 % injuries occurring in the first 100 hours of exposure. Further analysis showed athletes with hamstring: quadriceps peak torque ratio less than 0.60 at angular speed of 180 °/sec have a 17-fold increase risk of hamstring injury (Yeung *et al.*, 2009; Reurink *et al.*, 2012).

Data from one of the longest injury survey study (from 1992 – 2012) found hamstring injury as the most frequent and prevalent injury among Australian Football League players. They estimated six new hamstring injury occurs per club per seasons, resulting in 20 missed matches each season. Surprisingly, the incidence of hamstring injury has not declined in recent decades (Orchard *et al.*, 2008; Orchard *et al.*, 2013).

Posterior thigh strains were reported as the most common injury among professional football players (Ekstrand *et al.*, 2012; Reurink *et al.*, 2012). Seven players are expected to suffer new hamstring muscle injury in a team of 25 per season. Further, approximately 12 % of athletes who suffered hamstring injuries require more than 28 days to achieve full recovery (Wright-Carpenter *et al.*, 2004; Askling, 2006; Ekstrand *et al.*, 2011). In a self-reported hamstring injury study among ballet dancers, Askling *et al.* (2002) found 34 % of dancers reported history of acute and 17 % had history of chronic

hamstring injuries over the past 10 years. They noted majority of hamstring injuries occurred during flexibility training activities such as splits while only a few (12 %) during powerful movements (Askling *et al.*, 2002). Researchers also reported hamstring injuries mainly occurred during sprinting, kicking or jumping as muscle develops tension while lengthening (Zarins *et al.*, 1983; Kujala *et al.*, 1997; Wright-Carpenter *et al.*, 2004a).

1.4.2 Anatomy of hamstring muscles

The hamstring muscle group is located in the posterior compartment of the thigh (Figure 1.5). This muscle primarily involved in hip extension and knee flexion. In addition, hamstring muscle also medially (semimembranosus) or externally rotates (biceps femoris) the knee while in flexion. The hamstring's muscle group consists of semimembranosus (SM), semitendinosus (ST) and biceps femoris muscles. These muscles are long, and predominantly biarticular, hence higher susceptibility to strain injury (Heiser *et al.*, 1984; Bennell *et al.*, 1998; Orchard & Seward, 2002; Slavotinek *et al.*, 2002; Engebretsen *et al.*, 2010). In addition, hamstring muscles contain significantly high proportion of Type II fibres compared with quadriceps or adductors (Garrett *et al.*, 1984; Brooks, 2006; Marieb, 2009; Elliott *et al.*, 2011; Orchard & Seward, 2011; Tate, 2011; Ekstrand, 2012).

1.4.2 (a) Semimembranosus (SM)

The SM has the longest proximal tendon (72.7 % of total SM length) of all the hamstring muscles. The proximal tendon attaches to the lateral part of the upper half of

the ischial tuberosity. From here the tendon widened becoming broad, expansive and aponeurotic, being thick and rounded at its lateral border and flattening into a thin membrane medially (Komi *et al.*, 1977; Woodley & Mercer, 2005; Reurink *et al.*, 2012). The distal tendon of semimembranosus is thicker and shorter and attaches into the posterior part of the medial condyle of the tibia. The tibial division of the sciatic nerve usually innervates the SM muscle.

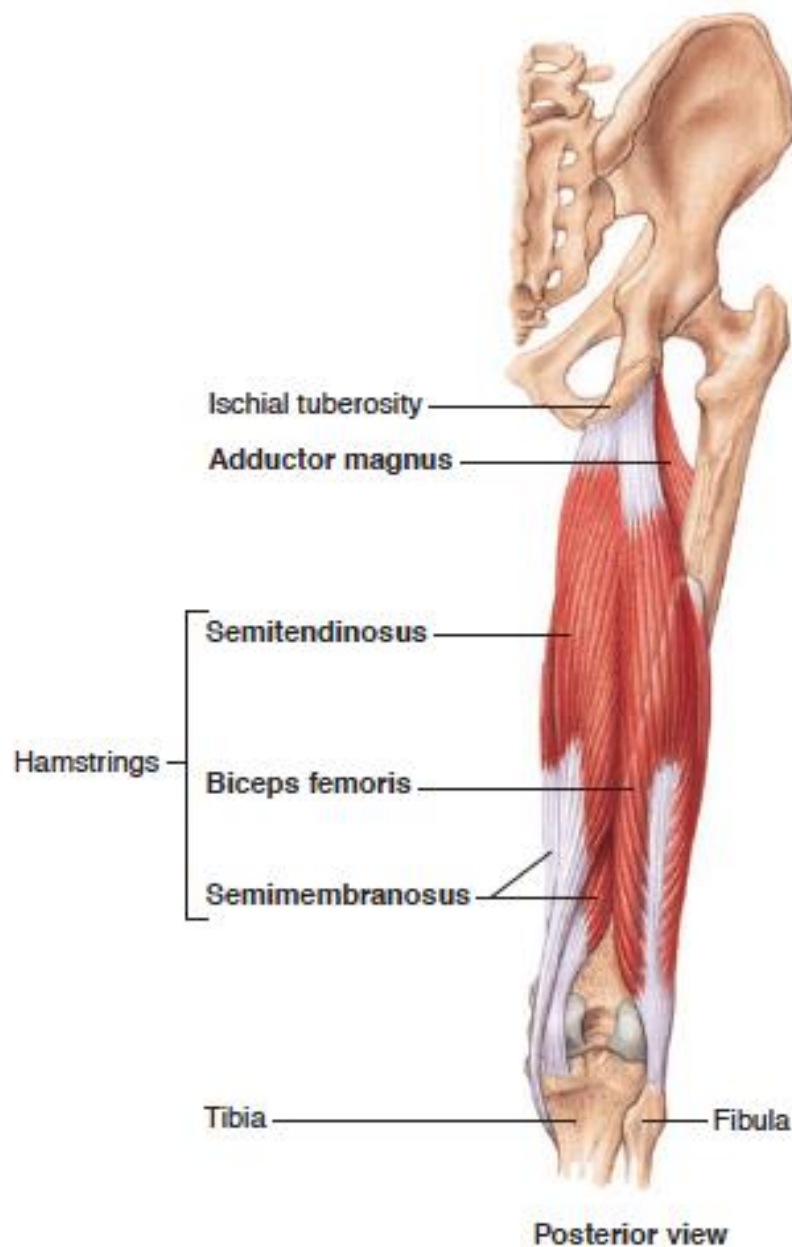


Figure 1.5. Right posterior thigh (hamstring) muscle (Source: Seeley's Principles of Anatomy & Physiology 2nd Edition 2012, p. 259).

1.4.2 (b) Semitendinosus (ST)

The proximal ST muscle arose from distinct locations: the posteromedial parts of the medial portion upper half of the ischial tuberosity and the medial border of BF long head tendon. The distal tendon is long and thin and passes along the medial aspect of the knee joint (Friden *et al.*, 1983; Jones *et al.*, 1986; Woodley & Mercer, 2005; Orchard *et al.*, 2008; Reurink *et al.*, 2012). The distal ST tendon on the other hand attaches to the medial surface of proximal tibia. The ST muscle receives nerve supply from the tibial division of the sciatic nerve.

1.4.2 (c) Biceps femoris (BF)

The BF has two distinct portions; the BF long head (BF_{lh}) and short head (BF_{sh}). The BF_{lh} has a fusiform muscle belly, and attaches to the medial portion of the upper half of the ischial tuberosity by means of a thick, round tendon (Patel & Cuizon, 1998; Woodley & Mercer, 2005; Askling, 2006). BF_{lh} distal tendon is flattened on the sides, with muscle fibres of the short head inserted into its deep surface. The tendon passes downwards and forwards directly inserted into the head of the fibula, the lateral ligament of the knee and lateral condyle of the tibia. In addition, the tendon blends anteriorly with the ilio-tibial tract and gives off expansions to the crural fascia covering the anterior, lateral and posterior compartment of the leg. The BF_{lh} is supplied by one muscle nerve that branches off the sciatic nerve (Woodley & Mercer, 2005; Koh, 2008; Tate, 2011).

The short head of BF (BF_{sh}) arose from three locations: (i) the linea aspera of the femur, (ii) the upper two thirds of the lateral supracondylar line and (iii) the lateral

intermuscular septum (separating BFsh from the vastus lateralis muscle). The muscle belly of BFsh is relatively thin, but broad and long. The distal tendon of BFsh inserted into the tendon of BFllh and innervated by two nerve branches: (i) the nerve branch from the sciatic nerve and (ii) the nerve branch from the common peroneal nerve.

The dual innervation of the BF muscle might lead to asynchronous stimulation of the two heads (Sutton, 1984; Koh, 2002; Woods *et al.*, 2004; Wright-Carpenter *et al.*, 2004). It had been suggested that mistimed contraction of the different parts of hamstring muscles (BFllh and BFsh) leads to reduced capacity to produce effective tension to control imposed loads of the muscle (Zuluaga, 1995; Järvinen *et al.*, 2000; Brooks *et al.*, 2005; Ekstrand, 2008; Engebretsen *et al.*, 2010).

1.4.3 Mechanism of hamstring injury

Previous researches noted hamstring injury often occurred during eccentric muscle contraction (Zarins & Ciullo, 1983; Garrett *et al.*, 1989; Brockett *et al.*, 2004; Schiaffino & Partridge, 2008; Tiidus, 2008; Marieb, 2009; Tate, 2011). Eccentric contraction occurs during many sport activities including running, sprinting, jumping, kicking and during passive hamstring stretching (Komi *et al.*, 1977; Järvinen & Lehto, 1993; William, 1999; Seward, 2003; Woods *et al.*, 2004; Askling, 2006). A total of 587 hamstring injuries were diagnosed over seven-year follow-up period among European male professional footballers. Majority of these injuries occurred while running or sprinting (Friden *et al.*, 1983; Jones *et al.*, 1986; Junge *et al.*, 2004; Järvinen, 2005; Junge & Dvorak, 2010; Ekstrand, 2012). Hamstring injury was also the most prevalent injury in Gaelic football; the author described noncontact nature, including sprinting,

turning and landing from a jump as the principal mechanism of injuries (Murphy *et al.*, 2012). Most (76.9 %) hamstring injury observed by Gabbe *et al.* (2004) occurred in competition, with the rest occurred at training sessions. The majority (80.8 %) of hamstring injury occurred while performing rapid acceleration movement during running or sprinting (Gabbe *et al.*, 2004). Kicking was responsible for hamstring injury less often (19.2 %) (Jones *et al.*, 1986; Schultz, 1989; Garrett, 1996; Gabbe *et al.*, 2004; Lieber & Friden, 2007; Koh, 2008; Tiidus, 2008; Flann *et al.*, 2011). Others, however, observed acute hamstring injury occurred more often (88 %) during slow, controlled, voluntary stretching activities with remaining 12 % occurred during a powerful and energetic movement such as a 'grandejeté' (split jumping) (Patel & Cuizon, 1998; Scott *et al.*, 2001; Askling *et al.*, 2002; Koh, 2002).

Askling *et al.* (2000) reported two cases of acute hamstring injury with different aetiologies. The magnetic resonance imaging (MRI) performed in both cases demonstrated injury occurred at two different locations, suggesting different tissues involvement. In artistic dancer, hamstring injury occurred during slow stretching activities and involved proximal semimembranosus tendon. In sprinter however, MRI changes mainly observed in the lateral and anterior part of the semitendinosus muscle belly. Further, the recovery period was significantly longer for the dancer (18 months vs. 6 months) than the sprinter. They demonstrated the importance of defining the exact anatomical localisation of injured tissue and the duration of recovery is also affected by type of tissue involved (Askling *et al.*, 2000).

A laboratory study conducted by McCully & Faulkner (1985) concluded lengthening (eccentric) contraction causes significant skeletal muscle injury compared with isometric or shortening contraction (McCully & Faulkner, 1985). During eccentric

exercises, the contracting muscles are forcibly stretched which might disrupt cellular structure and function (Jones *et al.*, 1986; Garrett, 1996; Järvinen *et al.*, 2000; Schiaffino & Partridge, 2008; Tiidus, 2008; Tate, 2011; Liu *et al.*, 2012). Such contraction occurs during downhill running when the contracting quadriceps muscle forcibly lengthened against the force of gravity with each step (Järvinen & Lehto, 1993; William, 1999; Proske & Morgan, 2001). Chumanov *et al.* (2012) observed that peak hamstring musculotendinous stretch occurred during late swing of the gait cycle. They also shown that peak hamstring force and negative musculotendinous work increased significantly with speed (Junge *et al.*, 2004; Järvinen, 2005; Thelen *et al.*, 2005; Junge & Dvorak, 2010; Chumanov *et al.*, 2012).

1.4.4 Location of hamstring injury

Radiological imaging modalities including ultrasonography (US), MRI and computed tomography (CT) allow accurate assessment of injury location, extent and severity (Crisco *et al.*, 1994; Garrett, 1996; Järvinen, 2005; Wong, 2005). Studies have consistently showed that forceful stretching injury usually occurs near the muscle-tendon and bone-tendon junction (Schultz, 1989; Garrett *et al.*, 1984; Jones *et al.*, 1986; Garrett, 1990; El-Khoury, 1995; Garrett, 1996; Lieber & Friden, 2007; Tiidus, 2008; Flann *et al.*, 2011; Silder *et al.*, 2013). A study on posterior thigh muscle injury in elite track and field athletes, found majority of hamstring (85 out of 90 cases) injuries affected the musculotendinous junction (Patel & Cuizon, 1998; Scott *et al.*, 2001; Malliaropoulos *et al.*, 2010). Similarly, Connell *et al.* (2004) found 62.2 % of hamstring injuries occurred at the musculotendinous junction on ultrasound (US) assessment (Connell *et al.*, 2004).

Researchers also showed most hamstring injuries affected the musculotendinous of the biceps femoris tendon (Friden *et al.*, 1983; Jones *et al.*, 1986; Garrett, 1996; Järvinen *et al.*, 2000; Connell *et al.*, 2004; Brooks, 2006; Cohen *et al.*, 2011; Liu *et al.*, 2012; Silder *et al.*, 2013). Up to 84 % of hamstring injuries among professional footballers affected the biceps femoris muscle (Garrett, 1996; Patel & Cuizon, 1998; Junge *et al.*, 2004; Ekstrand, 2006; Ekstrand *et al.*, 2012; Eirale *et al.*, 2013). Similarly, in a retrospective study of Australian Football League and Rugby league athletes, Comin *et al.* (2012) found majority of hamstring injury involved the bicep femoris muscle (45 of 62 hamstring injury cases). Hamstring injury involving the central tendon have significantly worse prognosis than those that only involve muscle fibres, epimysial fascia or the musculotendinous junction (Kalimo *et al.*, 1997; Hurme *et al.*, 2006; Koh, 2008; Comin *et al.*, 2012). In contrast, Askling *et al.* (2008) found semimembranosus muscle was most commonly injured in 83 % of athletes from various sports (Askling *et al.*, 2008).

1.4.5 Diagnosis of a hamstring injury

In most instances diagnosing a hamstring injury is easy and straightforward. Diagnosis begins with careful history of the injury as most athletes presented with sudden onset posterior thigh pain and tenderness sustained during activities (Huard *et al.*, 2002; Malliaropoulos *et al.*, 2010; Ekstrand *et al.*, 2011). Brooks *et al.* (2005) found most hamstring injuries resulted from noncontact injury during running and cutting movements (Brooks *et al.*, 2005; Järvinen, 2005). A thorough clinical examination (inspection and palpation) combined with functional hamstring muscle testing (flexibility and strength testing) is usually performed to diagnose hamstring injury

(DeLee, 2003; Järvinen, 2005; Gielen *et al.*, 2007; Järvinen *et al.*, 2007; Brukner & Khan, 2010). The diagnosis of hamstring injury is easy when a typical history of strain is accompanied by objective evidence of swelling and ecchymosis (bruising) just distal to the injured site (Hurme *et al.*, 1993; Järvinen *et al.*, 2007). In some cases, however, swelling and ecchymosis are less prominent especially when haematoma developed intramuscularly, where the extravasation of blood (from torn muscle fibres and blood vessels) are contained within the intact muscle fascia, limiting the size of swelling (Kalimo *et al.*, 1997; Järvinen *et al.*, 2000; Hurme *et al.*, 2006). In such cases, palpation of the affected site while athlete is in supine will usually elicit pain. In addition, athletes often have reduced flexibility (including the active knee extension test) and reduced strength of the injured hamstring (including 15 ° resisted knee flexion in supine test) (Lehto *et al.*, 1986; Hurme *et al.*, 1991; DeLee, 2003; Tornese *et al.*, 2006; Brukner & Khan, 2010; Malliaropoulos *et al.*, 2010). Based on hamstring clinical assessment several injury classifications were proposed (Table 1.2).

Unfortunately, clinical based classification does not accurately reflect the anatomy of the injury and has not been shown to reliably predict prognosis and time to return to sport (Best, 1995; Kalimo *et al.*, 1997; Tiidus, 2008). Therefore, radiological assessment including US and MRI has been recommended in assessing hamstring injuries (Table 1.3). The availability, low cost and ease of examination suggested that US might be superior to MRI (Peetrons, 2002; Järvinen *et al.*, 2007). Both US and MRI allow better understanding of the injury location, extent and severity of injury, which are relevant prognostic factors for predicting recovery time, return to pre-injury sport activity and risk of recurrence (Vaittinen *et al.*, 2002; Askling *et al.*, 2006; Guillodo *et al.*, 2011; Ekstrand *et al.*, 2012). Hence a classification system that combines both clinical and radiological imaging (MRI or US) of acute muscle injuries was proposed

(Hurme *et al.*, 2006; Cohen *et al.*, 2011; Chan *et al.*, 2012; Mueller-Wohlfahrt *et al.*, 2013).

Table 1.2 : Overview of clinically based muscle injury classification systems.

Author/s	Brukner & Khan (2010)	Järvinen <i>et al.</i> (2007)	DeLee & Drez (2003)	Malliaropoulos <i>et al.</i> (2010)
Injury severity				
Grade I	Strain involves a small numbers of muscle fibres with localized pain but no loss of strength.	Tear of only few muscle fibres with minor swelling and discomfort accompanied with no or minimal loss of strength and restriction of movement (ability to mobilise)	Tearing of few muscle fibres with mild and minimal loss of strength	AROM deficit < 10°
Grade II	Tear of significant number of muscle fibres with associated pain and swelling.	Greater damage to the muscle with clear loss of function (ability to contract)	Increased tearing of muscle fibres with some strength loss	AROM deficit 10° - 19°
Grade III	Complete tear of the muscle. Pain is reproduced on muscle contraction. Strength is reduced and movement is limited by pain	Tear extending across the entire cross-section of the muscle with virtually complete loss of muscle function	IIIA: Tearing of the entire muscle with complete loss of strength. IIIB: Avulsion fracture of at the tendon's origin or insertion site (more commonly in adolescent and small subset of adults)	AROM deficit 20° - 29°
Grade IV	NA	NA	NA	AROM deficit > 30°

AROM = active range of motion; NA = not applicable.

Table 1.3 : Overview of radiological muscle injury classification systems.

Author	Ultrasonography based		MRI based	
	Peetrans (2002)	Lee & Healy (2004)	Pathria & Boutin (2005)	Stoller (2007)
Injury severity				
Grade I	Minimal elongation with < 5 % of muscle involved	Normal appearance or focal or general areas of increased echogenicity.	Normal muscle morphology with mild abnormalities of muscle signal, particularly in the region of the myotendinous junction	MRI negative, no structural damage. Hyperintense oedema with or without hemorrhage
Grade II	Lesion involving from 5 to 50 % of the muscle volume or cross-sectional diameter	Hypervascularity around disrupted muscle fibres with intramuscular fluid collection with surrounding hypoechoic halo	Signal changes and mild alterations in muscle morphology. T2 weighted images may show irregularity, thinning, and mild waviness of the tendon fibres.	MRO-positive with tearing up to 50 % of the muscle fibres. Possible hyperintense focal defect and partial retraction of muscle fibres.
Grade III	Complete muscle tear with complete retraction	Complete myotendinous or tendooosseous avulsion	More significant alterations in the muscle morphology representing complete rupture of the myotendinous junction. Large amounts of haemorrhage with obvious tendons retraction	Muscle rupture or 100 % structural damage. Complete tearing with or without muscle retraction

1.4.6 Severity and recurrence

Currently there is no standard classification for muscle injury severity.

Researchers use several approaches in describing the severity of muscle injury. Injury severity often described by the number of days absent from games or training sessions in epidemiological studies. An injury is given an A grade if athlete missed less than 7 days; B if missed 7 to 14 days; C if missed more than 14 days and D if missed more than 8 weeks (Järvinen *et al.*, 1997; Feeley *et al.*, 2008). Other researchers classify

hamstring injury into minor (≤ 1 week), moderate (>1 to 3 weeks), and major (> 3 weeks) injuries based on time absence from training or competition (Askling *et al.*, 2004; Brooks *et al.*, 2005; Feeley *et al.*, 2008b; Shariff *et al.*, 2009; Ekstrand *et al.*, 2011a; Orchard & Seward, 2011; Murphy *et al.*, 2012).

Only few studies used specific clinical evaluation to decide on athlete's readiness to return-to-play (Askling *et al.*, 2002; Brooks *et al.*, 2005; Ekstrand, 2008; Feeley *et al.*, 2008b; Shariff *et al.*, 2009; Malliaropoulos *et al.*, 2010; Warren, *et al.*, 2010). Further, the criteria used for determination of athlete's readiness to return to sports vary across studies, as there is no consensus for safe return to sport following muscle injury (Askling *et al.*, 2004; Woods *et al.*, 2004; Brooks, 2006; Ekstrand, 2008; Feeley *et al.*, 2008b; Yeung *et al.*, 2009b; Warren *et al.*, 2010; Murphy *et al.*, 2012). In an attempt to standardise injury definitions and data collection, the FIFA Medical Assessment and Research Centre defines injury severity slight (0 - day); minimal (1 - 3 days); mild (4 - 7 days); moderate (8 - 28 days); severe (> 28 days) based on time loss from participation (Fuller *et al.*, 2006; Orchard *et al.*, 2013).

By combining anatomical diagnosis, physical examination and radiology imaging (including ultrasonography or MR imaging), the severity of hamstring injury can be categorised as Grade I: mild strain injury with minimum tear and minor loss of strength, Grade II: moderate strain injury with partial tear and significant loss of muscle strength that results in significant functional limitations, and Grade III: severe injury with complete rupture and associated with severe functional disability. Such grading is useful for standardising muscle injury severity and to forecast time-lost from participation (Tornese & Melegati, 2006; Järvinen *et al.*, 2007; Ekstrand *et al.*, 2011; Eirale *et al.*, 2013). The average time losses for different grades of hamstring injury

among professional soccer players was 17 days for grade I, 22 days for grade II and 73 days for grade III (Askling *et al.*, 2002; Ekstrand *et al.*, 2011).

Despite of various injury grading systems, it is comforting to know that majority of hamstring injuries were minor or moderate injuries. Brooks *et al.* (2006) found 74 % of hamstring muscle injuries occurred among professional rugby players were recorded as either minor or moderately severe injury (Brooks, 2006). Similarly, Malliaropoulos *et al.* (2011) also noted most hamstring injury falls under grades I to II injury severities (Malliaropoulos *et al.*, 2011; Tate, 2011). During the 1998/99 football seasons, 158 hamstring injuries recorded with 151 injuries were classified as grades I and II (Heiser *et al.*, 1984; Bennell *et al.*, 1998; Orchard & Seward, 2002; Slavotinek *et al.*, 2002; Dadebo *et al.*, 2004). In a prospective study of professional football players, only 31 (18.3 %) cases of major injuries were diagnosed between 1989 and 1996 (Elliott *et al.*, 2011). Further, Ekstrand *et al.* (2011) found severe hamstring injuries (causing absence of > 28 days) among professional soccer players represent only 16 % of total hamstring injuries (Ekstrand *et al.*, 2011).

Apart from causing considerable time lost from training and competition, another significant sequelae of hamstring injury are recurrent injuries (reinjuries). A recurrent injury is defined as an injury of the same type (diagnosis), which occurred after a player's full return to participation. A recurrent injury occurring within 2 months of a player's return to full participation is referred to as an "early recurrence" while those occurred between 2 to 12 months as "late recurrence". A recurrent injury occurring after 12 months is referred as "delayed recurrence" (Fuller *et al.*, 2006).

Analysis of recurrence data from the Australian Football League injury surveillance showed higher proportion of hamstring injury recurred during the first week after return to sport. Further, the risk of recurrence remains elevated for many weeks after return to play (Table 1.4) (Orchard & Best, 2002). Reduced tensile strength of the scar tissue, reduced muscle strength because of disuse atrophy, reduced flexibility of the muscle-tendon unit and adaptive changes in biomechanics following injury were suggested to be responsible of increased risk of recurrences (Orchard & Best, 2002).

The incidence of hamstring recurrent injury is estimated between 12 to 48 % of athletes (Hawkins *et al.*, 2001; Woods *et al.*, 2004; Brooks, 2006; Warren *et al.*, 2010; Malliaropoulos *et al.*, 2011; Ekstrand *et al.*, 2011; Murphy *et al.*, 2012; Eirale *et al.*, 2013). An audit of injuries in professional soccer in from 1997 to 1999 reported 202 (48 %) cases of recurrent hamstring injuries. Athletes who reinjured his hamstring within the same season were found to have a more severe injury than the previous injury and needed significantly longer recovery period (31.8 vs. 27.3 days) (Hawkins *et al.*, 2001). A prospective study of 51 football teams (2299 players) saw 16 % cases of recurrent hamstring muscle injury with reinjuries causes 30 % longer absences than did non-reinjuries (Ekstrand *et al.*, 2011).

Volpi *et al.* (2004) reported the lowest incidence of recurrent injury from a five-year injury survey of an Italian major league soccer team. Only one (3 %) case of recurrent hamstring injury was reported during the period of 1995 - 2000. The author suggested that constant and well coordinated monitoring of injured athletes by team doctor and other consultants form various areas including physiotherapists and trainers are responsible for such lower recurrent injury rate (Volpi *et al.*, 2004).

A steady decline in hamstring recurrence rate was observed over the past 10 years in Australian Football League. The decline in injury recurrences was attributed to more conservative approaches in managing hamstring injury and more conservative decision on return-to-play (Orchard & Seward, 2011; Orchard *et al.*, 2013).

Table 1.4 : Chance of hamstring injury recurrence after return from injury (1992 - 1998 Australian Football League) (Source: Orchard & Best 2002, pg:4)

Weeks after return from initial injury	Weekly percentage risk of injury recurrence (%)		
	Hamstring injury (n = 858)	Quadriceps injury (n = 251)	Calf injury (n = 217)
1	12.6	9.0	7.8
2	8.1	4.7	5.7
3	6.8	3.3	3.3
4 - 5	4.7	3.7	0.0
6 - 8	3.1	3.3	2.8
9 - 14	2.7	0.5	1.1
15 - 22	1.4	2.2	2.1
Cumulative risk of recurrence	30.6	22.9	12.2

Hamstring injuries affect both the team performance as well as the club financial state. A football league average club loss due to injury during the season of 1999-2000 was estimated to be £74.7 million (Woods *et al.*, 2004). At elite level, a quick and accurate determination of hamstring injury severity is important, as this would allow coaches to instil appropriate plan for the team while the injured player is recovering (Woods *et al.*, 2004; Järvinen, 2007). Medical imaging is a useful tool when combined with thorough clinical assessment (clinical history and medical examination) for accurate assessment of injury severity (Peetrons, 2002; Wong, 2005; Woodhouse & McNally, 2011). Radiological examination including US and MRI however would be a costly exercise. The cost of plain single part MRI and musculoskeletal US in the University Malaya Medical Centre is estimated around USD170.00 and USD 19.00 respectively. Further, the management for athletes with confirmed hamstring often involved physiotherapy sessions, which results in added expenses for the club.

Hamstring injury may result in loss of playing time for up to eight weeks (Warren *et al.*, 2010) and affect an average of six out of a team of 38 players each season (Orchard & Seward, 2002). Therefore hamstring injuries represent a substantial percentage of the season that may be lost. The effect can be more devastating for the team, should hamstring injury occurred on key player (primary goal scorer or playmaker), as this might disrupt team dynamic and indirectly affect success. In addition the loss of up to five players at one time could potentially affect team earnings through reduce gate receipt income as supporters might lose interest in attending matches (Seward, 2003). Hamstring injury may also impact on the player's individual income. In professional sports individual player wage structure often related to performance and the number of games played. Further, athlete with history of hamstring injury has higher risk of future hamstring injury and this may influence on on-going contract offered to the athlete.

1.4.7 Management of hamstring injury

Despite its frequency, the best treatment for hamstring injury is not clearly defined. The effectiveness of current interventions is still inconclusive (Copland *et al.*, 2009; Heiderscheit *et al.*, 2010; Mendiguchia & Brughelli, 2010; Petersen *et al.*, 2010; Reurink *et al.*, 2012). Guided by the pathophysiology understanding of muscle injury, current approaches in injury management are targeted according to phases of muscle repair. The main goal of treatment in the acute phase of muscle injury is to limit extent of injury by controlling haemorrhage, oedema and pain (Järvinen *et al.*, 2007). The conservative approach of rest, ice, compression and elevation (RICE) with short period of immobilisation is frequently used. Scientific evidence to support RICE approach is

largely based on experimental and animal studies (Drezner, 2003; Jarvinen, 2005; Järvinen *et al.*, 2007; De Carli *et al.*, 2009; Prior *et al.*, 2009; Ropiak & Bosco, 2012).

The use of non-steroidal anti-inflammatory drugs (NSAIDs) in the acute phase of muscle injury is controversial, as researchers found conflicting results. Laboratory studies showed short term gains in contractile and histologic properties of healing muscle but negative effects were reported with prolonged NSAIDs used (flubiprofen and piroxicam) (Obremsky *et al.*, 1994; Mishra *et al.*, 1995). In contrast, a double-blind placebo controlled study showed no additive effect of meclofenamate or diclofenac when combined with standard physiotherapeutic modalities (Reynolds *et al.*, 2008). In addition, higher frequency of adverse events was reported in both treatment groups (meclofenamante: 38 %, diclofenac: 35 %) than control (14 %).

While short-term (first few days after injury) immobilisation reduces haemorrhage and limits the extent of injury, longer period (beyond the acute phase) is associated with significant muscle atrophy of healthy muscle fibres, excessive deposition of scar tissues and substantially retard recovery of muscle strength (Järvinen, 2005). Therefore more active treatment is recommended once the acute phase of injury has passed, gradual active exercises including isometric training, isotonic training and isokinetic dynamic training (Järvinen, 2005). Generally these active exercises are combined with flexibility (stretching) exercises in the various active rehabilitation exercise programs (Malliaropoulos *et al.*, 2004; Gabbe *et al.*, 2006; Malliaropoulos *et al.*, 2010).

Recent study demonstrated nearly 50 % of the British team physiotherapist's time was spent administering therapeutic massage on muscles of team athletes

(Robertson *et al.*, 2004). Despite its wide use, there is relatively little research available on the effect of massage therapy on damaged muscle (Tiidus, 2008). Electrotherapeutic modality is another form of treatment that is used commonly for muscle injury. Therapeutic ultrasound (US) is an example of an electrotherapeutic modality widely used despite lacking scientific evidence (Markert *et al.*, 2005).

It should be reminded that most physiotherapeutic techniques for rehabilitation of hamstring injuries have not been assessed in randomised clinical trials (Mason *et al.*, 2007). A recent systematic review concluded that at this moment there is limited evidence to suggest that the rate of recovery is influenced by the frequency of hamstring stretching exercises. In addition there is limited evidence to suggest that exercises to correct movement dysfunction could reduce time to return to play and risk of reinjury. Based on these until further evidence is available, current published rehabilitation regimens cannot be supported or refuted (Mason *et al.*, 2007).

While majority of hamstring injury responded well to conservative approach, it often requires significant recovery time and period of increased susceptibility for recurrent injury (Gabbe *et al.*, 2006; Orchard & Seward, 2011). A cohort study of hamstring injured athletes treated with rehabilitation protocol, found the number of days lost from training and competition ranged from 4 to 74 days. More severe injury required significantly longer rehabilitation and recovery time (Malliaropoulos *et al.*, 2010).

In attempt to hasten recovery various treatment alternatives are being explored by researches. These include hyperbaric oxygen therapy, sclerosing therapy, injection of various substances into the injured area including mixed traumeel (a homeopathic anti-

inflammatory) and actovegin (protein-free extract from filtered calf blood), corticosteroids and local anaesthetic injection. It should be reminded that most of these treatment alternatives lack clinical scientific evidence and their uses are still controversial (Bennett *et al.*, 2005; Järvinen *et al.*, 2007; Stevens *et al.*, 2010; Franklyn-Miller *et al.*, 2011).

More recently, administration of biological substances has gained a lot of attention. Substances like autologous blood and blood products including autologous condition serum (ACS) and platelet-rich plasma (PRP) are used for soft tissues (muscles, tendons and ligaments) injuries despite limited clinical evidence (Engebretsen *et al.*, 2010).

Following muscle strain, injured muscle usually goes through the initial phase of destruction, where all injured myofibres including the intramuscular nerve branches undergoes necrosis. This phase is followed by repair and remodelling phase, in which undifferentiated reserve cells known as the satellite cells, in response to various growth factors and cytokines proliferates and differentiates into mature myoblasts (Järvinen *et al.*, 2000). These growth factors and cytokines play a significant role in myoblast proliferation and differentiation (Creaney & Hamilton, 2008). Inflammatory response that occurred following muscle injury leads to accumulation and activation of platelets at the injured site. Activated platelets degranulates releasing various growth factors including, platelet derived growth factors (PDGF), vascular endothelial growth factors (VEGF), epidermal growth factor (EGF), fibroblasts growth factors (FGF), insulin like growth factor-1 (IGF-1) and tumor growth factor beta-1 (TGF-B1) (Menetrey *et al.*, 2000). Researchers have showed that IGF-1 and FGF have the ability to speed up healing following muscle and tendon injury (Sanchez *et al.*, 2005; Hammond *et al.*,

2009). Hammond *et al.* (2009) in an animal model study demonstrated autologous PRP injection of an injured tibialis anterior muscle significantly accelerate duration to fully functional recovery from 21 to 14 days (Hammond *et al.*, 2009a). Sanchez *et al.* (2005) noted athletes with small hamstring tear obtained full recovery within half of the expected time when treated with PRP (Sanchez *et al.*, 2005). Even though there is increasing popularity to use PRP for muscle injury. The scientific evidence to support such used is based on limited human clinical study. Currently, there are two case reports, single case series and a case control study to support the use of PRP for muscle injury (Wright-Carpenter *et al.*, 2004; Sanchez *et al.*, 2005; Loo *et al.*, 2009; Hamilton & Best, 2011; Rettig *et al.*, 2013). A study with robust study design and method is needed to evaluate the effect of PRP for hamstring injuries.

1.5 Problem statement

Limited information is available on the epidemiology of acute muscle injuries among Malaysian athletes (Shariff *et al.*, 2009). In addition information on how these injuries are managed in Malaysian is not available. Consequently, time taken to fully recover and the duration return-to-play (DRP) among Malaysian athletes is inconclusive. It is possible that wide spectrums of treatments are being employed to treat muscle injuries in the local setting. Therefore, a cross-sectional study was conducted to study the patterns of muscle injury, current treatment regimens and the effectiveness of treatment among Malaysian athletes. Further, potential predictors of DRP after muscle injury were examined.

An accurate assessment in diagnosing and grading of muscle injury severity is important especially during on-field setting where immediate decision needed whether to allow athlete to continue with the games or otherwise. Ideally this can be achieved with a thorough clinical assessment and early radiological investigation using MRI or US. In practice though, physician would base their decision on clinical judgement, as radiological tools are rarely available on field. The severity of muscle injury was based on subjective feedback from athletes on pain intensity during clinical examination. The other objective assessment to determine injury severity is the active range of movement of the affected knee. As there are several methods of assessing AROM available, questions on the reliability of the different assessment methods arose (Bohannon, 1982; Booher & Thibodeau, 1985; Baltaci *et al.*, 2003). Active knee extension (AKE) test is one of the more popular methods for assessment of knee AROM. The AKE test aided by metal rig showed a high intrarater correlation coefficient when conducted within 30 minutes (Gajdosik & Lusin, 1983; Kuilart *et al.*, 2005). Others questioned the practicality of this method, as the apparatus used were complicated and rarely available in clinic setting. Also more than one assessor is needed to conduct the test (Worrell *et al.*, 1990; Rakos *et al.*, 2001). Hence a simple, reliable and practical method of knee ROM assessment is proposed.

The best treatment for sports related muscle injuries is still inconclusive (Prior *et al.*, 2009). More recently, autologous biological substances including PRP injection have received much attention as treatment alternatives for acute muscle injury. Interestingly, despite limited clinical evidence PRP are currently used by health care providers for soft tissue injuries including muscle injuries (Engebretsen *et al.*, 2010b; Filardo & Kon, 2012; Engebretsen & Schamasch, 2012; “Athletes Using PRP,” 2012; “What is blood spinning,” 2013). More robust clinical trials to evaluate the effect of

PRP for sports related muscle injury is needed (Engebretsen *et al.*, 2010b; Taylor *et al.*, 2011).

1.6 Conceptual framework

Figure 1.6 illustrates the conceptual framework of this study. The primary objective of this study was to examine the safety and effectiveness of intralesion autologous PRP injection in hastening muscle recovery.

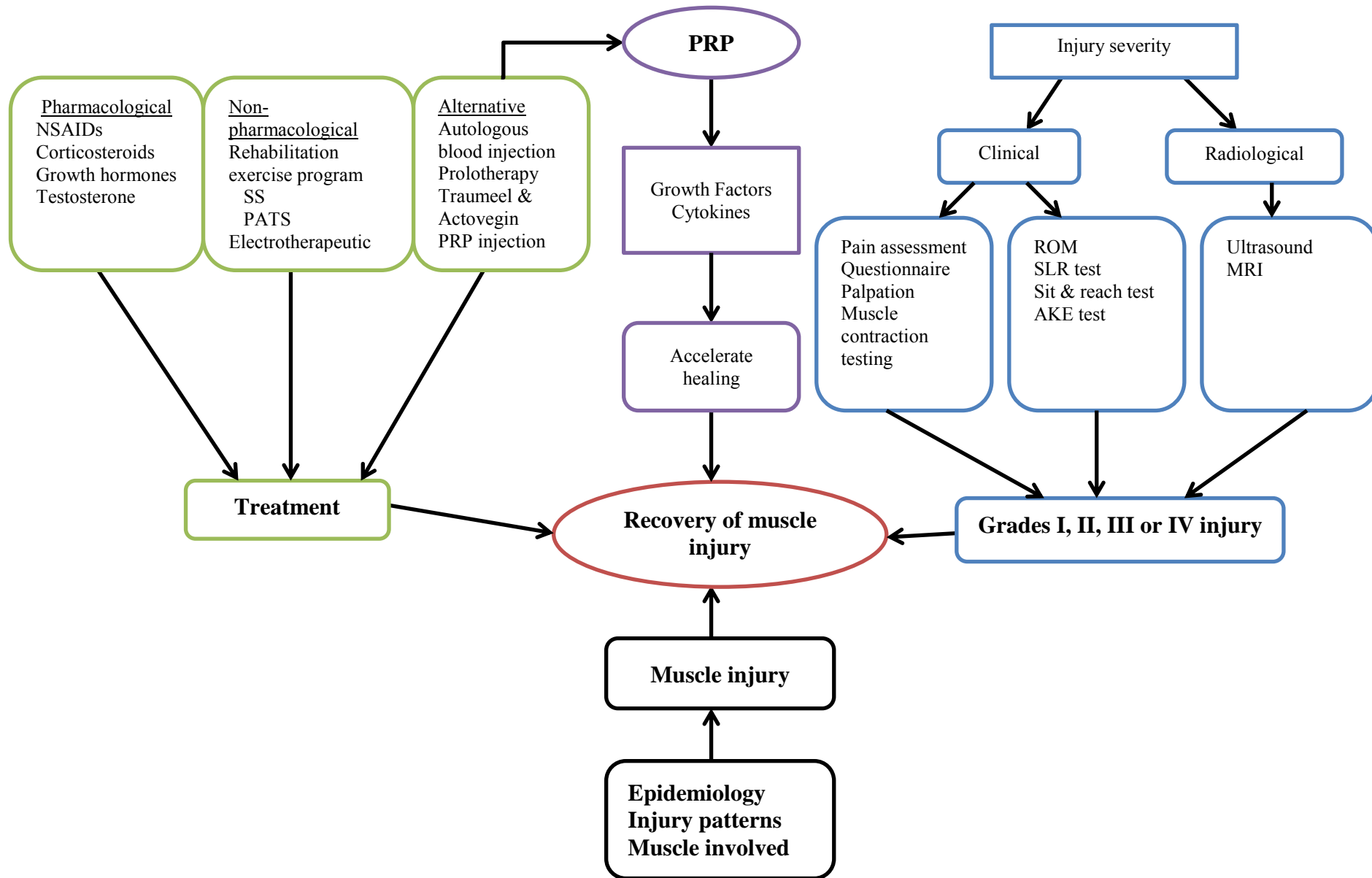


Figure 1.6. Study’s conceptual framework for recovery of muscle injury.

1.7 Research objectives

- to explore the patterns of muscle injury and explore predictors of duration to return-to-play among Malaysian athletes.
- to design and construct a simple, easy to use and reliable method of assessing knee active range of movement (AROM).
- to investigate the effect of single intralesional PRP injection combined with hamstring rehabilitation program on the DRP of patients with grade-2 hamstring muscle injury.

1.8 Research questions

Question 1.1 What is the pattern of muscle injury among Malaysian athletes?

Question 1.2 What factors predicts the duration to return-to-play (DRP) among Malaysian athletes?

Question 2.1 What is the interater reliability of the active knee extension (AKE) test among healthy adults?

Question 2.2 What is the intrarater reliability of the active knee extension (AKE) test among healthy adults?

Question 3.1 Is there a difference in the DRP on patient with grade-2 hamstring muscle injury receiving single PRP injection combined with hamstring rehabilitation compared to patient receiving hamstring rehabilitation program alone?

Question 3.2 Is there a difference in injury symptoms (pain intensity and interference) and sign (active knee extension angle) over time between patients receiving single PRP injection combined with hamstring rehabilitation compared to patient receiving hamstring rehabilitation program alone?

1.9 Research hypothesis

To answer research question 3, the study set out the following research hypotheses respectively;

Hypothesis 1 Patient group receiving PRP combine with hamstring rehabilitation program will demonstrate significantly shorter DRP (faster recovery) compared to group receiving hamstring rehabilitation program alone.

Hypothesis 2 Patient group receiving PRP combine with hamstring rehabilitation program will demonstrate significantly faster improvement in pain severity score (BPI-SF)

compared to group receiving hamstring rehabilitation program alone.

Hypothesis 3 Patient group receiving PRP combine with hamstring rehabilitation program will demonstrate significantly faster improvement in pain interference (BPI-SF) compared to group receiving hamstring rehabilitation program alone.

Hypothesis 4 Patient group receiving PRP combine with hamstring rehabilitation program will demonstrate significantly faster improvement in active knee extension (AKE) test compared to group receiving hamstring rehabilitation program alone.

1.10 Overview of methodology

A quantitative design was used for this research, and conducted in three phases:

Phase 1 is a cross-sectional study aimed to examine the pattern of muscle injury among Malaysian athletes. The types of muscle injury including injury severity, muscle commonly injured and treatment employed were explored. Factors that predict DRP were also studied. This study is presented in Chapter 3.

Information gathered from this study is used as a guide for subsequent study design of phase 3 – the randomised controlled trial (RCT).

Phase 2 is a cross-sectional study to determine the interater and intrarater reliability of an active knee range of movement (AROM) test that was designed and constructed. This study is presented in Chapter 4.

Phase 3 study is the randomised controlled trial that was conducted to investigate the effect of a single intralesional injection of platelet-rich plasma (PRP) combined with standard rehabilitation program for treatment of muscle injury. This study is presented in Chapter 4.

1.11 Significance of study

To our knowledge this is the first RCT conducted to examine the effect of a single intralesion administration of PRP combined with hamstring rehabilitation program on the DRP for a grade-2 hamstring muscle injury. Findings from this study could establish the effect of PRP and guide practitioners on deciding whether to include this treatment as one alternative for grade-2 hamstring muscle injury. Further, researchers could also use results from the current study as a reference point on further exploring the best methods of PRP use (dosages, frequency of administration, method of administration, etc.).

Chapter 2 Systematic Review on Platelet-Rich Plasma (PRP) Therapy for Muscle Injury

2.1 Introduction

To explore the role of platelet-rich plasma (PRP) for acute muscle injury a systematic review of literature was conducted. Acute muscle injury specifically the hamstring muscle is one of the commonest types of injury seen in athletes. Hamstring muscle injury often results in loss of training and competition time (Askling & Karlsson, 2004; Feeley *et al.*, 2008; Shariff *et al.*, 2009; Ekstrand *et al.*, 2011; Orchard & Seward, 2011; Murphy *et al.*, 2012). Despite its frequent occurrence, the best treatment for hamstring injury is not fully understood. Common treatments usually involve rest, ice, compression and elevation (RICE) especially in the early stage following injury (Kujala *et al.*, 1997; Huard *et al.*, 2002; Jarvinen, 2005; Gielen *et al.*, 2007; Järvinen *et al.*, 2007; Yeung *et al.*, 2009). Additional treatment modalities include painkillers, rehabilitative exercises, electrotherapeutic modalities, hyperbaric oxygen therapy and prolotherapy (Almekinders, 1999; Harrison *et al.*, 2000; Mason *et al.*, 2007; Banffy & ElAttrache, 2012; Orchard *et al.*, 2013). Clinical evidence to support the use of these modalities however is limited.

More recently, injection of autologous platelet-rich plasma (PRP) have gain a lot of attention for the treatment of sports injury. Despite limited clinical evidence to suggest effectiveness, PRP injection are used for various sports related injuries including acute muscle injury (Kaspriske, 2010; Ekstrand *et al.*, 2011; Engebretsen *et al.*, 2011; “Athletes Using PRP,” 2012). The objective of this review is to explore the current evidence on PRP use for acute muscle injury.

2.2 Methods

A systematic review using qualitative synthesis method was conducted to retrieve and review the findings of previous literatures on PRP use for acute muscle injury. The process started with a search question: Is the use of platelet-rich plasma effective for treating acute muscle injury? The question was developed using the PICOS (Participant, Intervention, Control, Outcomes, Study design) approach as in Table 2.1. The objective, characteristics of the study, contents of the intervention, targeted outcome and major findings for each of the selected study were assessed in this review.

Table 2.1 : Formulating the search questions.

Question		Element
Who are the group of patients?	Participants	Adults aged ≥ 18 years with muscle injury
What intervention to evaluate?	Intervention	PRP/PRFM/ACS/platelet concentrate/ platelet gel injection/PRGF/platelet releasate/ platelet lysate/ L-PRP/P-LRP
What is the main or usual alternative?	Comparison	Rehabilitation exercise program/ physiotherapy treatment
What could the intervention offer?	Outcomes	The length of DRP
What is(are) the study design(s)?	Study design	Randomised controlled trial

PRP = platelet rich plasma; PRFM = platelet rich fibrin matrix; ACS = autologous conditioned serum; PRGF = preparation rich in growth factors; L-PRP = leucocyte platelet rich plasma; P-LRP = platelet leucocyte rich plasma.

This qualitative systematic review includes the description of the criteria for study selection and the search methods for identification of studies, detailed qualitative synthesis of the selected studies and to discuss the findings from this review.

2.2.1 Criteria for study selection

The criteria for considering studies in this review include types of study, types of participants, types of interventions and types of outcome measures.

Types of study participants

Studies that include adults (≥ 18 years) diagnosed with acute hamstring muscle injury are considered for this review.

Types of interventions

This review includes studies with interventions to promote early recovery; shorter duration to return-to-play (DRP) among adults with acute muscle injury. The interventions may include one or combination of:

- Rehabilitation exercise program
- PRP injection therapy

No restrictions are defined regarding the type and contents of the control group. The interventions can be compared with no intervention control, group assigned to a waiting list, or minimal intervention control group.

Types of outcome measures

The primary outcome measures in the selected studies to include the DRP from acute muscle injury.

2.2.2 Search methods for identification of studies

The search is conducted according to the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) guideline (Liberati *et al.*, 2009). The process of this search method included describing the data sources, search strategy, data extraction and quality assessment.

2.2.3 Data sources and search strategy

The studies were searched electronically using the following databases: OvidMEDLINE, PubMed, EMBASE, SPORTDiscus and CINAHL. The reference lists of review articles and included studies were hand searched for other potentially eligible studies using the same selection criteria as described earlier. Published systematic reviews on PRP were used as a source of randomised controlled trials. Peer-reviewed published articles until December 2012 were searched. In view of limited resources for translation, only articles published in English were considered. No attempts were made to contact authors for additional information, however, cross-referencing on related previously published study is performed to obtain additional information. The search strategy used for OvidMEDLINE (Appendix A) is described in Table 2.2. Comparable searches were made for the other databases (Appendices B, C, D and E). In addition, search through a local library for archived articles from the South-East Asian region using the earlier described selection criteria were also performed.

2.2.4 Data extraction and quality assessment

The titles and abstracts of all studies retrieved from the search were reviewed following study selection criteria to decide whether the full-text manuscripts were needed for further evaluation. Each full-text manuscript retrieved were evaluated systematically according to the study's: 1) objective(s), 2) characteristics of the study (study design, participants, age and sample size), 3) contents of intervention (intervention strategies, intervention provider, duration of intervention and follow-up contacts), 4) targeted outcome(s) and 5) major findings. The outcomes extracted from the selected study were neither combined nor reanalysed due to the nature of this qualitative systematic review.

Table 2.2 : Search strategy for OvidMEDLINE.

Dates: Jan 1946 – Dec 2012		Result
1	Exp Platelet-Rich Plasma/	1338
2	platelet rich fibrin matrix.mp	16
3	autologous conditioned serum.mp	22
4	platelet concentrate.mp	602
5	platelet gel.mp	173
6	autologous growth factors.mp	52
7	preparation rich in growth factors.mp	14
8	platelet releasate.mp	101
9	platelet lysate.mp	281
10	leucocyte platelet rich plasma.mp	1
11	platelet leucocyte rich plasma.mp	0
12	muscle injury.mp	1738
13	1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11	2417
14	12 and 13	5

Data was retrieved on 29th June 2013.

Each selected article was evaluated for their methodological quality. Two investigators independently graded the methodological quality of each eligible article. The initial intention was to use the Physiotherapy Evidence Database Scale (PEDro)

(Zarins & Ciullo, 1983; Kujala *et al.*, 1997; Sherrington *et al.*, 2000) for evaluating randomised controlled trials (RCT) and the Newcastle-Ottawa Scale (NOS) (Heiser *et al.*, 1984; Bennell *et al.*, 1998; Wells *et al.*, 2000; Slavotinek *et al.*, 2002) for prospective studies. Since the literature search was unable to identify any RCT, only NOS were used in the final study evaluation.

The Newcastle-Ottawa Scale is a 9-point scale used to grade case control or cohort studies on their methodological quality of selection, comparability, exposure, and outcome of the study participants (Table 2.3). A quality score of ≥ 7 was chosen to represent high quality study. Studies with score of 5 or 6 were considered to be of moderate quality, and those with score of ≤ 4 were considered to be of low quality (Woodley & Mercer, 2005; Orchard *et al.*, 2008; Simunovic *et al.*, 2010; Reurink *et al.*, 2012; Sheth *et al.*, 2012).

Table 2.3 : Newcastle - Ottawa quality assessment scale case control studies.

No.	Criteria
Selection	
1)	Is the case definition adequate? <ol style="list-style-type: none"> yes, with independent validation yes, eg record linkage or based on self reports no description
2)	Representativeness of the cases <ol style="list-style-type: none"> consecutive or obviously representative series of cases potential for selection biases or not stated
3)	Selection of controls <ol style="list-style-type: none"> community controls hospital controls no description
4)	Definition of controls <ol style="list-style-type: none"> no history of disease (endpoint) no description of source
Comparability	
1)	Comparability of cases and controls on the basis of the design or analysis <ol style="list-style-type: none"> study controls for _____ (Select the most important factor.) study controls for any additional factor <input type="checkbox"/> (This criteria could be modified to indicate specific control for a second important factor).
Exposure	
1)	Ascertainment of exposure <ol style="list-style-type: none"> secure record (eg surgical records) structured interview where blind to case/control status interview not blinded to case/control status written self report or medical record only no description
2)	Same method of ascertainment for cases and controls <ol style="list-style-type: none"> yes no
3)	Non-response rate <ol style="list-style-type: none"> same rate for both groups non respondents described rate different and no designation

Note: A study can be awarded a maximum of one star for each numbered item within the Selection and Exposure categories. A maximum of two stars can be given for Comparability.

2.3 Results

2.3.1 Study selection and characteristics

The initial search identified 1016 potential articles from the databases search and another 3 were found through cross-referencing. After removing duplicates, 883 articles were assessed based on titles and abstracts against the selection criteria. A total of 842

articles were excluded because the studies were not on platelet-rich plasma and muscle injuries. Of the 41 full-text articles retrieved for further evaluation, only four articles were included in the final qualitative synthesis. The remaining 37 articles were excluded because 35 of them were review articles (including systematic reviews) and the remaining two were case reports. Figure 2.1 describes the PRISMA flow diagram for the study selection.

2.3.2 Data extraction and synthesis

All articles were in English language published between year 2000 and 2012.

2.3.2 (a) Selected studies

Table 2.4 describes the characteristics of selected studies. Out of the four studies selected for the review, one was a case control study (CC) (Wright-Carpenter *et al.*, 2004a) while the remaining three were *in vivo* laboratory studies (Wright-Carpenter *et al.*, 2004b; Hammond *et al.*, 2009; Gigante *et al.*, 2012). Therefore the discussion on the pilot human clinical trial and laboratory studies were conducted separately.

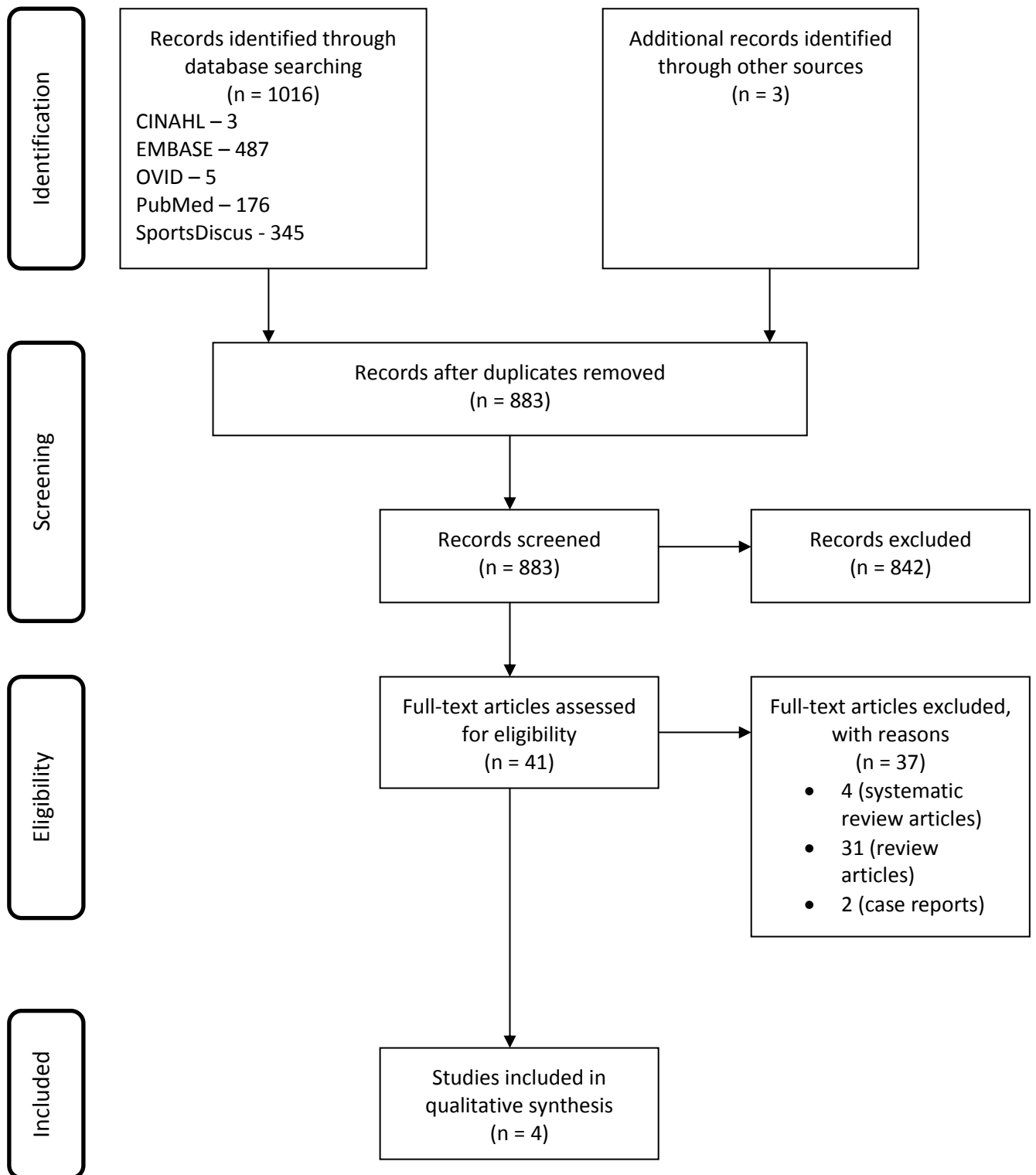


Figure 2.1. PRISMA flow diagram for the study selection.

2.3.2 (b) Description of studies

Clinical study

This pilot case control study was conducted in a clinic setting (Clinic for Sports Medicine & Orthopaedic). The participants in this study were professional sportsmen diagnosed with acute “moderate strains” (second degree) muscle injury. The diagnoses of injury were based on clinical assessment and magnetic resonance imaging (MRI) examinations (detection of bleeding of the involved muscle). The mean age of participants in the control and intervention groups and other demographics were not available for comparisons (Wright-Carpenter *et al.*, 2004a).

The intervention used in this study was intra-lesional injection of 2.5 ml autologous conditioned serum (ACS) combined with 2.5 ml of saline. The method of ACS preparation was adequately described. The ACS was injected to the affected area, guided only by palpation. Prior to administration of ACS, 5 ml local anaesthetic (Meaverin 0.5 %) was injected in portion of 1 ml to minimise the tonus of muscle. The ACS injection started two days after diagnosis and was repeated every second day until full recovery was achieved. The mean number of ACS injection per patient was 5.4.

Interestingly the control group in this study was a retrospective analysis of 11 patients who had been treated with local injection of Actovegin[®]/Traumeel[®] (3:2) combination therapy. Actovegin[®] is a deproteinised dialysate of bovine blood, while Traumeel[®] is a homeopathic formulation containing both botanical and mineral ingredients. This treatment combination is purported to suppress the release of inflammatory mediators and stimulate the release of anti-inflammatory cytokines

(Franklyn-Millet *et al.*, 2011). Local injection of Actovegin[®]/Traumeel[®] is considered a standard treatment of muscle strain in the centre. The method of Actovegin[®]/Traumeel[®] administration was similar to the ACS injection technique. The mean number of treatments in the control group was 8.3 Actovegin[®]/Traumeel[®] injections per patient. Participants in both groups underwent a standard rehabilitation program. In addition participants in both groups were given oral antiphlogistics. The frequency and dosages of the rehabilitation sessions were not specified.

The severity of muscle tears between intervention and control groups was comparable. Most tears were located in the hamstring and adductor muscles (12 in the ACS and 9 in the control group). The main outcome measured was the time required to resume full sporting activities. Return to full sporting activities was determined based on participant's subjective impression of readiness to resume activities and physiotherapist's standard examination. Athletes were allowed to resume full activities only when they were pain free and hamstring strength restored to at least 90 % of that of the unaffected limb muscle. The method of strength assessment was unclear, as isokinetic strength was not performed for fear of reinjury during testing.

The mean recovery time for participants in the ACS group (16.6 days) was significantly shorter compared to participants in the control group (22.3 days). In addition, MRI scans on day 16 demonstrated faster regression of the oedema/bleeding in the ACS group. Both treatments were considered safe, as there were no local or systemic side effects reported.

***In vivo* laboratory studies**

All studies were controlled animal studies conducted on different species of syngeneic rodents (Garrett *et al.*, 1989; Wright-Carpenter *et al.*, 2004b; Hammond *et al.*, 2009; Marieb, 2009; Gigante *et al.*, 2012). Studies differ in their methods of inducing muscle injuries. In one study muscle contusion was induced by dropping a stainless steel ball on the animal's hind limb from the height of 100 cm (Wright-Carpenter *et al.*, 2004b). In contrast, Hammond *et al.*, (2009) induced eccentric muscle injury over the tibialis anterior muscle by superimposing a lengthening contraction onto a maximally isometric contraction, using either a single repetition (large strain) or multiple repetitions (small strain) (Friden *et al.*, 1983; Jones *et al.*, 1986; Hammond *et al.*, 2009; Ekstrand, 2012). Gigante *et al.* (2012) on the other hand cause a bilateral muscle tear in on the longissimus dorsi muscle using a standard pincer technique.

As myogenesis relies upon satellite cells activation, proliferation, differentiation, and fusion between damaged muscle and maturation (increased myofibre diameter) (Patel & Cuizon, 1998; Jarvinen, 2005; Murphy *et al.*, 2012), majority of studies used objective assessment of muscle regeneration via immunohistochemical staining as one of their outcome measures. Wright-Carpenter *et al.*, (2004), used Ki-67 labelled antibody as marker of satellite cells proliferation (Wright-Carpenter *et al.*, 2004b). While, Hammond *et al.*, 2009 and Gigante *et al.*, 2012 both assayed the level of MyoD and Myogenin as markers of muscle regeneration (Hammond *et al.*, 2009; Gigante *et al.*, 2012). In addition, both studies also assessed the percentages of centrally nucleated fibres (CNFs) presence in the injured area as an additional measure of myogenesis.

Surprisingly, only one study assessed the functional recovery of the injured muscle. The assessment was done using maximal isometric torque test on the tibialis anterior muscle (Hammond *et al.*, 2009)

Characteristics of interventions

The interventions used in each study vary markedly. Wright-Carpenter *et al.* (2004), utilised blood from 20 syngenic mice to produce autologous conditioned serum (ACS) using a method originally developed for human blood (Meijer *et al.*, 2003). Animals in the intervention group received 10 µl of PRP at days 0, 3, 5 and 7. While the controls was injected with same volume of saline at the same intervals (Wright-Carpenter *et al.*, 2004)

Hammond *et al.*, (2009) used 20 ml of blood collected from five adult male Sprague-Dawley rats to produced autologous platelet-rich plasma (PRP) using a commercial kit. The autologous PRP was conditioned using high-frequency ultrasound to lyse platelets and release growth factors thus enriching the PRP prior to injection. Animals in the intervention group were injected with 100 µl of PRP into the injured tibialis anterior and the controls received same amount of platelet-poor plasma or no treatment. All injections were administered on days 0, 3, 5 and 7 (Hammond *et al.*, 2009).

In the study by Gigante *et al.*, (2012), platelet rich fibrin matrix (PRFM) was prepared using a commercial kit. The lesion in one side of the body was filled with

PRFM while the contralateral injured muscle (control) left untreated. The PRFM was given once throughout the study (Gigante *et al.*, 2012)

Effectiveness of interventions

The outcome measures and results of interventions promoting earlier muscle recovery are presented in Table 2.4. The primary outcome in all studies was quantification of muscle regeneration (myogenesis). In two studies this was achieved by immunohistochemical detection of Myogenin and MyoD (markers of muscle regeneration) (Hammond *et al.*, 2009; Gigante *et al.*, 2012). Whereas Wright-Carpenter *et al.* (2004) used Ki-67 markers as indicator of satellite cells proliferation (Wright-Carpenter *et al.*, 2004b). Only one study assessed muscle functional recovery in addition to the tests mentioned above. Hammond *et al.* (2009), measured maximal isometric contraction of the dorsiflexors before and four minutes after inducing injury to assess associated muscle strength lost. The maximal isometric force was retested at days 3, 5, 7, 14 and 21 after treatment commenced (Hammond *et al.*, 2009).

All studies demonstrated greater muscular regeneration in intervention group compared to controls. Significantly higher level of MyoD and Myogenin detected in autologous PRP and PRFM treated muscles compared to controls (Gigante *et al.*, 2012; Hammond *et al.*, 2009). In addition Wright-Carpenter *et al.* (2004) observed increased satellite cells activation as early as 30 to 40 hours after injury. Accordingly, significantly higher number of CN myofibres (larger diameter fibres) was found in PRP and ACS treated rodents (Wright-Carpenter *et al.*, 2004b)

Interestingly Hammond *et al.* (2009) found PRP therapy had little effect on muscle healing following single-repetition injury protocol. In the multiple-repetition injury protocol however, PRP treatment significantly improved contractile function at 2 time points and effectively shortened the time to full recovery from 21 to 14 days (Hammond *et al.*, 2009).

2.3.3 Studies methodological quality

Extensive search only resulted in a single human case control study; the particular study demonstrated several limitations including the use of retrospective data of athletes treated with Actovegin[®]/Traumeel[®] as controls. In addition the baseline participant's characteristics (including age) was not available for analysis. Using the Newcastle Ottawa scale this study score a total of 4 of maximal 9 and represented a study of low quality.

Interestingly our search did not found any human randomised controlled trial on PRP for acute muscle injuries. Furthermore only three *in vivo* laboratory studies were retrieved by the search.

Table 2.4 : Study characteristics and outcomes: Clinical and laboratory

Studies	Study design/target population	Treatment	Type of injury/location	Outcome measures	Results
Case control study					
Wright-Carpenter <i>et al.</i> 2004	Pilot controlled-clinical trial n = 16 (ACS); n = 11(control)/ professional sportsmen	ACS (combined with LA) injections vs. Traumeel/Actovegin (controls) injections both repeated every second day	2 nd degree muscle tears (MRI confirmed) most injuries to the hamstring and adductors muscle group	Time to recovery based on the participant's subjective judgement of readiness	Time to recovery was significantly shorter in ACS (16.6 days) than control groups (22.3 days) No side effects of treatment
Laboratory studies					
Wright-Carpenter <i>et al.</i> 2004	Controlled laboratory study n = 39(ACS) n = 39 (control)/syngeneic C57B1/6 mice	ACS vs. saline injections at 2, 24 & 48 hrs. after contusion impact	Iatrogenic contusion injury of the gastrocnemius muscles	Regeneration quantification <ul style="list-style-type: none"> Activated satellite cells Size of regenerating myofibres 	Significant increased in satellite cells activation at 30 & 48 hrs. after injury Larger diameter of CN cells in ACS group after 1 week
Hammond <i>et al.</i> 2009	Controlled laboratory study n = 72 Adult male Sprague-Dwaley rats	No treatment vs. PRP vs. PPP injections at Days 0, 3, 5 & 7 after induced eccentric injury	Iatrogenic eccentric injury of tibialis anterior muscle <ul style="list-style-type: none"> Single repetition (large strain) Multiple repetition (small strain) 	Functional recovery <ul style="list-style-type: none"> Maximal isometric contraction Muscle regeneration <ul style="list-style-type: none"> MyoD & myogenin markers 	Large strain injury PRP significantly improve contractile function at Day 3 Small strain injury PRP significantly improved contractile function at Days 7 and 14. Full recovery at Day 14 Muscle regeneration MyoD and myogenin significantly increased in PRP treated Significantly higher number of CN cells in PRP group compared with PPP and no treatment
Gigante <i>et al.</i> 2012	Controlled lab study n = 20 male Wistar rats	PRFM vs. No treatment (control). Random allocation.	Iatrogenic tear (pincer technique) bilateral longissimus muscle	Blind assessment <ul style="list-style-type: none"> Vascularization & muscle regeneration Inflammation & fibrosis 	PRFM group More muscle regeneration at D5 & D10 More neovascularization at D40 & D60 Less fibrosis at D10 No differences in inflammation

ACS = autologous conditioned serum; LA = local anaesthetic; MRI = magnetic resonance imaging; CN = centronucleated.

2.4 Discussion

2.4.1 Summary of main results

From the available evidences presented in this review, the number of well-designed trials on use of PRP therapy for acute muscle injury is limited. Only one human study was identified while the remaining three studies were *in vivo* laboratory studies on rodents. All studies reviewed suggested positive effects of PRP on muscle recovery following injury.

In vivo study on rodents induced with either single repetition (large strain) or multiple repetition (small strain) injury on to the tibialis anterior muscle found PRP therapy effective in reducing the time to full recovery only in the multiple-repetition injured animals. The author suggests PRP therapy is effective only when injury repair requires muscle regeneration such as those occurring following multiple-repetition contraction injuries. Since single large strain injury only requires sarcolemmal repair without muscle regeneration the effect of PRP for such injury might not be clinically evident.

All *in vivo* studies evaluated the amount and size of regenerated myofibres (histologically) as indicator of healing. Despite different histological methods used to quantify muscle regeneration, all studies showed significant acceleration of muscle regeneration in the intervention groups (ACS, PRP and PRFM) compared with controls. Whether such finding is also applicable in humans is uncertain, as evidence to support similar observation is not available.

In the pilot study on professional sportsmen diagnosed with second-degree muscle strain, athletes treated with repeated injections of ACS reported earlier subjective readiness to resume exercise at competitive level than those treated with Actovegin[®]/Traumeel[®]. Result from this trial must be interpreted cautiously as the study demonstrated several limitations including, lack of randomisation, non-blinded and atypical control group.

Based on this review there is no evidence with good methodological quality to suggest that PRP therapy is effective in accelerating functional muscle recovery after injury.

2.4.2 Applicability of evidence

This review identified a single case control and three *in vivo* laboratory studies that evaluated PRP therapy effect on muscle injury. All *in vivo* studies were conducted on rodents.

The methodological quality of the pilot case control study was rated as poor quality based on Newcastle-Ottawa scale with a score of 4/10. Several limitations were detected including lack of randomisation, absence of concealment of treatment allocation, absence of baseline data characteristics between subjects in both groups, and lack of blinding (subjects, therapists or assessors). Further, trials with inadequate methodological approach are known to be associated with bias (Schulz *et al.*, 1995). Professional sportsmen receiving ACS therapy reported significantly faster subjective impression of readiness to resume exercise at competitive level than controls. This

finding must be interpreted cautiously as the conclusion was based only on 18 participants. Also lack of assessors blinding might lead to reporting biases. A more objective assessment such as validated symptoms questionnaires and functional assessment (muscle strength assessment) might be useful in these circumstances, as it allows more objective and standardise assessment of readiness to participate in pre-injury level activities.

The methodological quality of the *in vivo* studies varies. Studies differ in methods of preparation of injectable (ACS, PRP & PRFM), as well as the nature of injury induced onto the animals. Using immuno-histochemical marker to quantify muscle healing/recovery, all *in vivo* studies demonstrated significant acceleration of muscle regeneration. Only one study however showed concurrent significant improvement in contractile function and faster time to full recovery in animals with small strain injury treated with ACS injection (Hammond *et al.*, 2009). Whether similar cellular changes observed in these studies would occur in humans remains unanswered. Replicating such study in humans will be challenging in view of substantial ethical consideration on the need to biopsy the injured muscle especially involving competitive athletes. In conducting such study one must also consider the importance between cellular recoveries versus functional recovery.

2.4.3 Limitation of review

There are limitations from this review. Only peer-reviewed papers published until December 2012 and published in English were included in the data extraction, hence a possibility of selection bias may exist. Even though the searches were done

thoroughly through multiple major databases with cross-referencing; there is a possibility that some papers were not included due to the criteria used.

2.5 Conclusion

In conclusion, there are limited studies on the effects of PRP therapy for acute muscle injury. Three *in vivo* studies that vary in methodology were reviewed. Despite this, significantly faster muscle recovery among animals in the experimental group was reported. Whether such findings could be translated into humans, remain to be answered.

Only one pilot human case control study was available at the time of review (Wright-Carpenter *et al.*, 2004). Although this study found significant reduction in DRP among athletes treated with ACS, the study design is questionable (Engebretsen *et al.*, 2010; Andia *et al.*, 2011; Hamilton & Best, 2011) as it lacks robustness that restricts generalisation of the findings.

2.6 Summary

Based on the systematic review conducted, the efficacy of PRP therapy on muscle recovery in humans is still unanswered. There is some evidence to suggest acceleration of muscle recovery (both histologically and functionally) with local injection of ACS, PRP and PRFM from *in vivo* laboratory studies.

More studies using robust clinical design are needed (Engebretsen *et al.*, 2010; Andia *et al.*, 2011) to demonstrate the efficacy or otherwise of PRP for the treatment of muscle injury. Hence a randomised controlled trial (RCT) to study the effect of PRP on muscle recovery was performed as phase 4 of this research project.

Chapter 3 Pattern of Muscle Injuries and Predictors of Return-to-Play among Malaysian Athletes: A Retrospective Study

3.1 Introduction

Muscle injury is one of the most common injuries affecting athletes (Järvinen, 2005; Orchard *et al.*, 2010; Liu *et al.*, 2011). They account for up to 36.4 % of injuries in sports events (Garrett *et al.*, 1989; Woods *et al.*, 2004; Volpi *et al.*, 2004; Eirale *et al.*, 2013; Ekstrand *et al.*, 2013). Contusion and strain are two common causes of muscle injuries. Muscle strain often occurs especially during sprinting or jumping when muscle is under tension while lengthening (eccentric contractions) (Zarins & Ciullo, 1983). Earlier studies identified several factors that predisposes to muscle injury including, history of muscle strain, increasing age and dominant leg (Orchard, 2001; Engebretsen *et al.*, 2010; Warren *et al.*, 2010). Muscle injuries often occur at the muscle-tendon (myotendinous) junction, affecting mainly muscles that span across two joints such as the rectus femoris, semitendinosus, and gastrocnemius. The diagnosis and grading of muscle injuries are usually made through clinical assessment (Woods *et al.*, 2004). In addition, ultrasonography is recommended in localising and characterising injury severity (Aspelin *et al.*, 1992).

In professional sports, muscle injuries can lead to significant pain and disability resulting in time away from participation (training and competition) and increased medical costs (Schmikli *et al.*, 2009). Not surprisingly, athletes and coaches are concerned about the time taken to achieve full recovery. Unfortunately, issues on duration to return-to-play (DRP) often not directly discussed during consultation with the medical team (Fisher, 1988). Predicting DRP not only important for planning of

rehabilitation program but also allows the coaching staff to restructure team for competition.

Studies have identified several factors that may help in estimating DRP. An observational study of 59 players from 10 Victorian-based Australian Football League clubs showed, time taken to walk pain-free was a significant predictor of DRP after hamstring injury (Warren *et al.*, 2010). Unfortunately, the authors did not discuss severity of muscle injury sustained by their athletes and the details of rehabilitation program used. In a prospective study among athletes with grades-1 to -4 hamstrings injury, researchers found the active knee range of motion deficit provides accurate prediction of DRP (Aspelin *et al.*, 2005; Warren *et al.*, 2010; Valle, 2011).

Identifying the pattern of muscle injury including the extent of the problem is an important early step in any injury prevention program (Figure 3.1). While it is recognised that muscle injury particularly hamstring muscle group is one of the most common injury affecting athletes (Table 3.1) (Brooks *et al.*, 2005; Ekstrand, 2008; Ekstrand *et al.*, 2011c; Orchard & Seward, 2011; Eirale *et al.*, 2013). It is reminded that current available data on muscle injuries were gathered from population outside Malaysia. Local information on pattern of injuries, current treatment and the DRP after muscle injury among Malaysian athletes is limited (Shariff *et al.*, 2009). Differences in physical build, climate, dietary intake, training regimen and injury management between local and foreign athletes might influence the pattern of muscle injury and DRP observed between different populations. Therefore a cross-sectional study was conducted to examine pattern of muscle injuries in Malaysian athletes. Information gathered in this study was used in designing the RCT component of this research. The

findings from this study were published in the Singapore Medical Journal recently (Shariff *et al.*, 2013) (Appendix F).

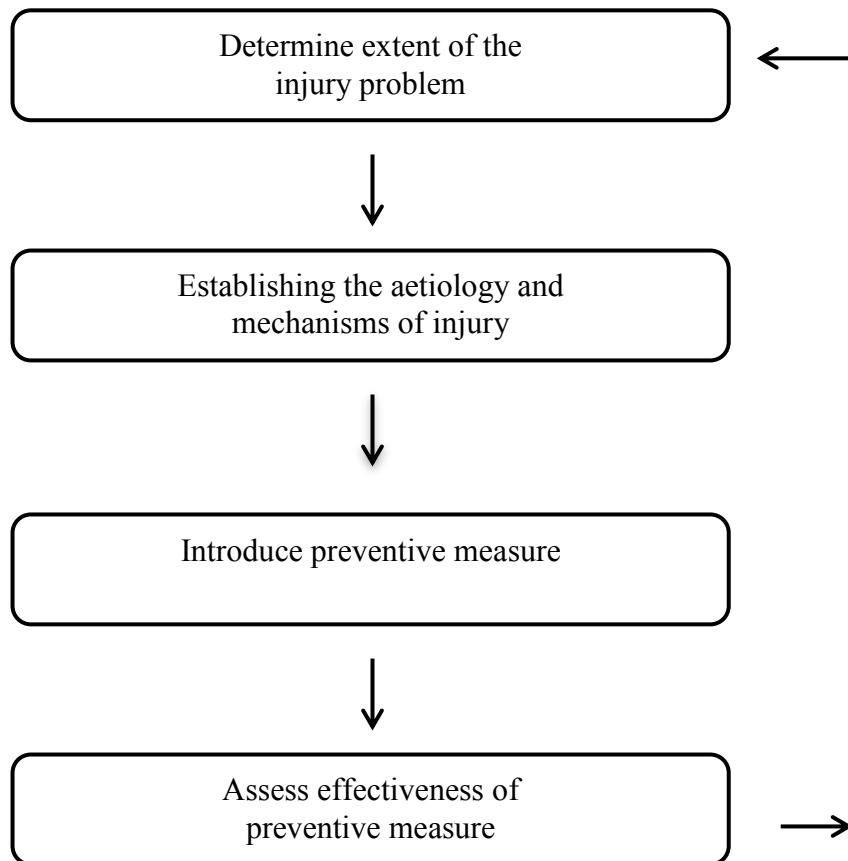


Figure 3.1. Typical flow in an injury preventive scheme (Adapted from van Mechelen *et al.*, 1992, p. 94)

Table 3.1 : A summary of Patterns of muscle injuries.

Author	Sports	Country	Common injuries
Askling <i>et al.</i> , 2004	Soccer		Hamstring injuries: 13/30 (43 %)
Brooks <i>et al.</i> , 2006	Rugby union	United Kingdom	Muscle and tendon injuries: 203/395 (51.4 %) Ligamentous injuries: 150/395 (38.0 %)
Feeley <i>et al.</i> , 2008	American football	America	Knee sprain: 120/728 (16.5 %) Hamstring injuries: n = 85 (21.8 %)
Alonso <i>et al.</i> , 2010	Track & field	Germany	Muscle injuries: 54/247 (21.8 %)
Cloke <i>et al.</i> , 2012	Soccer	United Kingdom	<ul style="list-style-type: none"> • Hamstrings: n = 37 (14.9 %) Muscle injuries: n = 1288 <ul style="list-style-type: none"> • Hamstrings: n = 525 (40.8 %) • Quadriceps: n = 442 (34.3 %) • Adductors: n = 266 (20.7 %)
Murphy <i>et al.</i> , 2012	Gaelic football	Ireland	Muscle injuries: n = 1014
Orchard & Seward, 2013	Australian football	Australia	<ul style="list-style-type: none"> • Hamstrings: n = 432 (42.6 %) Muscle injuries: n = 2841
Ekstrand <i>et al.</i> , 2013	Soccer	Europe	Muscle injuries: n = 1791/8029 (22.3 %) <ul style="list-style-type: none"> • Hamstrings: n = 1025 (12.8 %) • Quadriceps: n = 404 (5.0 %) • Calf: n = 362 (4.5 %)
Eirale <i>et al.</i> , 2013	Soccer	Qatar	Muscle injuries: 79/217 (36.4 %) <ul style="list-style-type: none"> • Hamstrings: n = 17 (21.5 %)

3.1.1 Specific objective of study

1. to examine patterns of muscle injury, and
2. to explore predictors of DRP among Malaysian athletes

3.2 Methods

3.2.1 Study design

A retrospective study was performed to examine the pattern of muscle injuries and factors that predict duration to return-to-play (DRP) among Malaysian athletes.

3.2.2 Study setting

The study was conducted at the National Sports Institute (NSI) Clinic, Kuala Lumpur, Malaysia from January to May 2010. Set up in 1992, the NSI primary role is to provide health care for national athletes, at both elite and developmental levels. In addition, the clinic acts as a referral centre for the nearby Bukit Jalil Sports School and for the Bandar Penawar Sports School in southern Malaysia.

3.2.3 Data source

The ultrasound registration records from June 2006 to December 2009 were reviewed. Medical records of athlete diagnosed with muscle injuries on ultrasonography were evaluated. Clinical information on muscle injuries including date of injury, date of first consultation, injury severity, events leading to injury, injury mechanism and date of return-to-play were extracted. In addition, athletes' socio-demographic background such as age, gender, playing level (school, club, state or national) and sports were recorded in a structured form (Appendix G).

Athletes were under the care of sports medicine specialists at the NSI. A visiting musculoskeletal radiologist with fourteen years' experience performed all ultrasonography assessments. Ultrasonography assessments were performed using Siemens Acuson Alcantares ultrasound system with a 4 cm linear transducer set at 8 to 10-MHz. Severity of muscle injury was described based on ultrasonography appearance using Peetron's grading system (2002) (Table 3.2). The University of Malaya Medical

Centre Ethics Committee and the National Sports Institute of Malaysia approved the study (Appendices H and I).

Table 3.2 : Grading of muscle injuries on ultrasound (Peetrons, 2002).

Grade	Ultrasonography findings
0	No ultrasound features of muscle injury
1	Small foci of muscle disruption (< 5 % area) appearing as low reflectivity areas within muscle
2	Partial tear with muscle fibre disruption (> 5 %) but not affecting the whole muscle. Associated with hematoma formation
3	Complete tear of the muscle with bunching of muscle on dynamic stress and frayed margins.

3.2.4 Statistical analysis

Data were analysed using the Statistical Package for Social Science (SPSS Inc., version 19.0 for Mac, Chicago). Data were described descriptively, and normality test performed with the Shapiro-Wilk test. Duration to return-to-play (DRP) of less than 6 weeks was used as the cut-off value for adequacy of DRP. This definition of adequacy was based on a recent systematic review conducted by Prior *et al.* (2009). Also, athletes who return-to-play more than 6-weeks following injury had significantly lesser chance (3.1 %) of sustaining injury recurrence compared to those who resumed at 2-weeks (8.1 %) or 3-weeks (6.8 %) (Orchard & Best, 2002).

The association between DRP with gender, age group (≥ 18 vs. < 18 years), and duration before first consultation (≤ 1 week vs. > 1 week) were assessed using Mann-Whitney U test. Meanwhile, the association between DRP and sports, playing level (national, state, school and others), new vs. recurrent injury, region of injury (upper

limb, lower limb and trunkal muscles) and ultrasound grading of injury (grades 0 – 3) were determined using the Kruskal-Wallis test.

A stepwise logistic regression analysis was performed to identify predictors of DRP. Variables that were less than 0.25 on univariate testing were included in the multivariate logistic regression model as recommended by previous researchers (Orchard & Best, 2002; Bursac *et al.*, 2008). Adjusted odds ratios (AORs) and 95 % confidence intervals (CIs) of the ORs were calculated, with significance level set at $p < 0.05$.

3.3 Results

A total of 562 medical records of athletes with suspected muscle injuries were screened. Only 360 medical records (237 men and 123 women) were included in the final analysis. The remaining 202 medical records were excluded for reasons including; incomplete medical information (n = 25); missing ultrasound report (n = 75) and injuries involving structures other than muscles (ligaments and tendons)(n = 102).

Three hundred ninety-seven (397) cases of muscle injuries were diagnosed among 360 athletes. The median age of athletes at time of injury was $20.0 \pm$ interquartile range (IQR) 6.0 years. Most injuries occurred among national-level athletes (Table 3.3).

Table 3.3 : Muscle injury characteristics among athletes.

Factor	No. (%)
Gender	
• Men	237 (65.8)
• Women	123 (34.2)
Sports Category	
• Track and Field	109 (30.3)
• Hockey	64 (17.8)
• Racket Sports	41 (11.4)
• Martial arts	24 (6.7)
• Soccer	20 (5.6)
• Weightlifting	18 (5.0)
• Swimming	15 (4.2)
• Gymnastics	17 (4.7)
• Others	52 (14.4)
Level of play	
• National	324 (90.0)
• University/School	17 (4.7)
• State	10 (2.8)
• Others	9 (2.5)
New vs. recurrent	
• New	218 (60.6)
• Recurrent	142 (39.4)
Injured region	
• Lower limb	253 (70.3)
• Upper limb	64 (17.8)
• Back	29 (8.1)
• Abdomen	14 (3.9)
Injuries event	
• Nontraumatic	338 (93.9)
• Traumatic	20 (5.6)
• Others	2 (0.6)
Activitiy	
• Training	297 (82.5)
• Competition	55 (15.3)
• Others	8 (2.2)
Ultrasound grading of injury	
• Grade 1	24 (6.7)
• Grade 2	333 (92.5)
• Grade 3	3 (0.8)
Management	
• Conservative	357 (99.2)
• Surgical	3 (8.0)
Frequency of physiotherapy session	
• Daily	236 (65.6)
• Once a week	65 (18.1)
• Twice a week	49 (13.6)
• Thrice a week	10 (2.8)

Muscle injuries chiefly diagnosed among track and field (30.3 %) athletes, followed by hockey (17.8 %), racket sports (11.4 %), martial arts (6.7 %), soccer (5.3 %), weightlifting (5.0 %), gymnastics (4.7 %) swimming (4.2 %) and other sports (14.4 %). Majority (60.6 %) of muscle injuries classified as new injury. Injuries often affected the lower limb muscles especially the hamstring and adductors muscle groups (Table 3.4). Clinical assessments performed on athletes with a primary complain of lower back pain (n = 29). In addition, a plain radiography of the lumbosacral region performed to rule out any bony pathology. Magnetic resonance imaging (MRI) was performed only when clinical assessment suspected neurological involvement (n = 3) as suggested by The American College of Physician and American Pain Society (Chou *et al.*, 2007). Of these three cases, the MRI was normal in two athletes and a sacrospinalis tear found in the third. Later all athletes underwent ultrasonography assessment of the lumbosacral region using a simple grading for severity (Schwartz *et al.*, 1999; Peetrons, 2002). The reliability and accuracy of ultrasound in diagnosing acute back muscle strain however has not been documented (Brandt *et al.*, 1996). Therefore, possibly other conditions such as intervertebral discs and facet joints' abnormalities might have been missed or overlooked.

The median time to first consultation was 7.0 days \pm IQR 12.0 after injury onset and median time for ultrasonography evaluation was 17.0 days \pm IQR 29.0 after initial injury. Majority of muscle injury were grade-2 (n = 368) muscle injury, followed by grade-1 (n = 26) and less often grade-3 (n = 3).

Most (93.9 %) muscle injuries related to sporting activities, with majority (82.5 %) occurred during training or practice sessions. Majority of the track and field athletes (69.7 %) injured during sprinting, and less often during jumping (13.8 %) and weight

training (5.5 %). A similar finding noted among hockey players with 75 % of muscle injuries occurred while sprinting. In contrast only 40 % of racket sports players' sustained injury during jumping smash.

Table 3.4 : Muscle injury according to body region.

Body region	Muscle group	No. of injuries (%)
Lower limb	Hamstrings	145 (36.5)
	Adductor	43 (10.8)
	Calf	49 (12.4)
	Quadriceps	31 (7.8)
	Others (anterior tibialis, posterior tibialis, peroneal muscles)	11 (2.8)
Upper limb	Deltoid	15 (3.8)
	Biceps	6 (1.5)
	Triceps	4 (1.0)
	Rotator cuff	15 (3.8)
	Others (pectoralis, rhomboids, small muscles of the hand)	35 (8.8)
Abdomen	Rectus abdominis	12 (3.0)
	Others (external obliques, transversus abdominis)	2 (0.5)
Back	Muscles of the back (erector spinae, quadratus lumborum)	29 (7.3)

Nearly all muscle injuries (99.2 %) were treated conservatively. Most athletes (66.4 %) received a short course (less than 1 week) of analgesia (for example Non-steroidal anti-inflammatory drugs) combined with at least one form of electrotherapeutic modality. Electrotherapeutic used include therapeutic ultrasound, interferential current, transcutaneous nerve stimulation and short wave diathermy. A physiotherapy session typically started with range of motion stretching exercises followed by progressive muscle strengthening. In addition various combination of electrotherapeutic modalities often incorporated by the treating physiotherapists during these sessions. Most session ended with 20 to 30 minutes of cryotherapy. Only three athletes with complete muscle rupture underwent surgical intervention.

Dates of the return-to-play were documented only in 168 athletes. The remaining 192 athletes were lost to follow up; hence the dates of return-to-play were not available. Approximately 40 % ($n = 67$) of athletes allowed full RTP within six weeks after injury. Athletes DRP ranges from 1 to 72 weeks, with a median of $7.4 \pm \text{IQR } 8.5$ weeks. No significant difference in DRP found ($H(26) = 25.32, p = 0.50$) across the different sports. Interestingly, the DRP was not affected by the frequency of weekly physiotherapy session ($H(3) = 0.44, p = 0.93$).

Further analysis revealed athletes loss to follow-up were significantly older ($U = 13197, z = -3, p = 0.03$). A moderate, significant positive relationship between time to first consultation and DRP ($U = 2023, p < 0.001$) noted. In addition, significant relationship between DRP and muscle region (limb versus trunkal) demonstrated ($\chi^2 = 6.8, p = 0.04$) (Table 3.5).

Gender, duration to first consultation, injury classification (new versus recurrent), injury severity, number of muscle involved, and side of injury, met the criteria for inclusion in a multivariate model. A delay of the first consultation of more than 1 week, recurrent muscle injury and women athletes identified as significant predictors of DRP more than six weeks (Table 3.6). No interactions found between predictors. All other variables were eliminated by the stepwise procedure.

Table 3.5 : Factors associated with duration to return-to-play (DRP).

Factor	No. (%)	<i>U</i> */ χ^2 §	<i>p</i> value
Gender*			
• Men	107 (63.7)	2898	0.23
• Women	61 (36.3)		
Age group (years)*			
• < 18	52 (31.0)	2730	0.32
• Above 18	116 (69)		
Duration before consultation (week)*			
• 1 week or less	91 (54.2)	2023	< 0.001
• > 1 week	77 (45.2)		
Injury type*			
• New	104 (61.9)	2908	0.17
• Recurrent	64 (38.1)		
Injurious event*			
• Traumatic	10 (6.0)	646	0.34
• Non-traumatic	158 (94.0)		
Injury grade (Ultrasound)*			
• Grade 1	12 (7.1)	681	0.12
• Grade 2	156 (92.9)		
No. of muscle injured*			
• One	147 (87.5)	1144	0.07
• Two	21 (12.5)		
Activity leading to injury§			
• Training	140 (83.3)	0.69	0.71
• Competition	26 (15.5)		
• Others	2 (1.2)		
Affected side§			
• Right	73 (43.5)	3.50	0.18
• Left	84 (50.0)		
• Bilateral	11 (6.5)		
Region affected§			
• Upper limb	31 (18.5)	6.8	0.04
• Lower limb	121 (72.0)		
• Trunkal	16 (9.5)		
Level of play§			
• National	155 (92.3)	0.24	0.97
• State	3 (1.8)		
• School	7 (4.1)		
• Other	3 (1.8)		
Physiotherapist session§			
• Daily	119 (70.8)	0.60	0.90
• Once a week	21 (13.1)		
• Twice a week	21 (12.5)		
• Thrice a week	6 (3.6)		

* Mann-Whitney U test

§ Kruskal Wallis test

Table 3.6 : Predictors of duration to return-to-play more than six weeks after muscle injury.

Determinant	<i>B</i> (SE)	<i>p</i>	AOR	95 % CI
Duration to consultation > 1 week	1.29 (0.32)	< 0.001	3.63	1.80 – 7.30
Recurrent injury	0.76 (0.37)	0.038	2.14	1.04 – 4.38
Women	0.74 (0.37)	0.048	2.09	1.01 – 4.34

SE = standard error, AOR = adjusted odds ratio, CI = confidence interval.

3.4 Discussion

The pattern of muscle injury among Malaysian athletes is comparable to earlier studies (Garrett 1996; Dick *et al.*, 2007; Alonso *et al.*, 2010; Liu *et al.*, 2012; Ekstrand *et al.*, 2012).

In this study, most frequently diagnosed muscle injury of the lower limb is the hamstring muscle injury followed by the adductor and calf muscles. Similar findings were noted among intercollegiate hockey players (Dick *et al.*, 2007). In addition, lower extremity muscle strain was the most frequent injury diagnosed at the 2007 International Association of Athletics Federations World Athletics Championships (Alonso *et al.*, 2010). Further, an 11-year cohort study of 27 Union of European Football Associations (UEFA) soccer clubs (1743 players) reported hamstring and adductor muscles were the two most common muscles injured (Ekstrand *et al.*, 2013).

Excessive tensile forces on contracting muscles fibres (eccentric contraction) during fast bursts of speed suggested as the main cause of muscle injury (Garrett 1996; Liu *et al.*, 2012). Muscle injury especially affects muscles that cross two joints, such as the biceps femoris, semimembranosus, semitendinosus, gastrocnemius and rectus femoris (Anderson *et al.*, 2001; Orchard *et al.*, 2002; Miller, 2007).

Eccentric contraction that occurs during sports (including running and jumping) can overstretch muscle fibres leading to membrane, sarcoplasmic reticulum, transverse tubule and sarcolemmal damage (Proske *et al.*, 2001; Proske *et al.*, 2005). Most muscle injury observed in this study occurred while running or jumping. Similar observations were reported by previous studies (Verrall *et al.*, 2003; Woods *et al.*, 2004;

Gabbe *et al.*, 2004). Askling *et al.* (2007) noted all athletes (n = 18) in their case series suffered hamstring injury while sprinting at maximal or close to maximal speed. Interestingly in all 18 sprinters, injury primarily involved the long head of biceps femoris (Askling *et al.*, 2006). In a cohort study of 12 English Premiership rugby union clubs (546 players), Brooks *et al.* (2006) reported majority (68 %) hamstring injury was sustained during running activities (Brooks *et al.*, 2006).

Grade-2 muscle injury was the most common (92.9 %) injury diagnosed in this study. Using similar method of injury classification, a lower frequency (35.5 %) of grade-2 muscle injury was reported in a prospective cohort study among elite track and field athletes (Malliaropoulous *et al.*, 2010). In a muscle injury study among professional footballers, Ekstrand *et al.* (2011) found majority of injury was classified on MRI as grade-1 (57 %) followed by grade-2 (27 %) and grade-3 (3 %).

A possible explanation for the difference observed could be due to the different study designs. Current study was a retrospective where relevant information about muscle injury was extracted from the athlete's medical records in the NSI Clinic. Being the main referral centre for sports medicine, possibly only more severe injuries were referred to this clinic, and athletes with less severe muscle injury may have received treatment elsewhere.

Most muscle injuries noted in the current study was sustained during training rather than competition. As most athletes would spend more time training for competition, such observation is expected (Shariff *et al.*, 2009). Previous studies however reported the opposite (Gabbe *et al.*, 2004; Brooks *et al.*, 2006; Askling *et al.*, 2006; Dick *et al.*, 2007; Alonso *et al.*, 2010; Eirale *et al.*, 2013). Gabbe *et al.* (2006)

reported majority (76.9 %) of hamstring injuries among community level Australian footballers occurred during competition (Gabbe *et al.*, 2004). Woods *et al.* (2004), in a cohort of 91 professional football clubs noted approximately two third (67 %) of hamstring injuries were sustained during matches (Woods *et al.*, 2004). Brooks *et al.* (2006) reported hamstring injury rate of 0.27 injuries/1000 player hours during training and 5.6 injuries/1000 player hours in professional rugby players. The author attributed lower injury rate during training to high volume of low-risk training activities performed by their players (Brooks *et al.*, 2006).

Several explanations could be responsible for differences observed between the current and previous studies. First, the cross-sectional nature of the current study does not allow hamstring injury rate to be reported. Second, most athletes in the current study were non-professional athletes from various sports participating at different playing levels. Hence, training activities including duration of each training session, frequency of training (per week) and activities performed (high-risk versus low-risk) is expected to be different. A prospective cohort study design is recommended to better explore hamstring injury incidence. Further, since the current study was based on information available in the outpatient clinic, injuries treated during sports events and may have been missed.

The median DRP of $7.4 \pm \text{IQR } 8.5$ weeks noted among Malaysian athletes in this study is comparatively longer than earlier studies (Malliaropoulos *et al.*, 2010; Warren *et al.*, 2010; Silder *et al.*, 2013; Rettig *et al.*, 2013). A study conducted in Greece reported an average of 2.1 weeks lost from training and competition among elite-level track-and-field athletes. Shorter DRP reported by could be because of higher proportion (64.5 %) of low-grade muscle injury (grade 1) in their study (Malliaropoulos

et al., 2010). In a randomised clinical trial, Silder *et al.* (2013), reported a mean DRP of 3.8 weeks (ranges: 13 - 49 days) among participants with grade-II hamstring injury treated with two different rehabilitation programs (progressive agility and trunk stabilization (PATS) versus progressive running and eccentric strengthening (PRES). The previous study however defined DRP as the period from injury to completion of rehabilitation instead of return to competition or training (current study). The mean DRP of 3.1 weeks observed among professional football players diagnosed with acute grade-II hamstring injury (Ekstrand *et al.*, 2011). A study on hamstring injury among Australian footballers found median time to return to competition of 3.7 weeks. The authors however did not describe the severity of muscle injury sustained by their athletes (Warren *et al.*, 2010).

In this study, athletes who delayed medical consultation by more than one week (after the onset of injury) took significantly longer period to recover (more than six weeks) than those who seek treatment earlier. A study involving 30 elite-level Swedish athletes reported a median DRP of 31 weeks among athletes who presented 12 weeks after sustaining hamstring injury. Also, 47 % of athletes in the study decided to retire after a follow-up period of 63 weeks (Askling *et al.*, 2008). Early management of muscle injury were shown to affect the extent of injury and amount of scar tissue formation, which influences the duration of muscle healing (Hawkins *et al.*, 2001; Deal *et al.*, 2002; Järvinen, 2005). Early immobilisation (less than one week) following injury shown to limit the size of connective tissue (scar) formed within the site of injury in rat gastrocnemius muscle (Deal *et al.*, 2002). Further, early cryotherapy is associated with significantly smaller haematoma, lesser inflammatory reaction and tissue necrosis and hastens early recovery (Hocutt *et al.*, 1982; Järvinen, 2005). Increasing athlete and coaches' awareness on the importance of early medical consultation following injury

and improving medical accessibility (on-site medical team support) may shorten the duration between injury and first consultation period which might positively affect the DRP.

History of previous muscle injury is one of the most important risks for subsequent muscle injury. In this study more than one-third (38.1 %) of athletes diagnosed with hamstring injury had similar episode of in the past. Hamstring reinjury rate of 12 to 44 % has been reported by researchers (Woods *et al.*, 2004; Askling *et al.*, 2008; Petersen *et al.*, 2010; Elliott *et al.*, 2011; Ekstrand *et al.*, 2012). Athletes with history of muscle strain are two to six times more likely to experience recurrent strains (Orchard, 2001; Verrall *et al.*, 2006). Reduced tensile strength of scar tissue, reduced muscle strength, and reduced muscle flexibility suggested to explain these observations. In addition possible adaptive changes including altered biomechanics and motor patterns of movements after injury are responsible for increasing risk of injury recurrence (Dick *et al.*, 2007).

In this study athletes with history of muscle injury were more likely to take more than six weeks to return-to-play than those diagnosed with new injury. Similar observation reported in the National Football League (NFL) training camp injuries prospective study. Significantly longer recovery time was noted among athletes diagnosed with hamstring re-injuries (56 days) than those with first-time injury (16.5 days) (Rettig *et al.*, 2008). Laboratory study showed absence of satellite cells within the fibrotic tissue (scar) and failed migration of neighbouring myogenic satellite cells into the scare as the phenomenon responsible for the delay in healing of recurrent muscle injuries (Grefte *et al.*, 2009).

Female athletes with muscle injury took more than six weeks to recover compared with male. The reason for this association is unclear. One possible explanation could be related to the difference in the circulating sex hormones between female and male. Significantly less inflammatory cells (neutrophils and granulocytes) infiltrated the vastus lateralis of female university students after a standardised pain inducing eccentric exercise compared to men (Stupka *et al.*, 2000). Leucocytes and macrophages infiltration into injured muscle are important for satellite cells activation and initiation for muscle regeneration. Therefore, oestrogen-attenuating effects on leucocyte infiltration may delay important stages in muscle healing and recovery (Schneider *et al.*, 1999; Tiidus, 2000; Tiidus, 2005). Further, a study on 16 premenopausal university students showed significant hamstring muscle extensibility changes throughout different phases of menstrual cycle that increases the likelihood of sustaining acute hamstring injury (Bell *et al.*, 2009).

Interestingly, the frequency of weekly physiotherapy sessions did not affect the DRP. In contrast to our findings, a study involving 80 athletes with grade-2 hamstring injury noted significantly shorter recovery time among athletes who received more intensive stretching program (Malliaropoulos *et al.*, 2004). The attending physiotherapists in this study did not use a standard treatment protocols. Each physiotherapist has their own treatment regimen based on anecdotal report and personal experience. The treatment protocol differs on the types; sequence of exercises prescribed, duration of the treatment session and type/s of electrotherapeutic modalities, which further complicates comparisons between the different regimens.

The high loss to follow-up rate (53 %) is of major concern especially when it involved national-level athletes. There is a possibility that athletes' loss to follow-up in

this study have achieved full recovery and recommenced usual activities. It is also possible that these athletes decided to retire or sought treatment elsewhere. A prospective study to explore on factors associated with loss to follow-up is underway.

This study found the timing of first consultation, history of muscle injury (recurrence) and women gender were significant factors in predicting the DRP among Malaysian athletes.

3.5 Conclusion

Grade-2 lower limb muscle injury was the commonest injury diagnosed among national-level athletes. Athletes with muscle injury responded well with conservative treatment approach. The median DRP of athletes with muscle injuries in this study was 7.4 weeks. Athletes who waited more than 1 week before first consultation, athletes with recurrent muscle injury and female athletes were significantly more likely to take more than six weeks to return-to-play after muscle injuries. Incorporating these important parameters in early assessments of muscle injuries is useful in predicting recovery. Strategic steps needed to ensure early consultation and commencement of treatment as soon as an injury occurs. Increasing awareness on factors associated with DRP among athletes, coaches and to those involve in the care of athletes is recommended.

A prospective study with a larger sample size could better show the associations between clinical assessments and treatment outcomes including potential variables with small to moderate effects.

3.6 Summary

Limited information is available on muscle injury among Malaysian athletes. A retrospective study of the medical records of athletes with muscle injury was performed to explore pattern of muscle injury among local athletes. The pattern of muscle injury and injury management were comparable to previous studies. Lower limb muscle injury particularly the hamstring group was the commonest muscle injured among athletes. Despite these similarities, the DRP for Malaysian athletes is relatively longer compared to other studies. Duration before initial consultation, recurrent muscle injuries and female athletes can significantly predicts the DRP among Malaysian athletes. Incorporating these factors in clinical setting can be useful in predicting recovery after muscle injury.

Chapter 4 Reliability of active knee extension (AKE) test among healthy adults:

A cross sectional study

4.1 Introduction

Based on the current knowledge, risks of hamstring muscle injury include previous hamstring injury, muscle strength imbalance, increasing age, inadequate warm-up, and muscle fatigue (Gabbe *et al.*, 2004; Croisier *et al.*, 2010; Cross *et al.*, 2010). Other factor such as hamstring muscle tightness has also been suggested to contribute to hamstring injury. The interaction between hamstring flexibility and knee flexion angle-torque was examined in 20 healthy volunteers (Alonso *et al.*, 2009). This study noted the angle-torque curve shifted to the left in less flexible hamstring (Figure 4.1). The less flexible hamstring group had higher torque at shorter muscle length but lower torque at longer muscle length. Possibly lower torque produced by the less flexible hamstring may not be able to withstand the passive lengthening forces especially during eccentric muscle contraction and increases the risk of injury.

The relationship of hamstring flexibility with hamstring injury is still inconclusive, as studies have shown conflicting results (Burkett, 1970; Ekstrand & Gillquist, 1983; Hennessey & Watson, 2005; Jonhagen *et al.*, 2006; van Beijsterveldt *et al.*, 2012).

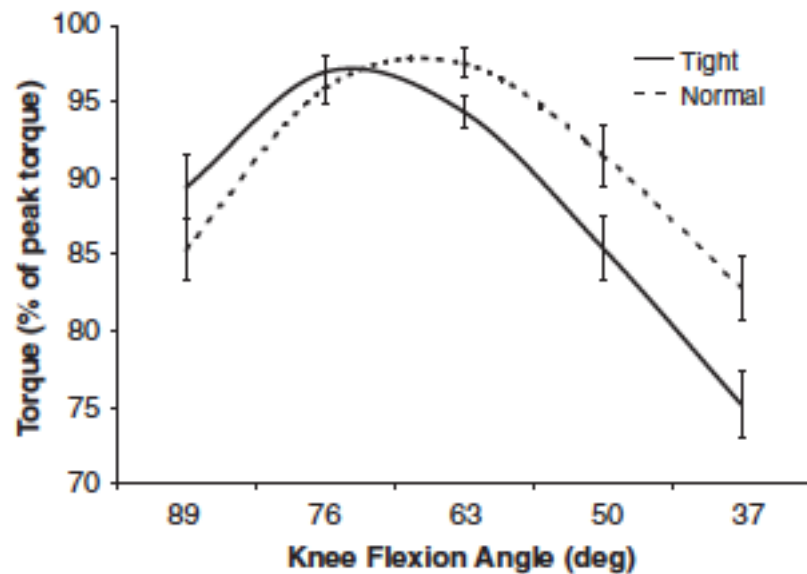


Figure 4.1. Angle-torque relationship for maximum isometric knee flexion contractions: tight vs. normal hamstrings. Mean standard error displayed (Source: Alonso *et al.*, 2009, p. 254).

Worrell *et al.* (1991) compares hamstring isokinetic strength and flexibility in 32 male athletes found athletes with history of hamstring injury had less hamstring flexibility than the noninjured group. They also noted that the injured extremity was significantly less flexible than the noninjured side within the hamstring injured group. A prospective cohort study of 146 professional soccer players found players with hamstring and quadriceps muscle injury had significantly lower flexibility in these muscles before the start of season. The author concluded soccer players with tight hamstring or quadriceps have a higher risk of injury (Witvrouw *et al.*, 2003).

In contrast, Burkett (1970) found no significant association between hamstring flexibility and hamstring injuries in a cross-sectional study of football players and track athletes. Similar observations were reported in more recent prospective cohort studies (Orchard *et al.*, 1997; Gabbe *et al.*, 2006; Yeung *et al.*, 2009; Engebretsen *et al.*, 2010).

Despite this, hamstring flexibility assessments are used regularly during routine preparticipation examination and in deciding athletes' readiness to return-to-play following an injury (Croisier *et al.*, 2002; Drezner, 2003; Mendiguchia & Brughelli, 2010). Thus, a simple and reliable method of hamstring flexibility assessment is still relevant.

There are several methods of hamstring flexibility assessments described in the literature. The three most common methods of flexibility assessments are the straight-leg-raising (SLR), sit and reach (SR) and active knee extension (AKE) tests (Gajdosik & Lusin, 1983; Booher & Thibodeau, 1985; Baltaci *et al.*, 2003). Each test has its own advantages and disadvantages.

The SLR test is usually performed with the patient lying in supine and both lower extremities extended. The examiner passively flexes the hip joint, while keeping the ipsilateral knee in extension. To avoid neural tension, the ankle is kept in slight plantarflexion throughout the test. At the end of passive movement, the hip joint angle is measured with either a universal goniometer or a gravity inclinometer (Figure 4.2) (Davis *et al.*, 2008). Despite the simplicity of performing the SLR test, questioned rose on the test specificity as this test is also widely used as a neurological test (Malanga & Nadler, 2006). Further, cinematographic on 11 healthy subjects (9 men and 2 women) showed significant pelvic rotation occurred during an SLR test. The authors concluded that when assessment of hamstring flexibility is made with SLR, pelvic rotation should either be prevented (stabilised) or accounted for (Bohannon, 1982).

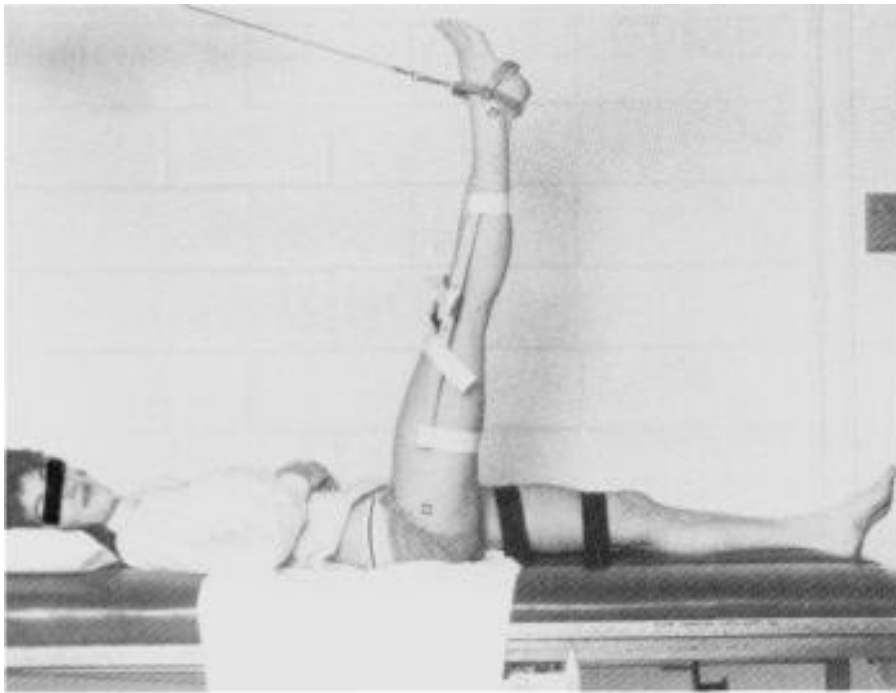


Figure 4.2. The straight leg raise test (Source: Bohannon, 1982, p. 1270).

Many variations exist in performing the SR tests. The classical SR test requires a measuring box with a mounted ruler. The subject sits on a flat surface (floor or plinth) with both knee extended and feet placed flat against the device (Figure 4.3). Subject is asked to reach forward slowly with both hands as far as possible and hold the position for 2 seconds. Using the plantar surface of the feet as reference point the most distant point reached with the fingertips is recorded.

Although hamstring flexibility assessment can easily be performed using the sit and reach (SR) test, the validity of this test was considered poor (Lemmink *et al.*, 2003; Clark, 2008; Mier, 2011). Moreover, the criterion-related validity of the SR test and modified SR test for estimating hamstring flexibility was found to be weak among children and adolescents aged 6 – 17 years (Pinero *et al.*, 2009).

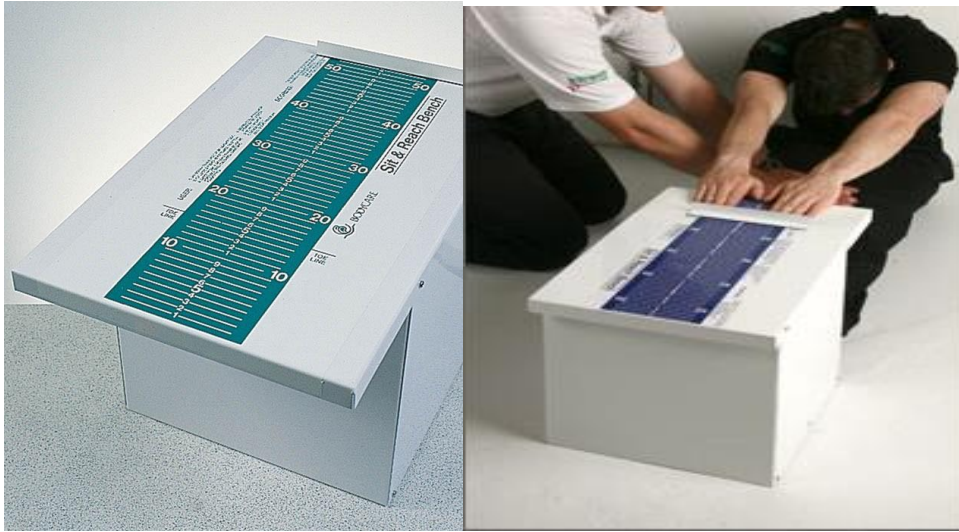


Figure 4.3. The sit and reach test (Sources: <http://www.habdirect.co.uk/products/fitness-testing/sit-reach-box-steel> and <http://www.fitnessbydesign.ie/images/sit-and-reach.jpg>).

The AKE test is an active test that involves movement at the knee joint. The test is performed while subject in supine with the leg not examined strapped on the examination couch. The centre of the knee joint is marked over the lateral joint line. Two lines are drawn from this point to the greater trochanter of the femur and another to the tip of the lateral malleolus. The subject is asked to bend the knee and hip to 90°. While keeping the hip joint in 90°, subject is instructed to extend the leg as far as possible and hold the position for a few seconds. The angle of the knee extension is measured using a universal goniometer positioned along the lines marked earlier (Figure 4.4). Most researchers consider this test safe as the patient dictates the end point of movement. Further, the AKE test aided by metal rig and straps (to limit pelvic and leg motion) showed good interater and intrarater reliability as demonstrated by previous studies (Gajdosik & Lusin, 1983; Kuilart *et al.*, 2005; Davis *et al.*, 2008; Reurink 2012). Others though, questioned the practicality of the AKE test, as the apparatus used by researchers are complicated and rarely available in clinic setting (Figure 4.5) (Worrell *et al.*, 1990). Therefore, a reliable and easy to perform method of hamstring flexibility assessment is much relevant.



Figure 4.4. The active knee extension (AKE) test (Norris & Matthews, 2005 p. 258).

In the Sports Medicine Clinic, University Malaya Medical Centre (UMMC), most patients are arranged to come in for their follow-up appointment once a week. At each follow-up session, a doctor or physiotherapist in charge attends to patients. Usually the same health care provider provides continuity of care for the patient. However, in some occasions this may not be feasible. Therefore developing an efficient, practical and reliable method of AKE assessment will be valuable under these circumstances.



Figure 4.5. The active knee extension (AKE) test using metal rig (Kuilar 2008 p. 92).

The interater and intrarater reliability of the AKE test used in this study was published in the Journal of Physical Therapy Science in 2013 (Hamid *et al.*, 2013) (Appendix J).

4.1.1 Specific objective of study

1. to design and set up a simple apparatus that would allow a single assessor to perform assessment of AKE easily
2. to determine the intrarater and interater reliability of proposed method of AKE assessment

4.2 Methods

4.2.1 Construction of AKE apparatus

A simple, portable and easy to use apparatus (to help stabilise the pelvic and hip motion) was designed and constructed to aid in AKE assessment. The apparatus is made of 15 mm polyvinyl chloride (PVS) pipe (STAR uPVC: BS3505 Class 7) (Figure 4.6), as it was cheap, easy to handle and lightweight, enabling the apparatus to easily transport to any location. The apparatus when used with a universal goniometer (Sammons Preston # 7514) (Figure 4.7) allowed a single assessor to perform AKE test easily.

The designed of the apparatus was based on those used in previous study (Gajdosik & Lusin, 1983). It consisted of a single horizontal bar attached to two vertical poles on either side. The vertical poles anchored to the sides of the plinth by means of four tubing clips. These clips were attached to the plinth by four screws (Figure 4.8). The vertical poles position can be adjusted proximally or distally by slotting the horizontal bar into the appropriate connection on the side of the plinth. The horizontal bar should be place aligning the patient's anterior superior iliac spines (ASIS) during measurement.



Figure 4.6. Apparatus used to aid active knee extension (AKE) test constructed from polyvinyl chloride (PVC) tubes.

4.2.2 Participants

Sixteen healthy participants (10 men and 6 women) volunteered in this study. Participants' age ranges from 28 to 39 years old. Participants were either Sports Medicine postgraduate students or the staffs at the Faculty of Medicine, University of Malaya. All participants were free from any orthopaedic or neurological disorders.

4.2.3 Determination of AKE measurement reliability

The University of Malaya Medical Centre Ethics Committee approved the study (MEC Ref no.835.11).

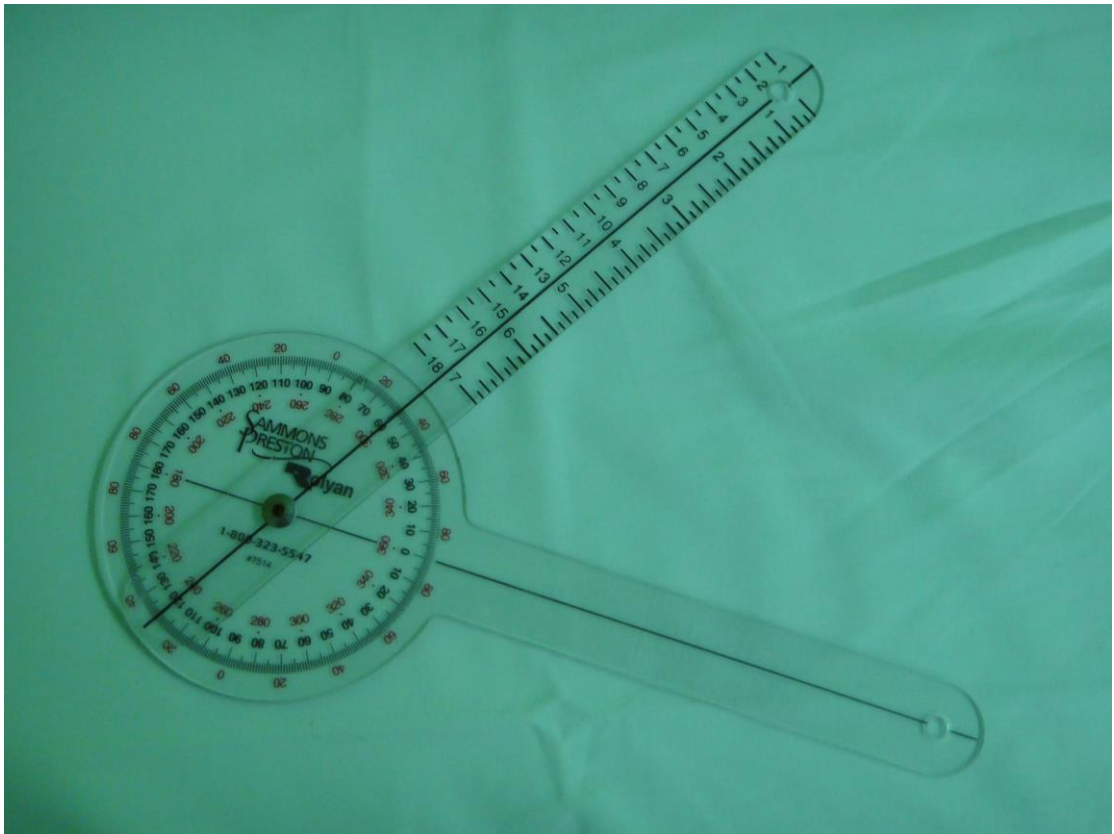


Figure 4.7. A standard perspex goniometer.



Figure 4.8. Horizontal poles anchored to the sides of the plinth.

All assessments were conducted at the Sports Medicine Clinic, University of Malaya Medical Centre. Prior to participation, the purpose of the study and testing procedure involved were explained to all participants, and each signed an informed consent. The preferred leg for physical activities (jumping or kicking a ball) was recorded as the participants' dominant leg. To ensure that all participants would be assessed in the standard manner, both assessors attended a half-day workshop on a standardised method of AKE test conducted by the main author.

A sport physician (SAH) and a physiotherapist (LPC) performed all the AKE tests independently. All assessments were conducted between 9.00 to 11.00 am at room temperature (22.7 ° C and 85.7 % humidity). All participants were asked to avoid from any physical activities on the day of AKE test and remain hydrated (Rakos *et al.*, 2001). This is because previous study showed changes in biomechanical characteristics of collagen and muscles viscoelastic properties after warming-up, and might affect AKE measurement.

Participants were assessed on a plinth in the supine position with both knees extended. Both anterior superior iliac spines were positioned aligning with vertical bars of the apparatus (Figure 4.9). The lower extremity not being measured was secured to the plinth with a strap across the lower third of the thigh. Each assessor marked the lateral knee joint line with washable ink. From here, two lines were drawn, first, to the greater trochanter and another to the top of lateral malleolus. Participants were told to flex his or her hip until their thigh touches the horizontal bar (Figure 4.10). While maintaining contact between thigh and the horizontal bar, the participant was asked to extend the leg as far as possible (point of discomfort), keeping their foot relaxed, and held the position for about 5 seconds.



Figure 4.9. Participant position at the beginning of active knee extension (AKE) test.

A standard universal goniometer was placed over the previously marked joint axis and the goniometer arms aligned along the femur and fibula (Figure 4.11). The AKE measurement was defined as the degree of knee flexion from terminal knee extension. Each knee was measured twice, and the mean angle of AKE test was used for analysis. All participants attended two testing sessions one week apart to allow for establishment of test-retest reliability of the method. The order in which the rater assessed the participants was randomly assigned in the first session and maintained thereafter.

4.2.4 Data analysis

Data were analysed using Statistical Package for the Social Sciences (SPSS Inc., version 20.0 for Mac, Chicago). The average AKE angles of two measurements for each knee were used for statistical analysis. Data were described descriptively, and normality testing performed using Shapiro-Wilks test. Paired *t*-tests were performed to compare differences between tests and retest measurement within and between raters.

Two different types of measures of reproducibility were assessed: measures of agreement and measures of reliability. The interater agreement quantified by calculating the mean difference between the two raters (raters 1 – 2) and the standard deviation (SD) of this difference. Further, the 95 % limits of agreement were calculated according to the method of Bland & Altman (Bland & Altman, 1986). These limits represent the range in which 95 % of the differences between the two raters fall.



Figure 4.10. Participant flexing the hip until thigh touches the horizontal bar.



Figure 4.11. Participant extending the knee while maintaining thigh position.

Plots of differences between raters against the corresponding mean of the two raters for each participant were produced to examine homoscedasticity as proposed by Bland-Altman (Bland & Altman, 1986). Further, the frequency of agreement of the raters within 5 and 10 ° was calculated. Differences exceeding 10 ° was determined as being unacceptable as they are likely to affect decisions on patient management as suggested by previous study (Weijer *et al.*, 2003).

The AKE test reliability was evaluated by computing the Intraclass Correlation Coefficients (ICC). The ICC analyses consistency of quantitative measurements. An ICC, two-way random model was used to establish reliability ICC, and one-way random model was chosen to evaluate reliability (Portney & Watkins, 2000). The ICC value of the standard error of measurement (SEM) was calculated to express magnitude of disparity between measurements (Shrout & Fleiss, 1979). The SEM was calculated using the formula; $SEM = SD_{avg}(\sqrt{1 - ICC})$ where SD corresponds to the pooled standard deviation and ICC is the reliability coefficient (Portney & Watkins, 2000). A smaller value of SEM suggests greater agreement between measurements (Atkinson & Nevill, 1998). The minimum detectable change (MDC) was calculated using the formula $MDC = (1.96) (SEM) \sqrt{2}$ (Macedo & Magee, 2008).

4.3 Results

Only 14 (8 male and 6 female) participants were able to complete the AKE test and retest sessions. Two participants were excluded from the final analysis as one suffered a hamstring injury, and the other had a road traffic accident. Table 4.1 summarizes the results of the normality testing of continuous variable. The median age

of the participants was 31.0 (range: 28.0 – 39.0). A significant difference in body weight between men (Mean = 77.69, SD =12.86) and women (Mean = 63.42, SD = 10.27) participants; $t = 2.23$, $p = 0.046$ was noted.

Table 4.1 : Test of normality for continuous variables.

Continuous variables	Shapiro - Wilks		
	Statistics	Degree of freedom	P value
Age (years)	0.873	14	0.046*
Weight (kg)	0.942	14	0.448
Height (cm)	0.950	14	0.556
BMI	0.945	14	0.487
AKE dominant knee	0.911	14	0.161
AKE non-dominant knee	0.908	14	0.145
AKE differences	0.954	14	0.629

Note: * $P \geq 0.05$ = Normal distribution.

General description of participant's physical characteristics is displayed in Table 4.2. The means AKE measurements of the dominant and non-dominant side were compared. No significant difference in AKE angle between both sides was found ($t = 0.59$, $p = 0.487$). Furthermore, no significant difference was observed between test and retest sessions for both dominant ($t = 0.77$, $p = 0.46$) and non-dominant ($t = -1.01$, $p = 0.33$) knees (Table 4.3).

Table 4.2 : Descriptive data participant's physical characteristics

Variables	All participants		Male	Female
	Mean (SD)/ Median (IQR)†	Range	Mean (SD)/ Median (IQR)†	Mean (SD)/ Median (IQR)†
Age (years)†	31.00 (2.50)	28-39	32.00 (3.01)	30.00 (2.43)
Body weight (kg)	71.57 (13.54)	54-100	77.69 (12.87)	63.42 (10.27)
Height (cm)	168.40 (6.06)	159-181	170.94 (5.82)	165.00 (4.90)
Body mass index (BMI)	25.09 (3.50)	19.13-31.83	26.49 (3.36)	23.23 (2.99)
AKE† dominant knee (°)	24.06 (7.43)	13.0-42.0	25.48 (8.90)	22.17 (5.01)
AKE non-dominant knee (°)	24.22 (9.72)	15.1-42.5	26.23 (8.62)	21.54 (4.41)
AKE difference (°)	-0.16 (2.41)	-3.38-4.75	-0.75 (1.81)	0.63 (3.03)

SD = Standard deviation; IQR = interquartile range; AKE = Active knee extension.

Table 4.3 : Active knee extension (AKE) measurements of dominant and non-dominant knees

Side	Mean AKE angle	SD	Range
Dominant	24.063	7.43	13.00 – 42.00
Non-dominant	24.223	7.29	15.13 – 42.50

AKE = active knee extension; SD = standard deviation.

4.3.1 Interater agreement and reliability

Results of raters 1 and 2 from the testing sessions were compared. Table 4.4 displays the results of these comparisons. No significant difference between raters was observed for both lower limbs AKE tests. A summary of the interater agreement observed in this study is displayed in Table 4.5. Both raters had similar measurements of AKE, with the limits of agreement of 0.1 ± 12.9 (SD) for the dominant and -1.1 ± 15.7 for non-dominant knees. The percentages of agreement within 10° for these measurements were 93 and 79 % for the dominant and non-dominant knee respectively.

Table 4.4 : Mean, standard deviation (SD), mean differences between raters and the frequency of agreement within 5 and 10 degrees.

AKE test	Rater 1 Mean (SD)	Rater 2 Mean (SD)	Rater 1 – 2 Mean (SD)	Upper and lower limit of agreement	% of agreement	
					5°	10°
Dominant knee (°)	24.9 (9.3)	24.8 (10.1)	0.1 (6.9)	-13.4 – 13.6	43	93
Non-dominant knee (°)	23.7 (8.1)	24.9 (7.2)	-1.1 (7.2)	-15.2 – 13.0	43	79

AKE = Active knee extension; SD = Standard deviation.

The AKE measurement differences between raters were plotted against the mean value of both raters for both the dominant and non-dominant knees (Figures 4.12 and 4.13). Errors of measurement for both knees were independent of the magnitude of the range of measurements demonstrating homoscedascity.

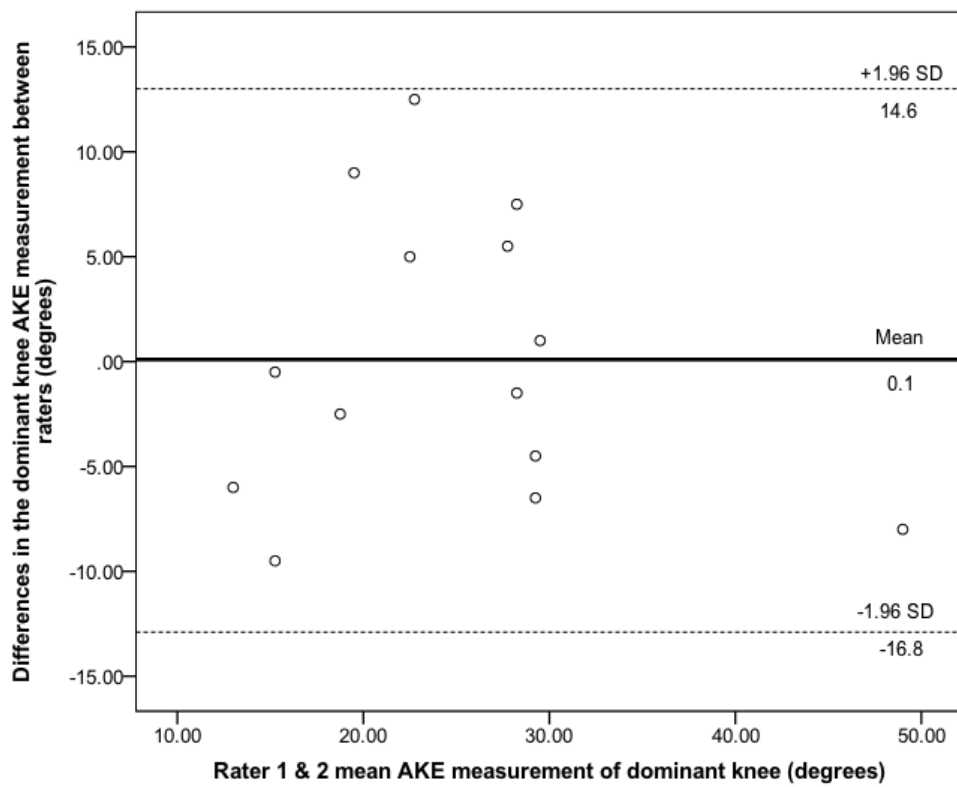


Figure 4.12. Bland-Altman plot of the differences versus means of the dominant AKE measurements.

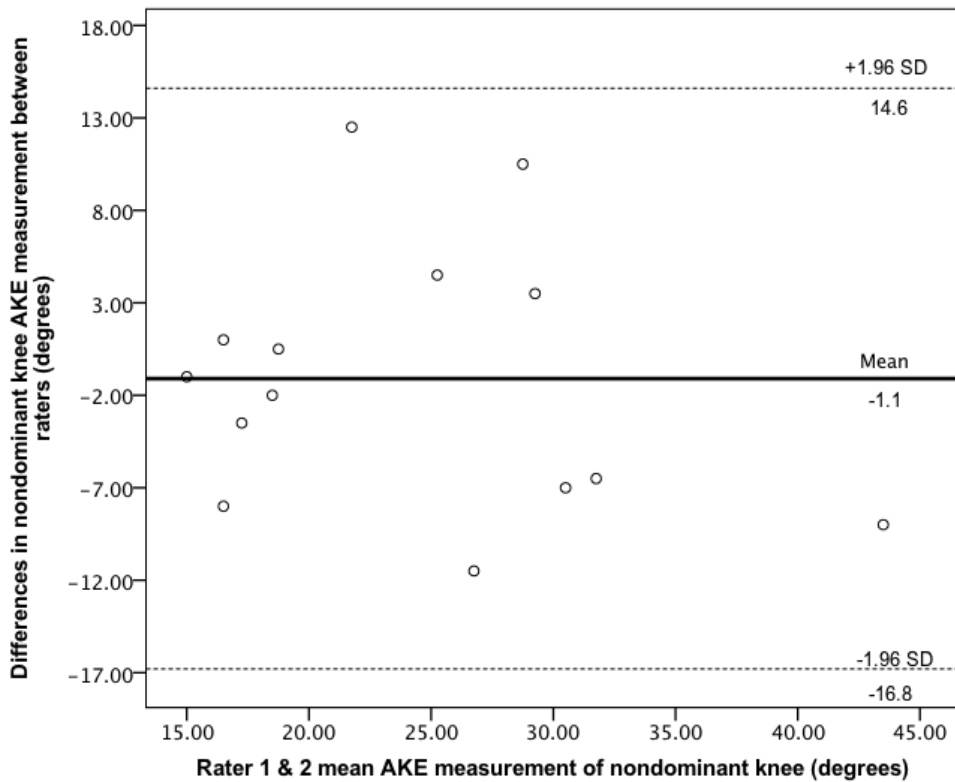


Figure 4.13. Bland-Altman plot of the differences versus means of the non-dominant AKE measurements.

The AKE measurements by raters 1 and 2 from the first testing session were compared (Table 4.5). No significant differences between raters were observed for both lower limbs. The AKE test interater reliability was excellent, with ICC_{2,1} values of 0.87 (0.58 – 0.97; 95 % CI) for the dominant and 0.81 (0.41 – 0.94; 95 % CI) for the non-dominant knees and standard error of measurement (SEM) values of 3.5 ° (18.0 – 31.7°; 95 % CI) and 3.8 ° (16.9 – 31.7 °; 95 % CI) respectively. Minimal detectable change was between 9.7 and 10.5 °.

Table 4.5 : Inter-rater reliability of active knee extension (AKE) test.

AKE measurements	Rater 1, mean (SD)	Rater 2, mean (SD)	<i>p</i> value	ICC _{2,1}	95 % CI ICC	SEM	95 % CI SEM
Dominant knee (°)	24.9 (9.3)	24.8 (10.1)	0.95	0.87	0.58 – 0.97	3.5	18.0 – 31.7
Non-dominant knee (°)	23.7 (8.1)	24.9 (9.5)	0.56	0.81	0.41 – 0.94	3.8	16.9 – 31.7

AKE = Active knee extension test; SD = Standard deviation; ICC = Intraclass correlation coefficient; CI = Confidence interval; SEM^l = Standard error measurement.

4.3.2 Test-retest reliability

The AKE measurements by raters 1 and 2 from the first testing session were compared. No significant differences of the mean AKE measurements between the first and second testing sessions were found for both raters (Table 4.6). The ICC_{3,1} values ranges from 0.78 to 0.92.

Table 4.6 : Active knee extension (AKE) test, test-retest reliability of both raters.

AKE test	Rater	Session 1, mean (SD)	Session 2, mean (SD)	<i>p</i> value	IC C _{3,1} ‡	95% CI [§] ICC	SEM	95% CI SEM
AKE dominant knee (°)	1	24.9 (9.3)	24.0 (6.8)	0.46	0.92	0.76 – 0.97	2.3	19.9 – 29.0
	2	24.8 (10.1)	22.3 (6.2)	0.21	0.78	0.32 – 0.92	3.9	16.0 – 31.2
AKE non-dominant knee (°)	1	23.7 (8.1)	25.2 (8.6)	0.33	0.88	0.45 – 0.92	2.9	18.8 – 30.2
	2	24.9 (9.5)	23.1 (5.9)	0.31	0.82	0.46 – 0.94	3.3	17.5 – 30.5

AKE = Active knee extension test; SD = Standard deviation; ICC = Intraclass correlation coefficient; CI = Confidence interval; SEM = Standard error measurement.

4.4 Discussion

Despite conflicting observation on the association of poor hamstring flexibility and the risk of hamstring injury, assessment of hamstring flexibility continues to be routinely done during athlete's physical assessment. Furthermore, hamstring flexibility is frequently used in deciding athlete's readiness to return-to-play following an injury (Croisier *et al.*, 2002; Drezner, 2003; Mendiguchia & Brughelli, 2010). The AKE test is considered by some to be the gold standard for hamstring flexibility assessment (Davis *et al.*, 2008).

With the aid of a simple stabilizing apparatus and a universal goniometer, we have shown that a single assessor could perform the AKE test easily. In uninjured participants we have demonstrated that the AKE angle between the dominant and non-dominant is comparable (range of differences -3.38 to 4.75 °). Further, no significant difference in AKE angle of the dominant and non-dominant knees between genders was observed. The interater reliability ICC_{2,1} values of 0.87 and 0.81 were found for the dominant and non-dominant knees respectively. In addition, the standard error of measurement were 3.5 ° for the dominant and 3.8 ° for the non-dominant knee. Hence, a good level of agreement between raters was established.

Test-retest reliability of the AKE test in this study was excellent with ICC_{3,1} values ranges from 0.78 to 0.92 for both raters. Our findings are in agreement with earlier studies. Gajdosik *et al.* 1983, reported the AKE test Pearson product-moment correlation coefficient of 0.99 for both lower extremities. Higher reliability coefficient value in the previous study could be explained by the different statistical methods used by the author. A very short interval between the first AKE test and retest sessions (30 minutes apart) used in previous study did not reflect true clinical setting and may have

introduced systematic bias and affected reliability (Askling *et al.*, 2004). In this study we chose an interval of one week between the test and retest sessions as it reflects our clinical practice, where majority cases are reviewed on weekly basis.

Using the mean value of both lower limbs AKE measurements, Gabbe reported excellent test-retest reliability with ICC_{3,1} values of 0.94 – 0.96 (Gabbe *et al.*, 2004). In contrast to Gabbe *et al.*, 2004, the current study evaluated each knee separately to explore any potential differences between the dominant and non-dominant knees, and such difference in method may explain the wider ICC_{3,1} values noted in this study.

A pilot study conducted by Davis *et al.*, (2008) reported an excellent intrarater reliability of knee extension angle (KEA) test, with ICC_{3,1} of 0.94. The method of hamstring flexibility assessment employed by the previous author differs from the current study. They measured the KEA using two gravity inclinometers placed immediately superior to the patella, and another was placed on the distal anterior tibia. Moreover the examiner performed both hip flexion and knee extension passively, whereas participants actively performed all movements in the current study.

Our findings on interater reliability was consistent to those reported by earlier studies (Sullivan & DeJulia, 1991; Gabbe *et al.*, 2004). Gabbe *et al.* (2004), reported an interater reliability ICC_{2,1} of 0.93 and 4 ° SEM for the AKE test in 15 healthy participants of comparable age group (mean age: 31.6 years). Similarly, in a study to determine the effect of pelvic positioning and stretching method on hamstring flexibility, Sullivan & DeJulia (1991) reported an interater reliability ICC_{1,1} and SEM of 0.93 and 4.81° respectively for the AKE test among 12 healthy subjects. On the other hand, Rakos *et al.*, (2001), performed the AKE test with the aid of an intricate

stabilizing apparatus demonstrated a good interater reliability with an $ICC_{2,1}$ of 0.79 among children age 10 to 13 years old.

Despite demonstrating excellent interater and test-retest reliability, a wide range of CIs was noted for some of the point estimates in this study. Such finding suggests that further research with larger samples size may be necessary to determine the reliability estimates with greater precision (Hopkins, 2000). Second, the reliability displayed in this study was based on assessment of healthy and non-injured volunteers.

4.5 Conclusion

The current study demonstrated that a single assessor could perform the AKE test easily using a simple, portable and inexpensive stabilizing apparatus and have excellent interater and intrarater reliability. The interater reliability intraclass correlation coefficients ($ICC_{2,1}$) were 0.87 for the dominant and 0.81 for the non-dominant knee. In addition, the intrarater (test-retest) reliability $ICC_{3,1}$ values' ranges between 0.78 - 0.92 and 0.75 – 0.84 for raters 1 and 2 respectively. Further this study demonstrated no significant differences of the AKE angles between the dominant and non-dominant sides in healthy individuals. A difference of less than 10 ° between the sides can be considered as healthy, and can be use to guide athlete recovery following injury. Therefore, we recommend the use of the AKE test (with apparatus) described in this study by health care providers for assessment of hamstring flexibility.

4.6 Summary

Hamstring flexibility assessment is performed during athlete's preparticipation evaluation. In addition this test is also used in deciding athlete readiness to return-to-play following an injury. Several hamstring flexibility assessment methods is currently available, each with their own advantages and disadvantages. A simple active knee extension (AKE) test aided by a cheap, portable and easy-to-use stabilising apparatus was proposed. This method demonstrated excellent interater and intrarater (test-retest) reliabilities with ICC values' ranges from 0.81 – 0.87 and 0.75 – 0.97 respectively. Further, a difference of $< 10^\circ$ in AKE angles between the dominant and non-dominant sides was noted in 14 healthy volunteers. The proposed AKE test is a reliable test that could be used to monitor hamstring flexibility and guide practitioners on the decision to allow return-to-play following an injury.

Chapter 5 Platelet-rich plasma (PRP) for the treatment of hamstring injury: A randomized controlled trial

5.1 Introduction

The cross-sectional study on pattern of muscle injuries among Malaysian athletes is in agreement with other studies (Garrett 1996; Dick *et al.*, 2007, Alonso *et al.*, 2010, Liu *et al.*, 2012; Ekstrand *et al.*, 2013). Hamstring is the commonest muscle injured among Malaysian athletes (Chapter 3). In addition, information on athlete's demographics, current injury management and duration to return-to-play (DRP) among local athletes were gathered. Further, the preliminary study revealed several factors that predict earlier DRP among Malaysian athletes (Shariff *et al.*, 2013).

Despite of it's frequent occurrence, the best treatment of hamstring injury is not known. Current approaches involve rest, intermittent cryotherapy (ice), compression and elevation (RICE) especially in the early stage following an injury. The objectives of the RICE treatment are to limit extent of injury and for pain control. In addition, short-term painkillers are often prescribed for pain control in this early stage. Following this acute phase, treatment usually focuses on pain control, regaining full range of movement and strength of the affected muscle. In most cases patients are prescribed with range of movement and strengthening exercises. These approaches are often combined with the various electrotherapeutic modalities such as transcutaneous electrical nerve stimulation (TENS), therapeutic ultrasound and interferential current to name a few. Clinical evidence to suggest efficacy of these treatments however is limited (Zuluaga, 1995; Reynolds *et al.*, 2008).

More recently (past two decades) autologous biological substances have received much attention for soft tissues injury. Biological substances such as autologous blood (AB), preparation rich in growth factors (PRGF), preparation rich in fibrin matrix (PRFM) and platelet-rich plasma (PRP) are currently used by researchers and physicians to hasten recovery (Wright-Carpenter *et al.*, 2004a; Kaspriske, 2010; “Tiger Admits to Platelet-Rich Plasma,” 2010; Ekstrand *et al.*, 2011; Eirale *et al.*, 2013).

Muscle tissue heals through intricate balance of scar tissue formation and muscle cells regeneration (Järvinen *et al.*, 2005). New muscle cells are formed from activation and differentiation of the dormant satellite cells found within the basal lamina (Tiidus *et al.*, 2008). Activated satellite cells proliferates and differentiates into mature myoblasts in response to various growth factors and cytokines. Autologous biological substances contain growth factors such as, insulin-like growth factor-1 (IGF-1), platelet derived growth factors (PDGF), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF) basic fibroblasts growth factors and transforming growth factor beta-1 (TGF- β 1) and various cytokines that are responsible for muscle healing (Järvinen 2005; Tiidus *et al.*, 2008). It is believed that increasing the amount of the various growth factors and cytokines in an injured area would speed up healing process allowing faster and more complete recovery. Currently there are some evidences demonstrating autologous biological substances efficacy for tendon injury (Mishra & Pavelko, 2006; deAlmeida *et al.*, 2010; Filardo *et al.*, 2010; Gaweda *et al.*, 2010; Peerbooms *et al.*, 2010; Mishra *et al.*, 2013). However, clinical evidence to suggest efficacy for muscle injury is limited to laboratory and case control studies (Zarins & Ciullo, 1983; Wright-Carpenter *et al.*, 2004a; Wright-Carpenter *et al.*, 2004b; Hammond *et al.*, 2009; Gigante *et al.*, 2012; Rettig *et al.*, 2013). Further, there is no randomised controlled clinical trial available on PRP use for muscle injury (Chapter 2).

The effect of autologous PRP injection for treating hamstring muscle injury was explored in this chapter. A randomised controlled clinical trial (phase 3) was conducted to examine the effect platelet-rich-plasma (PRP) injection on the duration of return-to-play (DRP) following an acute grade-2 hamstring muscle injury. This study protocol has been registered with the Current Controlled Trials (ISCR TN66528592) (Hamid *et al.*, 2012) (Appendices K and L).

5.1.1 Specific objective of study

1. to investigate the effect of single platelet-rich plasma (PRP) injection combined with progressive agility and trunk stabilisation (PATS) rehabilitation program on the duration to return-to-play (DRP) after grade-2 hamstring injury
2. to explore the effect of single PRP injection combined with PATS rehabilitation program on changes in signs and symptoms associated with grade-2 hamstring injury over time.

5.2 Materials and methods

A randomised assessor-blinded controlled trial was designed to explore the effect of single intralesional injection of autologous PRP combined with a standard rehabilitation program versus standard rehabilitation programme alone on the DRP after an acute grade-2 hamstring injury.

5.2.1 Study setting

The trial was conducted in the Sports Medicine Clinic, University Malaya Medical Centre (UMMC). This clinic is one of the pioneer Sports Medicine Clinics in providing sports related injury care in Malaysia. The Sports Medicine Clinic, UMMC is located in the heart of Kuala Lumpur, the capital city of Malaysia and bordering the State of Selangor, which is the most populous state in Malaysia. Federal Territory of Kuala Lumpur and the state of Selangor has a combine population of 8.13 million (25.2 %) of the total Malaysian population (Malaysia, 2011). The Sports Medicine Clinic, UMMC played a central role in providing medical services during the XVI Commonwealth Games, Kuala Lumpur in 1998, the 15th Asean University Games in 2008, Asia Cycling Tournament and International Rugby Tournament in 2012 including several other local and international sport events.

In addition, over the past years this clinic has developed strong working relationships with the National Sports Institute and the National Sports Council of Malaysia in Bukit Jalil, Kuala Lumpur.

5.2.2 Participants and recruitment

Study notice (Appendix M) and invitation (Appendix N) to take part in this study were distributed to all sports medicine practitioners (sports physicians and physiotherapists) within the Klang Valley, Malaysia between November and December 2011. Participant's recruitment began from January 2012 until May 2013. Patients with confirmed or suspected hamstring muscle injury were invited to take part in this study.

Sports medicine practitioners were encouraged to refer patients that fulfilled the clinical criteria of hamstring muscle strain as described by Askling *et al.*, (2006), these include:

- a) Acute (within seven days) onset of posterior thigh pain suffered during training or competition;
- b) Tenderness on palpation of the hamstring muscle;
- c) Pain with stretching of the hamstring muscle; and
- d) Pain with resisted contraction of the hamstring muscle.

All potential participants underwent a screening process conducted by the research team to determine eligibility and to ensure safety of participation in the study. This screening process was based on the predetermined inclusion and exclusion criteria using a structured clinical report form (Appendix O).

The screening process involved clinical and radiological assessment of the current injury. Potential participants were required to provide detail clinical history on the current injury. Following this, a sport physician and a physiotherapist examined and graded the severity of the injury using clinical grading as recommended by previous authors (Järvinen *et al.*, 2000; DeLee, 2003). Participants clinically diagnosed with grade-2 hamstring injury were referred to the Department of Radiology for confirmatory ultrasonography. An experienced musculoskeletal radiologist conducted all diagnostic ultrasound assessment using the Philips IU 22 ultrasound with 10 MHz, 14 cm linear probe to confirm the diagnosis. Severity of hamstring injury was determined using the ultrasonography grading system used in UMMC (Peetrons *et al.*, 2002) (Table 5.1). Diagnostic ultrasound assessment was conducted 24 to 48 hours after completion of physical examination (Figure 5.1). Ultrasonography findings were documented in a

standard ultrasound report form (Appendix P). Patients who met the criteria were invited to take part in the study.

Table 5.1 : Grading of muscle strain injuries on ultrasound used at UMMC.

Grade	Ultrasound findings
0	No ultrasound features seen
1	Muscle oedema only
2a	Partial tears of muscle fibres, disruption involving < 33 %
2b	Partial tears of muscle fibres, disruption involving ≥ 33 – 66 %
2c	Partial tears of muscle fibres, disruption involving ≥ 66 – 99 %
3	Complete tear of muscle



Figure 5.1. Typical ultrasonography appearance of a grade-2 muscle injury.

Before enrolment, potential participants were required to agree on the following requirements:

- to comply with the rehabilitation program prescribed and record sessions in the rehabilitation diary.
- to attend weekly clinical assessment and complete series of questionnaires throughout the study period.

- to avoid any sports participation or any other physical activities except for the rehabilitation exercise program prescribed until participants considered fit to return-to-play.

The selection criteria's during the screening process are listed in Table 5.2.

Table 5.2 : List of participants' selection criteria.

Inclusion criteria
1. Grade-2 hamstring injury diagnosed on clinical assessment and confirmed on ultrasonography
2. Aged \geq 18 years
3. Acute hamstring muscle injury (\leq seven days)
4. Able to understand and follow the study protocol and had completed the written informed consent form
Exclusion criteria
1. Had received any form of injection therapy for the current injury
2. Using anti-inflammatory drugs (NSAIDs) within one week before randomization
3. Unable to fulfil weekly follow-up appointments and comply rehabilitation program
4. Significant cardiovascular, renal, hepatic disease, malignancy, history of anaemia, and previous muscle surgery

5.2.3 Randomisation

Participants were randomly allocated into one of the two groups:

- i. Autologous platelet-rich plasma (PRP) group or;
- ii. Control group.

Platelet-rich plasma (PRP) was chosen as the intervention of choice as PRP has been shown to contain the highest amount of platelets (the active ingredients in accelerating cellular repair and regeneration). No placebo control was used in this study since withdrawing blood and discarding it has raised ethical concerns.

Randomisation was performed after eligible participants had read and understand the trial information (Appendix Q) and signed the written informed consent (Appendix

R). A computer generated block randomisation of four was used to create a randomisation schedule (Appendix S). Treatment assignment was conducted by the trial manager MS.

5.2.4 Intervention

5.2.4 (a) Platelet-rich plasma (PRP) group

Participants in the PRP group were prescribed with rehabilitation exercise programme at enrolment by a sports physiotherapist. Participants were advised to perform the exercises at home at least once a day. The rehabilitation programme prescribed in this study focused on progressive agility and trunk stabilisation (PATS) exercises (Table 5.3). The PATS programme is based on a set of exercises used in an earlier study (Sherry & Best, 2004). This rehabilitation programme was found to be more effective than a programme that only emphasised on hamstring stretching and strengthening in promoting earlier return-to-play and in preventing injury recurrence in athletes with an acute hamstring strain (Sherry & Best, 2004). Clarifications on some of the movements in the PATS exercise protocol were made through email communication with the original author. In addition, permission to use the PATS rehabilitation exercises in this clinical trial was obtained from the original author (Appendix T). An instructional video and booklet on the PATS rehabilitation exercises was made available and distributed to each participant at enrolment (Figure 5.2). All participants were asked to continue with their unsupervised daily home rehabilitation exercises as prescribed and to keep a record of these sessions.

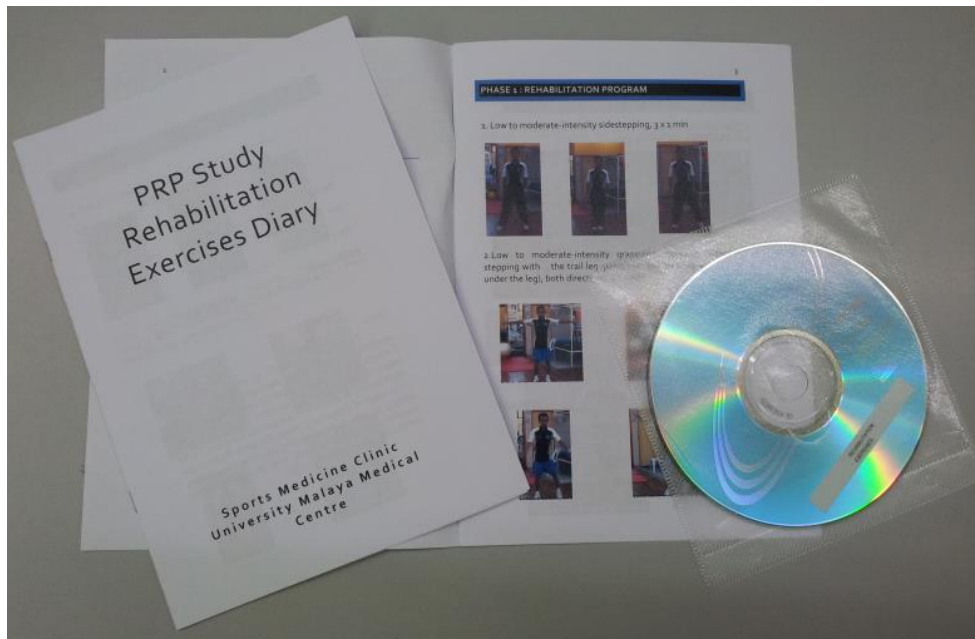


Figure 5.2. Rehabilitation booklet and instructional video.

Besides the rehabilitation exercises, participants in the PRP group also received a single injection of autologous PRP administered under ultrasound guidance. The injection was administered once, on randomisation of the treatment group (day 1 of the study).

Autologous PRP preparation

Autologous PRP was prepared using the Biomet GPS[®] III Platelet Separation System (Figure 5.3) in accord with the manufacturer guideline. With an 18 G needle, 54 millilitres (ml) venous blood were collected from the participants' cubital vein and transferred into a 60 ml syringe primed with 6 ml of anticoagulant citrate dextrose solution (ACD-A) (Figure 5.4). Another 2 ml of the venous blood were collected and sent to the hospital laboratory for determination of platelets and leucocytes (white blood cells) count.

Table 5.3 : Rehabilitation exercises prescribed to all patients.

Phase 1	
1.	Low to moderate-intensity sidestepping, 3 x 1 min
2.	Low to moderate-intensity grapevine stepping (lateral stepping with the train leg going over the lead leg and then under the leg), both directions, 3 x 1 min
3.	Low to moderate-intensity steps forward and backward over a tape line while moving sideways, 2 x 1 min
4.	Single-leg stand progressing from eyes open to eyes closed, 4 x 20 sec
5.	Prone abdominal body bridge (performed by using abdominal and hip muscle to hold the body face-down straight-plank position with the elbows and feet as the only point of contact), 4 x 20 sec
6.	Supine extension bridge (performed by using abdominal and hip muscles to hold the body in a supine hook lying position with the head, upper back, arms, and feet as the points of contact), 4 x 20 sec
7.	Side bridge, 4 x 20 sec on each side
8.	Ice in long-sitting position for 20 min
Phase 2*	
1.	Moderate to high-intensity sidestepping, 3 x 1 min
2.	Moderate to high-intensity grapevine sidestepping, 3 x 1 min
3.	Moderate to high-intensity steps forward and backward while moving sideways, 2 x 1 min
4.	Single-leg stand windmill touches, 4 x 20 sec of repetitive alternate hand touches
5.	Push-up stabilization with trunk rotation (performed by starting at the top of a full push-up, then maintain this position with 1 hand while rotating the chest toward the side of the hand that is being lifted to point toward the ceiling, pause and return to the starting position), 2 x 15 reps on each side
6.	Fast feet in place (performed by jogging in place with increasing velocity, picking the foot only a few inches off the ground), 4 x 20 sec
7.	Proprioceptive neuromuscular facilitation trunk pull-downs with Thera-Band, 2 x 15 to the right and left
8.	Symptom-free practice without high-speed manoeuvres
9.	Ice for 20 min if any symptoms of local fatigue or discomfort present

Key: Low intensity, a velocity of movement that was 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 from phase 1 to 2 when they could walk with a normal gait pattern and perform a high knee march in place without pain. (Source: Sherry & Best, 2004, p.119)

The collected blood was transferred into the disposable separation tube (Figures 5.5 & 5.6) and spun using a centrifuge (Biomet/Drucker Centrifuge 755VES - 230V, Biomet Biologics, Germany) at 3200 rpm at room temperature for 15 minutes.

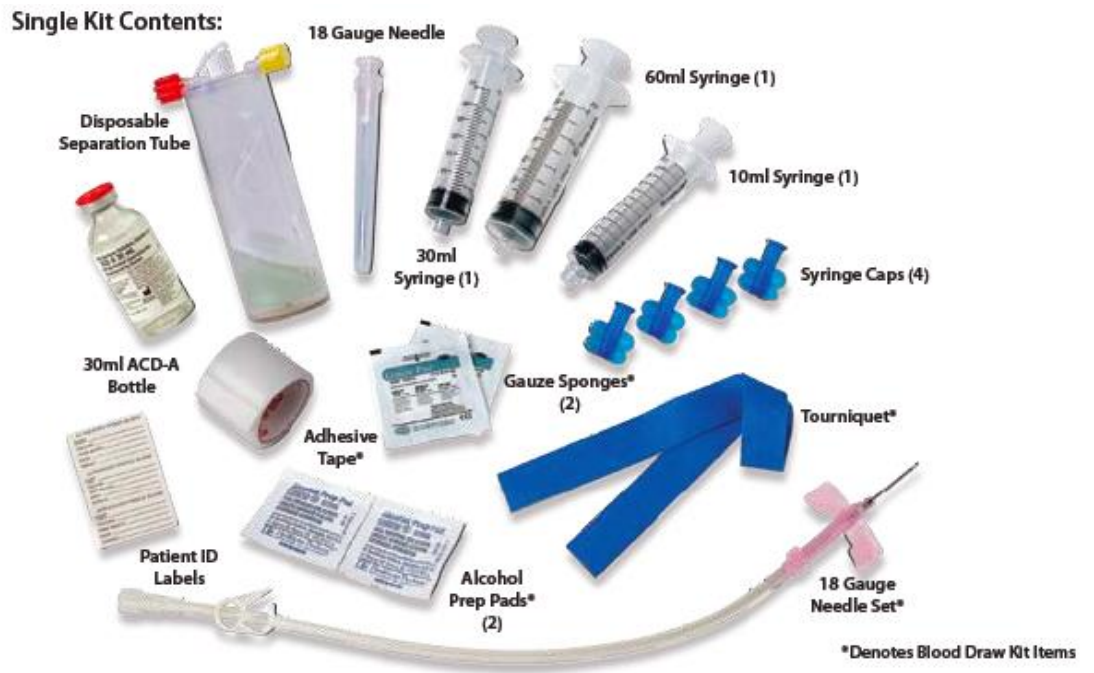


Figure 5.3. Biomet GPS III Platelet Separation System Kit.

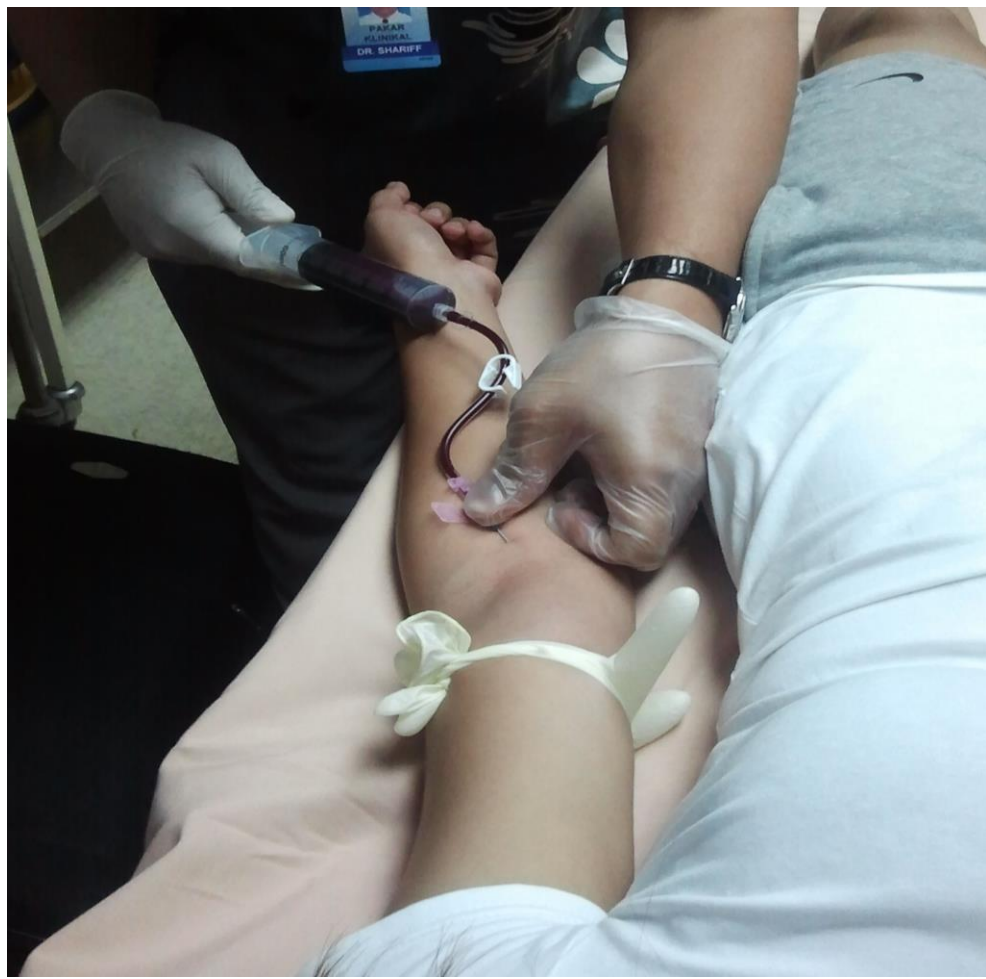


Figure 5.4. Venous blood collection from an antecubital vein.



Figure 5.5. The collected blood was transferred into a separation tube.



Figure 5.6. The collected blood was centrifuged at 3200 rpm for 15 minutes.

Centrifugal force separates the blood components into three distinct layers based on their particular densities (Marieb, 2001; Harmening, 2002). The heaviest particles, the red blood cells (erythrocytes) sunk at the bottom of the tube, the least dense

constituents the platelet-poor plasma (PPP) move to the top of the tube, while the platelet-rich plasma (PRP) remained at the centre (Figure 5.7).

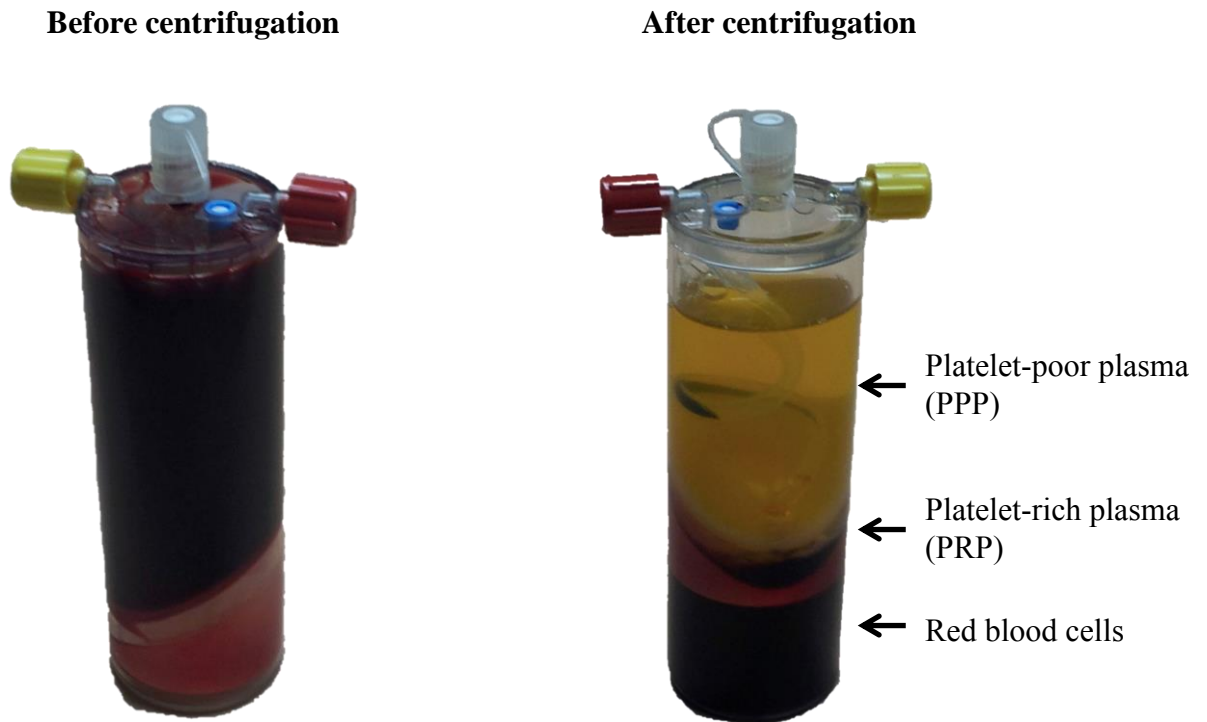


Figure 5.7. Platelet-rich plasma (PRP) preparation.

The whole PPP was extracted into a 30 ml syringe and discarded. Following this, PRP was extracted into a 10 ml syringe. Since an acidic anticoagulant (Anticoagulant Citrate Dextrose Solution – Solution A [ACD-A]) was added during the collection of venous blood, collected PRP was buffered to increase the pH to normal physiological levels, just before injection. This was accomplished by adding 8.4 % sodium bicarbonate solution in a ratio 0.05 ml of sodium bicarbonate to 1 ml of PRP. No activating agent was added to the PRP before administration. The time taken to prepare PRP was about 30 minutes. A standard 60 ml GPS[®] III kit produced roughly 6

ml of PRP. This method shown to produced PRP with platelets content five times higher than peripheral blood (Mishra & Pavelko, 2006; Thanasas *et al.*, 2011).

In this study, 3 ml of the extracted PRP were injected into the injured area under ultrasound guidance. One ml was sent to the hospital laboratory for platelets and leucocytes count. The remaining 2 ml were stored at - 20 ° C for analysis of growth factors; basic fibroblast growth factor (bFGF); insulin-like growth factor-1 (IGF-1); transforming growth factor- β 1 (TGF- β 1), which were performed later.

As a recent study showed local anaesthetic agent (lidocaine and bupivacaine) significantly decreases tenocyte proliferation and cell viability, no local anaesthetic agent was given before PRP injection in this study (Carofino *et al.*, 2012).

Platelet-rich plasma (PRP) injection technique

Based on previous studies 3 ml of PRP was injected direct into the injured area using a 21 G ultrasound needle (USB120-21) using peppering technique (Figures 5.8 & 5.9) (Sanchez *et al.*, 2005; Hamilton *et al.*, 2010). All injections were performed under aseptic condition. Immediately after injection, patients were kept in supine position for 10 to 15 minutes. Patients were advised to rest and limit their activities for the next 48 hours. Only acetaminophen was allowed for pain control, should participants need. Patients were advised to avoid non-steroidal anti-inflammatories (NSAIDs) medications as laboratory studies have showed delayed tibialis anterior and gastrocnemius muscle healing in animal models (Almekinders & Gilbert, 1986; Jarvinen *et al.*, 1992).

Following PRP injection, participants were reassessed for any adverse reactions (including increased pain and signs of infection over the site of injection) three days after receiving the PRP.

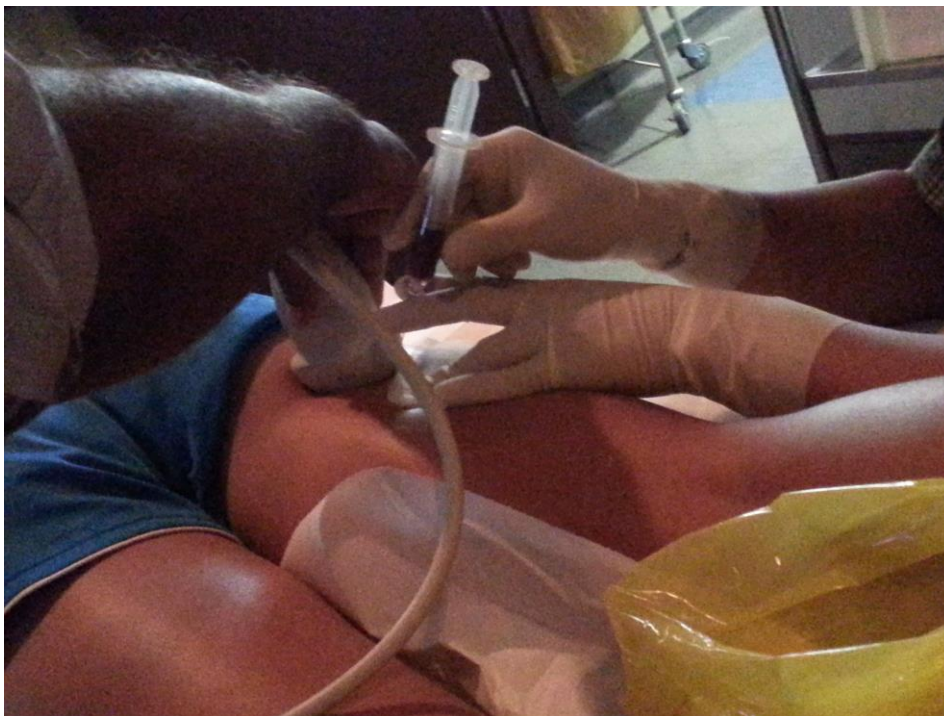


Figure 5.8. Platelet-rich plasma injection under ultrasound guidance.



Figure 5.9. Ultrasound guided PRP injection. White arrowheads: shadow of the ultrasound needle.

5.2.4 (b) Control group

Participants in the control group were prescribed with the same rehabilitation exercise programme as the PRP group. Participants in the control group however, did not receive any PRP injection into their injured area.

All participants (PRP and control groups) were asked to attend a weekly follow-up assessment and rehabilitation sessions with a physiotherapist until full recovery was achieved or the end of week-16. At each visit, patients completed the brief pain inventory scale - short form version (BPI - SF) (Appendix U). Permission to use BPI - SF questionnaire was obtained from the original author (Appendix V).

A physiotherapist who was blinded of the treatment allocation performed standard clinical examinations to assess participant's readiness to return-to-play (outcome measures). In addition, participant's rehabilitation exercise programme was reinforced under the same physiotherapist's supervision. Each follow-up session lasted between 45 – 60 minutes.

A physiotherapist (with five years of clinical experience) was trained to assess the outcome measures and deliver PATS rehabilitation exercise programme. The training involved a half-day course delivered by the principal researcher and a treatment manual was given to the physiotherapist. The manual contained a brief summary of the study, standard assessment methods and hamstring rehabilitation exercises (PATS) based on the protocols described by Sherry & Best, 2004.

Patients in both groups were asked to track their exercise compliance and report any difficulties or problems faced at each follow-up visit.

5.2.5 Blinding

A sports physiotherapist (LPC) acted as the outcome measure assessor for all participants. The assessor also involved in providing standard rehabilitation programme to all participants but was unaware (blinded) about the participants' group allocation. Participants were asked not to disclose details of their treatment and group. Assessor blinding was considered appropriate to reduce differential assessment of outcomes (ascertainment bias).

5.2.6 Potential risks discomfort and inconveniences

The risk of blood reaction is small since PRP was prepared from the patient's own blood (autologous). In addition an aseptic technique was used during PRP injection to reduce the risk of infection. Patients however were told to expect slight discomfort or pain during blood withdrawal and during injection of PRP into the injured area.

5.2.7 Potential benefits

For the participants:

Participants who received treatment for their injury were under constant supervision from the study team of experts and all treatment costs covered by the study.

For the researchers:

The study provides information on the clinical effectiveness of the intervention in hastening muscle recovery after injury.

5.2.8 Ethical consideration

The University of Malaya Medical Ethics Committee (Appendix W) approved the methods and materials set up for the randomised controlled trial. Verbal and written consents were obtained from all participants before the conduct of the study.

Confidentiality of the participants was ensured. All participants were assigned with non-identifiable identification codes for data entry and data analysis. All consent forms, clinical report forms, and completed questionnaires were stored in a locked filing cabinet accessible only by the researcher and the supervisors.

5.2.9 Study outcomes measures

5.2.9 (a) Primary outcome: Duration to return-to-play (DRP)

The primary outcome for this study was the duration of return-to-play (DRP). DRP was defined as the duration (days) from the date of injury onset until the participants fulfilled the return-to-play (RTP) criteria. As there are limited scientific

studies that examined the outcome of various RTP strategies (Orchard *et al.*, 2005; Engebretsen *et al.*, 2010), it was necessary to set criteria for RTP. The RTP criteria proposed in this study were adapted from recent clinical sports medicine recommendations (Croisier *et al.*, 2002; Drezner, 2003; Croisier, 2004; Brukner & Khan, 2010; Mendiguchia & Brughelli, 2010). The criteria were based on participant's symptoms of pain, full range of movement (flexibility) of the affected knee and hamstring strength (Table 5.4).

Table 5.4 : Criteria for return-to-play (RTP).

Sign	General Recommendation
Pain	Pain-free (on direct palpation) Pain free on hamstring contraction (resisted isometric hamstring muscle contraction)
Range of movement	Symmetrical with unaffected site (difference between affected and unaffected side of $\leq 10^\circ$)
Strength	Isokinetic strength within 5 % (Hamilton <i>et al.</i> , 2010; Harmon <i>et al.</i> , 2010) to 10 % (Drezner, 2003) of contralateral side

Symptom of pain

Direct palpation of the hamstring muscle was performed with participants in prone position. Palpation started over an area close to the injury site and gradually moved to the injured area (Figure 5.10). Pain elicited during this test was recorded in the participants' clinical research form (CRF). In addition, pain provocation test was also performed during physical assessment. This test was performed in prone lying position with the knee flexed at 15° (Figure 5.11) (Warren *et al.*, 2010). The participants were asked to actively contract the hamstring muscles against resistant. The test was considered positive if the participant reported pain while performing the movement.

Range of movement test (Flexibility)

Hamstring range of movement (ROM) was assessed using the active knee extension (AKE) test. The AKE test involves movement of the knee joint with the hip stabilised, unlike the straight-leg raise (SLR) test which involves movements of both hip and knee joints. AKE test is an active test and is considered safe as the participants dictate the end point. This test has been recommended and often used to measure hamstring tightness (Cameron & Bohannon, 1993; Gajdosik *et al.*, 1993; Gajdosik *et al.*, 2008; Alonso *et al.*, 2009). The AKE test used in this study has been described in detail in Chapter 4.



Figure 5.10. Direct palpation of hamstring.



Figure 5.11. Hamstring pain provocation test.

Hamstring strength assessment

Participants in both group whose symptom of pain have subsided and were pain free during the clinical assessment including attainment of full range of movement assessed for isokinetic muscle strength.

Hamstring muscle strength was assessed using an isokinetic dynamometer (System 4 Pro, Biodex Medical System, NY, USA). Assessment of hamstring and quadriceps muscles performed on the uninjured leg followed by the injured leg. The test started with two minutes slow cycling on a stationary cycle ergometer as warm up. Participants were allowed to familiarise with the experimental protocol before testing. During the familiarisation period, participants practised with submaximal effort. The participants' knee joint centre was kept aligned with the axis of the dynamometer crank arm. The dynamometer was set at 90° with the knee range of movement limit from 0 – 100°.

The testing protocol assessed maximum voluntary strength of both legs, with the

uninjured leg tested first. Muscle strength test was performed under concentric effort at three angular speeds of 60, 180 and 300°/second, representing slower, moderate and fast contraction speed respectively (Figure 5.12). Each participant performed five maximum contractions at angular speeds of 60°/s, ten maximum contractions at angular speeds of 180°/s, and fifteen maximal contractions at angular speeds of 300°/s. Participants were given a 60 seconds rest between each angular speed test. At each speed, quadriceps muscles were tested first followed by the hamstrings. The participants did not receive any visual feedback during the test; however, verbal encouragements were given to all.

Participants who have fulfilled all criteria for RTP were allowed to resume training activities and progressively increase their training load until reaching their preinjury levels. Participants were advised to take it easy over the next six weeks to reduce risk of injury recurrence (Orchard & Best, 2002; Peetrons, 2002). Participants who have not met the RTP criteria by the end of week 16 were allowed to continue their treatment in UMMC until fully recovered (Figure 5.13).

5.2.9 (b) Secondary outcomes

Brief pain inventory – Short Form (BPI-SF)

The BPI - SF is a self-reported or interview questionnaire that assesses the severity of pain and the impact of pain on daily functions. In addition, pain medications and amount of pain relief in the past 24 hours or the past week were also assessed.

The BPI - SF was validated in more than three-dozen languages (including Malay) by examining the consistency of its two-factor structures; the severity of pain

and the impact of pain. The Cronbach alpha reliability of BPI - SF ranges from 0.77 to 0.91 (Cleeland, 2009).

PRP classification

To allow comparison with other studies, the PRP prepared in this study was classified according to the current classification system described in literatures (Dohan *et al.*, 2009; DeLong *et al.*, 2012). The amount of platelets present in venous blood and PRP were determined using the Sysmex XN-10 and XN-20 (Sysmex Co, Japan) high performance automated haematology analyser in UMMC outpatient laboratory. The ratio of platelet levels in venous blood to PRP was calculated to determine the ability of GPS III[®] kit (Biomet Biologics, Warsaw, Indiana, USA) to concentrate platelets. Also the amount of leucocytes present in PRP was performed using similar method.



Figure 5.12. Isokinetic hamstring strength test using a Biodex System.

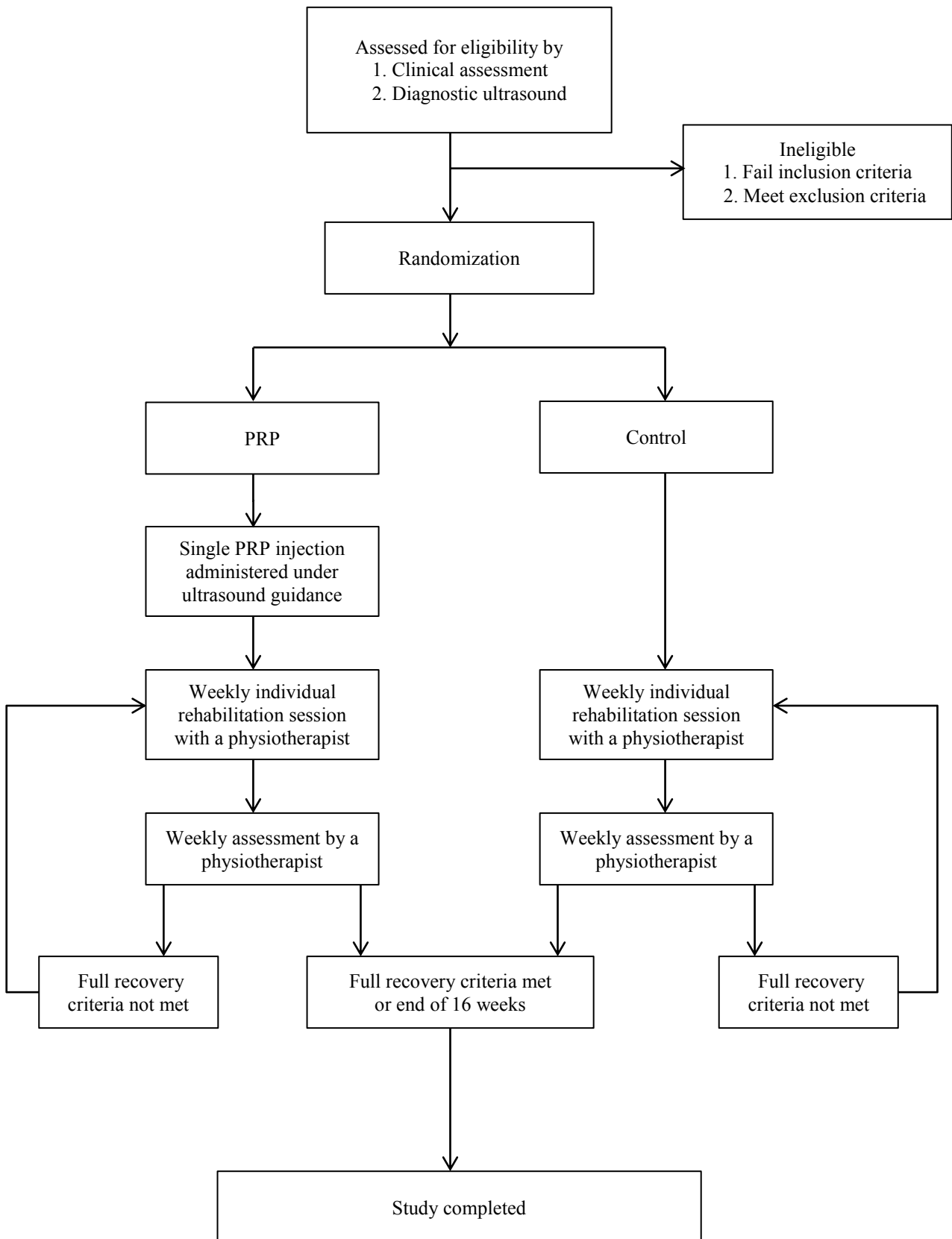


Figure 5.13. Summary of the trial design.

Growth factors

About 2 ml of PRP were stored in the freezer at – 20 ° C. These samples were used to quantify the level of three types of growth factors; insulin-like growth factor-1 (IGF-1); basic fibroblasts growth factor (bFGF) and transforming growth factor - β 1 (TGF- β 1). The level of each growth factors were determined using the Human Insulin-like Growth Factor-1 (IGF-1)(CSB-E04580h, Cusabio Biotech Co., Ltd, China), Human basic fibroblast growth factor (bFGF)(CSB-E08000h, Cusabio Biotech Co., Ltd, China) and Human Transforming Growth Factor β 1 ELISA (TGF- β 1) (CSB-4725h, Cusabio Biotech Co., Ltd, China) kits respectively.

5.2.10 Statistical

5.2.10 (a) Sample size

Sample size was determined using the formula as shown in Equation 5.1 (Lwanga & Lemeshow, 1991):

$$N = \frac{2 \times [z_{(1-\alpha/2)} + z_{(1-\beta)}]^2 \sigma^2}{[\mu_1 - \mu_2]^2} \dots\dots\dots \text{Equation 5.1}$$

Where,

N = the sample size in each of the groups

$z_{(1-\alpha/2)}$ of 0.05 = 1.96 (percentage points of the normal distribution for statistical significance level of 0.05)

$z_{(1-\beta)}$ of 80 % = 0.84 (percentage points of the normal distribution for statistical power of 80 %)

μ_1 = population mean in treatment Group 1

μ_2 = population mean in treatment Group 2

$\mu_1 - \mu_2$ = the mean difference

σ^2 = population [standard deviation (SD)] (Wright-Carpenter *et al.*, 2004)

The total number of participants required for this study was 24 (12 participants per group). After considering 30 % estimation of attrition rate, 14 participants in each group were required, giving a total of 28 participants for the study.

5.2.10 (b) Data analysis

The main researcher performed all data collection and recording in this RCT. The researcher checked and corrected (if needed) all data. Double entry was performed for data verification. Identification of data errors and outliers was conducted using descriptive techniques. Missing data were specified as 999.00 and participants who withdrew from the study contributed to the missing data in this study. No missing data was noted among the participants who completed the study. All analyses conducted were two-tailed with significant level set at p - value < 0.05.

5.2.10 (c) Descriptive analysis

Descriptive analysis of participants' characteristics was performed. Continuous variable including participant's demographic, clinical history and baseline variables were reported using means and standard deviations (SD) or median and interquartile range (IQR) depending on the data distribution based on the Shapiro-Wilk test of normality. Categorical data on the other hand were presented as frequencies and

percentages. Normal distribution of the data is assumed when the Shapiro-Wilk test has a p - value ≥ 0.05 .

Comparison between participants who completed and withdrew from the study was made using Chi-square or Exact test (for unbalanced data) for categorical variables and independent t -test for continuous data. Assumptions for independent t -test were checked, which include: 1) group independency, 2) data continuity, 3) normally distribution of data, and 4) homogeneity of data. A Mann-Whitney U tests were performed for variables that violated these assumptions.

The homogeneity of the participants' characteristics at baseline was determined using Chi-square or Exact test (for unbalanced data) for categorical variables and independent t -test for continuous data. Assumptions for independent t -test were checked as mentioned earlier.

5.2.10 (d) Duration to return-to-play (Primary outcome measure)

A survival analysis employing intention-to-treat principles was used to determine the effectiveness of the intervention across the study periods (Hamilton *et al.*, 2010; Tabachnick & Fidell, 2012). Also a survival analysis is more robust to the potential bias from missing and unbalanced data compared with the more conventional methods of imputation for missing data (Almekinders & Gilbert, 1986; Singer & Willett, 2003).

Participants who completed the study and achieved full recovery were identified as completers whereas those who withdrew from the study were censored. A Kaplan-

Meier survival curve was plotted to display graphic representation of survival functions of the two groups. Comparison of the survival functions between groups was performed using the Log-Rank test.

Cox regression survival analysis was performed to evaluate the effects of treatment and other covariates on DRP. Covariates including participants age, gender, duration of injury, pain score (at enrolment), length of injury (measured during MRI assessment), the AKE angle (at enrolment), and previous hamstring injury were analysed. These covariates were reported by previous study as significant predictors of muscle recovery (Verrall *et al.*, 2003; Schneider-Kolsky, 2006; Reynolds *et al.*, 2008; Warren *et al.*, 2010).

Covariates other than treatment were entered first into the Cox regression models, followed by treatment as this allow a likelihood-ratio test of the effect of treatment, after statistical adjustment for the other covariates (Sherry & Best, 2004; Tabachnick & Fidell, 2012). The survival analysis effect size was represented as R^2 and calculated using the formula as shown in Equation 5.2 (Heiser *et al.*, 1984; Tabachnick & Fidell, 2012):

$$R^2 = 1 - e^{(-G^2/n)} \dots\dots\dots \text{Equation 5.2}$$

Note: R^2 = effect size, G^2 = [- 2 log-likelihood for smaller model) – (- 2 log - likelihood for larger model)], n = number of participants.

5.2.10 (e) Secondary outcome measures

The secondary outcome was changes in participant's symptoms and signs associated with hamstring injury. This includes pain intensity, pain interference with daily activity and the range of movement (flexibility) of the affected knee. Participants' symptoms of pain severity were based on the mean scores of Q3 to 6 of the brief pain inventory scale – short form (BPI - SF). Meanwhile symptoms of pain interfering with daily activities were the composite mean of Q9A to 9G of BPI - SF questionnaire. Changes in the range of movement (ROM) of the knee were represented by the active knee extension (AKE) angle measured at each follow-up appointment. Analyses of symptoms and signs changes were performed with linear mixed model (LMM) using the principles of intention-to-treat (Singer & Willett, 2003). The LMM was chosen based on the nature of data collected (repeated measures) and to address potential missing data for this RCT. LMM is also more robust to the potential bias from missing data compared with conventional methods of missing data imputation such as the last observation carried forward (Mallinckrodt *et al.*, 2003). The models were used to assess the effectiveness of the interventions over time with two stages analysis of change. In the first stage of analysis, known as level 1, the main effects of between-group change and change over time across the study were determined. The second stage of analysis, known as level 2, determined the interaction between group and change over time across the study periods.

The assumptions for LMM were checked and fulfilled, which included: 1) linear relationship between residuals of different levels, 2) residuals are normally distributed, 3) equal variances of residuals at each levels), 4) no multicollinearity, and 5) no influential outliers. The models' overall fit was tested using the change in the Chi-

square likelihood ratio test and the critical values of the Chi-square were obtained from Tabachnick & Fidell, (2012). The adjusted R^2 was calculated to determine the cross validation. The statistical significance was set at $p < 0.05$. The effect sizes were presented as eta squared and were calculated using the formula shown in Equation 5.3 (Tabachnick & Fidell, 2012):

$$\eta^2 = \frac{s_1^2 - s_2^2}{s_1^2} \dots\dots\dots \text{Equation 5.3}$$

Note: η^2 = eta squared, s_1 = residual variance of null model, s_2 = residual variance of final model

The effect sizes were reported according to Cohen’s definition with $d = 0.20$, $d = 0.50$ and $d = 0.80$ as small, medium and large effect sizes respectively (Cohen, 1992). Data were analysed using the IBM Statistical Package for Social Sciences (SPSS) version 20.0 (IBM Corp. Armonk, NY, USA).

5.3 Results

5.3.1 Description of the study participants

Following the CONSORT guidelines on reporting findings from a randomised controlled trial; description of participants who completed and withdrew from the study is presented. More importantly descriptions of participants of both groups at baseline were compared.

5.3.2 Participants' recruitment and retention

Thirty-four patients with hamstring muscle injury were approached and screened in this study. Patients responded to a notice about this study, referred by other physicians or approached through personal communication by the clinic staff. Out of the 34 patients, two (5.9 %) patients were excluded, as they did not meet the study's criteria. While another four patients (11.8 %) refused participation. Twenty-eight (82.4 %) patients who fulfilled the study criteria were invited and subsequently agreed to participate in this study. Table 5.5 summarises the reasons for non-participation after initial screening. The twenty-eight patients enrolled in this study then underwent baseline assessment and randomisation.

Table 5.5 : Reasons for non-participation after screening.

Reasons for non-participation	
Not fulfilling study criteria, N = 2 (5.9 %)	<ul style="list-style-type: none">• Extensive tear that involved hamstring and adductor longus muscles• Severe grade 3 tear of the hamstring muscle
Refuse to participate, N = 4 (11.8 %)	<ul style="list-style-type: none">• Will be studying interstate University soon• Unable to comply with study protocol

A total of 24 participants remained at completion of the study representing 85.7 % retention from baseline. Two participants in each group withdrew from the study. Two participants in the control group did not attend their scheduled follow-up and cannot be contacted. One participant in the PRP group recommenced physical activities without prior clearance from the study protocol while the other received other form of physical therapy outside the study protocol. Figure 5.14 illustrates the flow of study participants' during this RCT.

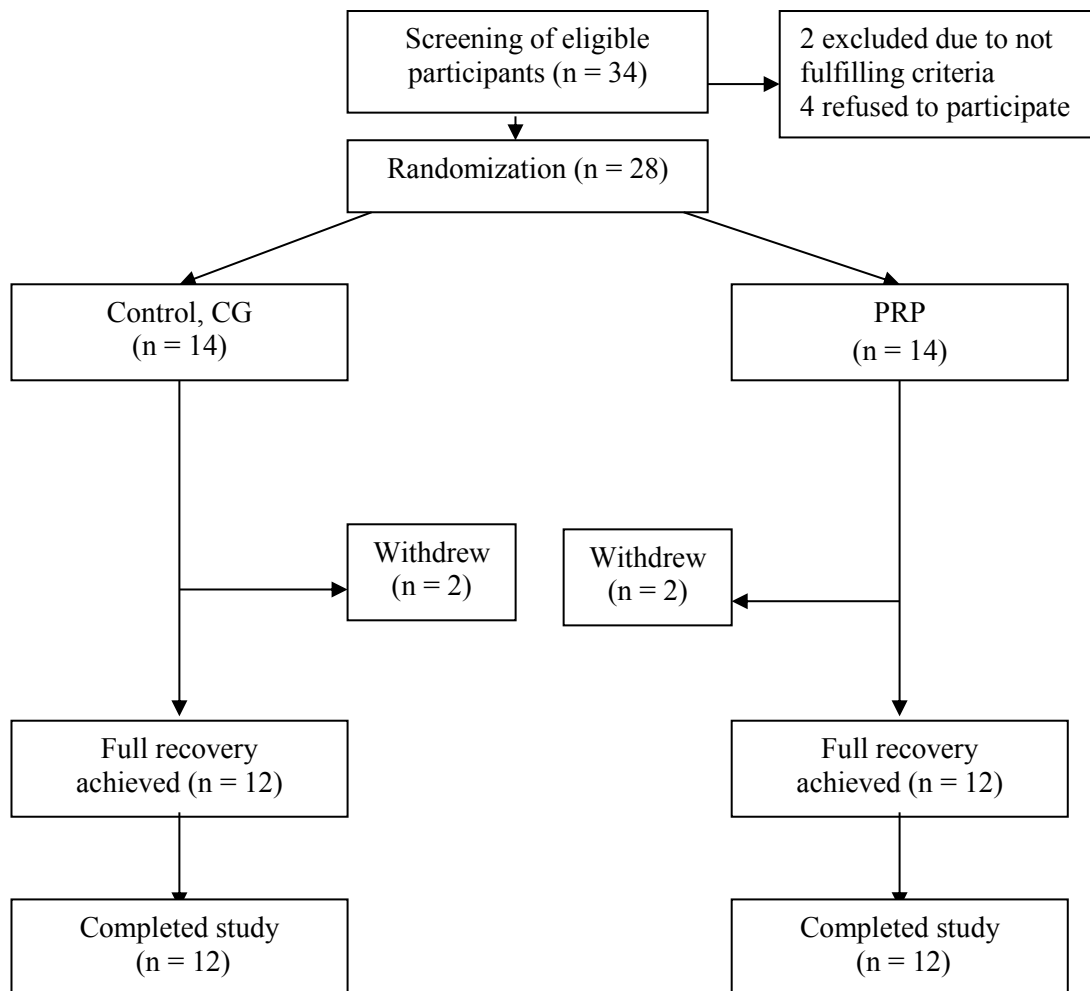


Figure 5.14. Flow diagram of randomised controlled trial (RCT) participants.

5.3.3 Test of normality

The baseline continuous variables were tested with Shapiro-Wilk test of normality to determine if the data has normal distribution meeting the assumptions of parametric tests such as independent *t*-test. Non-parametric tests such as Mann Whitney U or Kruskal Wallis tests were performed on not normally distributed data. Normal

distribution of the data was assumed when the *p*-value from Shapiro-Wilk test was 0.05 or greater. Variables that were normally distributed at enrolment were the duration of injury (in days), pain intensity score, pain interference scores (as assessed by the BPI - SF questionnaire), active knee range of movement of the non-injured side (as assessed by the AKE test), distance of injured site from ischial tuberosity, and length of injury (determined during ultrasound assessment). Table 5.6 summarises the results of the normality tests of the baseline continuous variables.

5.3.4 Characteristics of study participants

Participants' socio-demographic (ethnicity, sports, participation level, leg dominance), clinical characteristics and information on current injury is described below.

5.3.4 (a) Participants clinical characteristics

Twenty-four (85.7 %) participants achieved full recovery and completed this study. The median age of participants in this study was $21.00 \pm \text{IQR } 8.50$ (range: 18 - 49 years). More than two-third (71.4 %) of the participants were of Malay ethnicity. Majority of the participants were men (85.7 %). Most participants were national level athletes (53.6 %) while the rest were athletes at school (pre-university)(28.6 %), state (10.7 %) and club (7.1 %) levels.

The size of the injured area was examined using an ultrasound where the length, width and depth of the lesion were documented. With the assumption that the lesion is

ellipsoid in nature, the volume of the lesion was estimated using mathematical formula as below:

$$Volume\ of\ injured\ area = \frac{4}{3} \times \pi \times w \times l \times d \dots\dots\dots Equation\ 5.4$$

Table 5.6 : Normality testing of baseline continuous variables.

Continuous variables	Shapiro-Wilks		
	Statistics	Degree of freedom	P value
Age (year)			
• Control	0.703	14	< 0.001
• PRP	0.613	14	< 0.001
Weight (kg)			
• Control	0.963	14	0.779
• PRP	0.674	14	< 0.001
Height (cm)			
• Control	0.875	14	0.049
• PRP	0.937	14	0.381
BMI (kg/m ²)			
• Control	0.955	14	0.634
• PRP	0.720	14	0.001
Sporting experience (year)			
• Control	0.789	14	0.004
• PRP	0.699	14	< 0.001
Period of injury before study enrolment* (day)			
• Control	0.899	14	0.110
• PRP	0.921	14	0.231
Pain intensity (BPI-SF)*			
• Control	0.891	14	0.084
• PRP	0.956	14	0.656
Pain interference (BPI-SF)*			
• Control	0.935	14	0.357
• PRP	0.947	14	0.517
Knee ROM (AKET): Injured side (°)			
• Control	0.963	14	0.768
• PRP	0.789	14	0.004
Knee ROM (AKET): Non injured side*(°)			
• Control	0.961	14	0.742
• PRP	0.883	14	0.064
Knee ROM difference between injured and injured side (°)			
• Control	0.946	14	0.496
• PRP	0.769	14	0.002
Distance of injured site from ischial tuberosity* (cm)			
• Control	0.960	14	0.717
• PRP	0.950	14	0.568
Width (w) of injured area (cm)			
• Control	0.788	14	0.004
• PRP	0.835	14	0.014

Continuous variables	Shapiro-Wilks		
	Statistics	Degree of freedom	P value
Length (l) of injured area* (cm)			
• Control	0.973	14	0.914
• PRP	0.930	14	0.300
Depth (d) of injured area (cm)			
• Control	0.908	14	0.145
• PRP	0.785	14	0.003
Estimated volume of injured area [#] (cm ³)			
• Control	0.923	14	0.239
• PRP	0.849	14	0.022

Note: * $P \geq 0.05$ = Normal distribution; PRP = platelet-rich plasma; BMI = body mass index; BPI-SF = Brief Pain Inventory Scale-Short Form; ROM = range of movement; AKET = Active knee extension test.
[#]Injured area estimated volume based on Equation 5.4.

Most (92.9 %) athletes were right side dominant. Interestingly, 17.9 % of athletes in this study reported history of cigarette smoking. Table 5.7 summarises the study participants' socio-demographic.

5.3.4 (b) Characteristics of hamstring injury

Twelve (42.9 %) participants were soccer players; nine (32.1 %) track and field athletes, and three (10.7 %) hockey players. The remaining participants were netball, rugby, basketball and tennis players. The mean duration of injury before the athletes enrolled into the study was 4.6 ± 2.15 days (range: 0 - 7 days).

More than half (60.7 %) of athletes classified their current injury as a new injury, while the rest has had similar episode in the past (> 6 months ago). Most athletes reported that their injury occurred suddenly while engaging in sports. Most injuries occurred during training or practice sessions (64.3 %) while the remaining happened during competitions (35.7 %). Majority of the injury occurred while athletes were running (82.1 %), other injury mechanisms were jumping (7.1 %), stretching (3.6 %)

and kicking (3.6 %). Only one athlete injured his hamstring after a slipped while he was walking.

In this study, hamstring injury most often affected the biceps femoris (67.9 %), followed by semimembranosus (17.9 %), semitendinosus (10.7 %) muscles. While one athlete in the PRP group (3.5 %) injured the semimembranosus with slight semitendinosus extension. The summary of baseline injury characteristics is displayed in Tables 5.8 & 5.9.

Table 5.7 : Participant's socio-demographic characteristics.

Characteristics	Mean±SD [†] /Median±IQR ^{††} (range)	Frequency (%)
Age (year)	20.50 ± IQR7.00 (18 - 49)	
Sex		
• Men		24 (85.7)
• Women		4 (14.3)
Ethnicity		
• Malay		20 (71.4)
• Chinese		2 (7.1)
• Indian		3 (10.7)
• Others		3 (10.7)
Type of sports		
• Soccer		12 (42.9)
• Track and field		9 (32.1)
• Field hockey		3 (10.7)
• Netball		1 (3.6)
• Rugby		1 (3.6)
• Basketball		1 (3.6)
• Tennis		1 (3.6)
Experience (year)	8.50 ± IQR7.00 (2.5 - 38)	
Level of sports participation		
• National		15 (53.6)
• State		3 (10.7)
• Club		2 (7.1)
• School		8 (28.6)
Leg dominance		
• Right		26 (92.9)
• Left		2 (7.1)
Smoking		
• Yes		5 (17.9)
• No		23 (82.1)

Note: SD = standard deviation; IQR = interquartile range.

Table 5.8 : General injury characteristics.

Characteristics	Mean±SD [†] /Median ± IQR ^{††} (range)	Frequency (%)
Duration of injury before enrolment (day)	4.50 ± SD2.15 (0 - 7.0)	
Weight (kg)	66.02 ± IQR10.71 (50.70 - 127.25)	
Height (cm)	170.00 ± IQR11.88 (161.00 - 184.2)	
BMI	22.70 ± IQR2.07 (19.10 - 37.60)	
Current injury		
• New injury		17 (60.7)
• Recurrent injury		11 (39.3)
Injury circumstance		
• Training/practice		18 (64.3)
• Competition		10 (35.7)
Injury mechanism		
• Running		23 (85.7)
• Jumping		2 (7.1)
• Stretching		1 (3.6)
• Shooting		1 (3.6)
• Slipped		1 (3.6)
Injury involved specific muscle		
• Biceps femoris		19 (67.9)
• Semimembranosus		6 (21.4)
• Semitendinosus		3 (10.7)
• Semimembranosus & semitendinosus		1 (3.5%)

Note: SD = standard deviation; IQR = interquartile range; BMI = body mass index.

Table 5.9 : Participant's injury clinical characteristics.

Characteristics	Mean±SD [†] /Median ± IQR ^{††} (range)	Frequency (%)
Pain intensity (BPI-SF)	4.10 ± SD1.82 (0 - 6.75)	
Pain interference (BPI-SF)	3.32 ± SD1.91 (0.14 - 7.00)	
Distance of injured site from ischial tuberosity (cm)	19.15 ± SD6.66 (7.50 - 36.00)	
Width (w) of injured area (cm)	1.10 ± IQR0.71 (0.55 - 2.99)	
Length (l) of injured area (cm)	2.85 ± SD1.17 (0.52 - 4.83)	
Depth (d) of injured area (cm)	1.25 ± IQR0.86 (0.55 - 3.12)	
Estimated volume of injured area (cm ³)*	18.25 ± IQR23.87 (2.87 - 51.51)	
Side involved		
• Dominant		15 (39.3)
• Non-dominant		17 (60.7)
Knee ROM (AKET): Injured side (°)	30.00 ± IQR22.63 (12.50 - 78.00)	
Knee ROM (AKET): Non-injured side (°)	17.10 ± SD6.37 (5.00 - 30.00)	
Knee ROM (AKET) difference between injured and non-injured side (°)	12.50 ± IQR16.16 (0 - 54.50)	

Note: SD = standard deviation; IQR = interquartile range; BPI-SF = Brief Pain Inventory Scale-Short Form; ROM = range of movement; AKET = Active knee extension test.

*Injured area estimated volume based on Equation 5.4.

The mean duration of injury before enrolment into the study was $4.60 \pm \text{SD } 2.15$ days. The mean score of pain intensity and pain interference was $4.10 \pm \text{SD } 1.82$ and $3.32 \pm \text{SD } 1.91$ (out of maximum score of 10) respectively. The mean estimated volume of the injured area was $18.25 \pm \text{IQR } 23.87 (2.87 - 51.51) \text{ cm}^3$.

Most injury affected the non-dominant (60.7 %) than the dominant (39.3 %) leg. Higher AKE angle represent lesser ability of knee extension (greater limitation). A difference in AKE angle of $\geq 10^\circ$ between injured and non-injured side was considered a positive AKE test. Interestingly, the AKE test was only positive in 11 (39.3 %) participants with grade-2 hamstring injury. A significant difference in the AKE angle between the injured and non-injured side was found. The AKET angle of the injured hamstring was higher (median = 30.00) than the non-injured side (median = 17.50), $z = -4.38, p < 0.01, r = -0.59$.

5.3.5 Summary of study participants

Most participants in this study were Malay male athletes participating either in soccer or track and field events. More than half of injuries were new injury. Majority sustained the injuries while running during training or practice sessions. Injury often affected the non-dominant side of the leg. Biceps femoris was the most frequent muscle injured followed by semimembranosus and semitendinosus. The estimated mean volume of muscle tear was $18.25 \pm \text{IQR } 23.87 \text{ cm}^3$. The time that athletes waited before enrolling into the study ranges from 0 to 7 days (mean = $4.50 \pm \text{SD } 2.15$ days).

Despite suffering grade-2 muscle injury, athletes rated the pain severity and how much the pain interfered with their daily activities rather low. The mean score for pain severity and interference in daily life were 4.10 and 3.32 (out of maximum score of 10) respectively.

5.3.6 Baseline comparison between participants who completed and withdrew from study

Participants who completed and withdrew from the study were analysed and comparison made based on their baseline socio-demographic and injury characteristics.

5.3.6 (a) Comparison between participants completed and withdrew from the study by socio-demographic characteristics

No significant differences were observed between athletes who completed and those who withdrew on age, years of experience, gender, ethnic background, level of sports participation and leg dominance. Table 5.10 summarises these findings.

Table 5.10 : Comparisons between participants who completed and withdrew by socio-demographic characteristics.

Characteristics	Status of participants		Mann Whitney U [†] / Chi square test [‡]	
	Complete (N = 24)	Withdrew (N = 4)	<i>z</i> / χ^2	<i>P</i> value
Age [†] , median ± IQR (year)	21.00 ± 7.00	19.00 ± 6.50	-0.84	0.402
Sex [†] , n (%)				
• Men	22 (88.0)	2 (66.7)	0.99	0.382
• Women	3 (12.0)	1 (33.3)		
Ethnicity [†] , n (%)				
• Malay	18 (72.0)	2 (66.7)	3.96	0.304
• Chinese	1 (4.0)	1 (33.3)		
• Indian	3 (12.0)	0 (0)		
• Others	3 (12.0)	0 (0)		
Experience, median ± IQR (year) [‡]	8.5 ± 9.25	9.0 ± 8.13	-0.30	0.767
Level of participation [†] , n (%)				
• National	13 (52.0)	2 (66.7)	0.73	0.865
• State	3 (12.0)	0 (0)		
• Club	2 (8.0)	0 (0)		
• School	7 (28.0)	1 (33.3)		
Foot dominance				
• Right	23 (92.0)	3 (11.5)	0.26	0.611
• Leg	2 (8.0)	0 (0)		

Note: IQR = interquartile range; *P* value < 0.05 = statistically significant.

5.3.6 (b) Comparison between participants who completed and withdrew from the study by injury profiles

There was no significant difference between participants who completed or withdrew in the duration of injury before enrolment, weight, height, body mass index (BMI), injury history, injury circumstances, injury mechanisms and muscle injured.

Summary of these comparisons is displayed in Table 5.11.

Table 5.11 : Participant's injury profiles between completers and withdrawals.

Characteristics	Status of participants		<i>t</i> -test [†] / Mann Whitney U [‡] / Chi square test [#]	
	Complete (N = 24)	Withdrew (N = 4)	<i>t/z</i> / χ^2	<i>P</i> value
Duration of injury before enrolment (day) [†] median \pm SD	4.50 \pm 3.50	6.50 \pm 3.25	-	0.315
Weight (kg) [‡] , median \pm IQR	65.50 \pm 11.27	72.20 \pm 11.28	-	0.237
Height (cm) [‡] , mean \pm SD	170 \pm 11.63	173 \pm 14.00	1.18	0.664
BMI [‡] , median \pm IQR	22.50 \pm 2.20	23.30 \pm 1.70	-	0.139
Current injury [#] , n (%)			1.48	
• New injury	15 (60.0)	2 (66.7)	0.99	0.382
• Recurrent injury	10 (40.0)	1 (33.3)		
Injury circumstance ^{‡#} , n (%)				
• Training/practice	16 (64.0)	2 (66.7)	0.00	1.00
• Competition	9 (36.0)	1 (33.3)	8	
Injury mechanism [#] , n (%)				
• Jumping	20 (80.0)	3 (100.0)	0.73	1.00
• Running	1 (4.0)	0 (0)		
• Stretching	1 (4.0)	0 (0)		
• Shooting	1 (4.0)	0 (0)		
• Slipped	1 (4.0)			
Muscle injured [#] , n (%)				
• Biceps femoris	17 (68.0)	2 (66.7)	2.33	0.391
• Semimembranosus	6 (24.0)	0 (0)		
• Semitendinosus	2 (8.0)	1 (33.3)		

Note: SD = standard deviation; IQR = interquartile range; *P* value < 0.05 = statistically significant.

5.3.6 (c) Comparison between participants who completed and withdrew from the study by clinical injury characteristics

No significant difference in the pain intensity score, pain interference score, distance of injured site from ischial tuberosity, dimensions on injured area (width, length, depth and estimated volume of injured area) and ROMs of both knees between participants who completed and those withdrew from the study. Summary of these comparisons are displayed in Table 5.12.

Table 5.12 : Participant's clinical injury characteristics between completers and withdrawals.

Characteristics	Status of participants		<i>t</i> -test [†] / Mann Whitney U [‡] / Chi square test [#]	
	Complete (N = 24)	Withdrew (N = 4)	<i>t</i> / <i>z</i> / χ^2	<i>P</i> value
Pain intensity [†] , mean \pm SD (BPI - SF)	4.00 \pm 3.19	5.40 \pm 2.88	-1.08	0.292
Pain interference [†] , mean \pm SD (BPI - SF)	3.00 \pm 2.89	4.50 \pm 5.04	-0.89	0.382
Distance of injured site from ischial tuberosity [†] , mean \pm SD (cm)	18.90 \pm 9.38	22.50 \pm 11.25	-0.35	0.728
Width of injured area [‡] (cm), median \pm IQR	1.10 \pm 0.97	1.10 \pm 0.58	-0.33	0.743
Length of injured area [†] , mean \pm IQR (cm)	3.00 \pm 1.92	3.20 \pm 1.89	-0.42	0.675
Depth of injured area [‡] (cm), median \pm IQR	1.30 \pm 1.33	0.90 \pm 0.82	-0.99	0.325
Estimated volume of injured area (cm ³) [†] , median \pm IQR	19.50 \pm 24.53	14.30 \pm 24.16	-0.72	0.470
Side involved [#] , n (%)				
• Dominant	9 (36.0)	2 (66.7)	1.06	0.543
• Non-dominant	16 (64.0)	1 (33.3)		
Knee ROM (AKET): Injured side (°) [‡] , median \pm IQR	31.30 \pm 24.38	27.50 \pm 17.00	-0.40	0.693
Knee ROM (AKET): Non-injured side, mean \pm SD (°) [†]	17.50 \pm 9.00	15.00 \pm 10.25	1.32	0.198
Knee ROM (AKET) difference between injured and non-injured side (°) [‡] , median \pm IQR	12.30 \pm 24.63	20.00 \pm 15.75	-0.66	0.510

Note: SD = standard deviation; IQR = interquartile range; BPI – SF = Brief Pain Inventory Scale-Short Form; ROM = range of movement; AKET = Active knee extension test.

*Injured area estimated volume based of formula in Equation 5.4.

5.3.7 Summary of differences between participants who completed and withdrew from study

Three men and one-woman participants withdrew from the study, as they could not comply with the study protocol. No significant difference was found in all measured parameters between participants who completed and withdrew from the study.

5.3.8 Comparison between participants' baseline characteristics and the different groups

Homogeneity of the data at baseline between the control and PRP groups, including the participants' socio-demographic characteristics, injury characteristics, was analysed.

5.3.8 (a) Baseline socio-demographic characteristics in different groups

The baseline socio-demographic characteristics between the participants in the two groups were compared as shown in Table 5.13. There was no significant difference in the baseline socio-demographic characteristics across the two groups.

Table 5.13 : Baseline socio-demographic characteristics between intervention groups.

Characteristics	Study group		Mann Whitney U [†] / Chi square test [‡]	
	Control (N = 14)	PRP (N = 14)	<i>z</i> / χ^2	<i>P</i> value
Age [†] , median \pm IQR (year)	21.00 \pm 8.50	20.00 \pm 6.50	- 0.28	0.778
Sex [‡] , n (%)				
• Men	11 (78.6)	13 (92.9)	1.17	0.280
• Women	3 (21.4)	1 (7.1)		
Ethnicity [‡] , n (%)				
• Malay	9 (78.6)	9 (64.3)	0.87	0.833
• Chinese	1 (7.1)	1 (7.1)		
• Indian	1 (7.1)	2 (14.3)		
• Others	1 (7.1)	1 (7.1)		
Experience, median \pm IQR (year) [‡]	7.00 \pm 10.75	10.00 \pm 7.00	- 0.30	0.764
Level of participation [‡] , n (%)				
• National	7 (50.0)	8 (57.1)	0.90	0.825
• State	1 (7.1)	2 (14.3)		
• Club	1 (7.1)	1 (7.1)		
• School	5 (35.7)	3 (21.4)		
Foot dominance [‡] , n (%)				
• Right	13 (92.9)	13 (92.9)	<0.0001	1.000
• Leg	1 (7.1)	1 (7.1)		

Note: IQR = interquartile range; *P* value < 0.05 = statistically significant.

5.3.8 (b) Baseline injury profiles and characteristics

The length of injury (measured during ultrasonography assessment) was significantly longer in the PRP group ($p = 0.017$). However, the difference did not reach significance level when the volume of injured area was estimated (Equation 5.4). There was no statistically significant difference between the two groups for other variables. Tables 5.14 & 5.15 summarise the baseline injury profiles and characteristics across the different groups.

Table 5.14 : Baseline injury profiles between intervention groups.

Characteristics	Study Group		Mann Whitney U [‡] / Chi square test [#]	
	Control (N = 14)	PRP (N = 14)	z/χ^2	P value
Duration of injury before enrolment (day) [‡] , median ± IQR	5.00 ± 3.00	5.00 ± 3.00	-0.94	0.348
Weight (kg) [‡] , median ± IQR	66.50 ± 13.87	66.00 ± 10.21	-0.39	0.696
Height (cm) [‡] , median ± IQR	169.50 ± 19.38	171.00 ± 10.88	-0.21	0.836
BMI [‡] , median ± IQR	22.50 ± 2.63	22.80 ± 2.55	-0.89	0.370
Current injury [#] , n (%)				
• New injury	11 (78.6)	6 (42.9)	3.74	0.053
• Recurrent injury	3 (21.4)	8 (57.1)		
Injury circumstance [#] , n (%)				
• Training/practice	9 (64.3)	9 (64.3)	<0.001	1.00
• Competition	5 (35.7)	5 (35.7)		
Injury mechanism [#] , n (%)				
• Running	10 (71.4)	13 (92.9)	3.40	0.495
• Stretching	1 (7.1)	0 (0)		
• Jumping	1 (7.1)	1 (7.1)		
• Shooting	1 (7.1)	0 (0)		
• Slipped	1 (7.1)	0 (0)		
Muscle injured [#] , n (%)				
• Biceps femoris	11 (78.6)	8 (57.1)	3.47	0.176
• Semimembranosus	1 (7.1)	5 (35.7)		
• Semitendinosus	2 (14.3)	1 (7.1)		

Note: SD = standard deviation; IQR = interquartile range; P value < 0.05 = statistically significant.

Table 5.15 : Baseline injury characteristics between intervention groups

Characteristics	Study Group		<i>t</i> -test [†] / Mann Whitney U [‡] / Chi square test [#]	
	Control (N = 14)	PRP (N = 14)	<i>t/z</i> / χ^2	<i>P</i> value
Pain intensity [†] , mean \pm SD (BPI - SF)	4.30 \pm 1.85	3.90 \pm 1.83	0.54	0.595
Pain interference [†] , mean \pm SD (BPI - SF)	3.60 \pm 2.35	3.00 \pm 1.36	0.87	0.391
Distance of injured site from ischial tuberosity [†] , mean \pm SD (cm)	19.30 \pm 7.93	19.00 \pm 5.40	0.13	0.898
Width of injured area [‡] (cm) , median \pm IQR	1.20 \pm 0.98	1.00 \pm 0.63	- 0.78	0.435
Length of injured area [†] , mean \pm SD (cm)	2.30 \pm 1.04	3.40 \pm 1.09	- 2.56	0.017*
Depth of injured area [‡] (cm) , median \pm IQR	1.50 \pm 0.86	1.20 \pm 0.65	- 1.22	0.223
Estimated volume of injured area** (cm ³) [†] , median \pm IQR	19.50 \pm 23.14	15.30 \pm 34.24	- 0.37	0.713
Side involved [#] , n (%)				
• Dominant	5 (41.7)	4 (33.3)	0.18	1.000
• Non-dominant	7 (58.3)	8 (66.7)		
Knee ROM (AKET): Injured side (°) [‡] , median \pm IQR	35.80 \pm 21.75	25.00 \pm 15.63	-1.48	0.140
Knee ROM (AKET): Non-injured side, mean \pm SD (°) [†]	17.80 \pm 7.10	16.40 \pm 5.71	0.60	0.553
Knee ROM (AKET) difference between injured and non-injured side (°) [‡] , median \pm IQR	16.30 \pm 18.88	10.00 \pm 27.00	-1.13	0.259

Note: SD = standard deviation; IQR = interquartile range; BPI-SF = Brief Pain Inventory Scale-Short Form; ROM = range of movement; AKET = Active knee extension test.

**P* value < 0.05 = statistically significant.

**Injured area estimated volume based of formula in Equation 5.4.

5.3.9 Summary of differences in participants' baseline characteristics between groups

The control and PRP group were comparable in socio-demographic background, injury profiles and characteristics. The extent and severity of hamstring injury as measured by clinical assessment (AKE), diagnostic ultrasound as well as by BPI - SF questionnaire demonstrated no statistical significant difference between the two groups.

5.3.10 Overall summary of participants characteristics

Most participants in this study were national level athletes. The participants' median age was $21.00 \pm \text{IQR } 8.50$ (range: 18 - 49 years) with a median of $8.50 \pm \text{IQR } 7.00$ (range: 2.5 - 38 years) experience in their sports. The participants' mean duration of injury before enrolment was 4.50 ± 2.15 days (range: 0 - 7.0 days). Injury often affected the non-dominant leg. Biceps femoris was the most frequent muscle injured followed by semimembranosus and semitendinosus.

At baseline, no significant difference in the socio-demographic background as well as injury profiles and characteristics were noted between participants in the control and PRP groups.

5.3.11 Effectiveness of the Platelet-rich plasma

The primary outcome of interest in this study was the duration of return-to-play (DRP). In addition, potential predictors of DRP were analysed. Changes in clinical signs and symptoms associated with the injury assessed using the Brief pain inventory – short form (BPI - SF) questionnaire and the active knee extension (AKE) test was included as secondary objectives of this study. Characteristics of PRP produced were also examined and presented below.

5.3.12 Duration to return to play (DRP)

The effect of PRP on DRP was analysed using survival analysis statistical procedures. Survival functions of the two groups were compared using the log - rank (Mantel - Cox) test.

A Cox proportional hazard-model (Cox regression) was performed to explore the effect of covariates on the DRP. Previous literatures have identified several factors that predict (predictors) recovery following muscle injury. Dedrick & Clarkson, (1990) reported increasing age as significant predictors of muscle recovery. Our cross-sectional study among national level athletes found duration of injury before enrolment significantly predicts DRP (Shariff *et al.*, 2013). Other researchers also reported pain severity, length of injured area, the AKE angle, distance of injury from the ischial tuberosity, and previous hamstring injury as significant predictors of hamstring recovery (Verrall *et al.*, 2003; Connell *et al.*, 2004; Askling *et al.*, 2006; Schneider-Kolsky, 2006; Askling *et al.*, 2008; Malliaropoulos *et al.*, 2010; Warren *et al.*, 2010).

Before performing survival analyses the following assumptions were checked and fulfilled: 1) Normality of sampling distribution and missing data, 2) absence of influential outliers (univariate and multivariate), 3) equal variances of residuals at each levels), and 4) absence of multi-collinearity, and 5) absence of systematic differences between censored and completed cases. None of the covariates has missing data. All covariates, except for age, duration of injury before enrolment and AKE angle of injured side at enrolment were normally distributed (Table 5.16). Four participants were censored, as they were unable to comply with the study protocol or did not attend scheduled follow-up appointment. Regression analysis did not show any significant difference between completers and censored cases (Table 5.17).

Table 5.16 : Normality tests for covariates of interest.

Covariates	Mean (SD) /Median (IQR)*	Shapiro - Wilks		
		Statistics	Degree of freedom	P value
Age* (year)	20.50 (7.00)	0.821	28	< 0.001
Length of injured area (cm)	2.85 (1.17)	0.971	28	0.614
Duration of injury before enrolment* (day)	5.00 (3.75)	0.890	28	0.007
Pain intensity (BPI-SF)	4.10 (1.82)	0.959	28	0.332
Pain interference (BPI-SF)	3.32 (1.91)	0.971	28	0.621
AKE angle injured side at enrolment* (°)	30.00 (22.63)	0.891	28	0.007
Distance of injured site from ischial tuberosity (cm)	19.15 (10.44)	0.975	28	0.721

Note: *P ≥ 0.05 = Normal distribution; IQR = Interquartile range; BPI – SF = Brief Pain Inventory Scale-Short Form; AKET = Active knee extension test.

Table 5.17 : Regression analysis for differences between completers and withdrawals.

Model	Unstandardized coefficient		Standardized coefficient	<i>t</i>	<i>P</i>
	B	SE	Beta		
(Constant)	-0.068	0.906		-0.075	0.941
Age (year)	-0.002	0.011	-0.127	-0.215	0.832
Length of injured area (cm)	0.050	0.074	0.163	0.669	0.513
Duration of injury before enrolment (day)	0.013	0.044	0.073	0.299	0.769
Pain intensity (BPI-SF)	0.001	0.079	0.006	0.014	0.989
Pain interference (BPI-SF)	0.056	0.079	0.301	0.714	0.485
AKET angle injured side at enrolment (°)	-0.003	0.006	-0.125	-0.464	0.649
Distance of injured site from ischial tuberosity (cm)	0.002	0.013	0.030	0.119	0.906

Note: **P* < 0.05 = Significant difference; BPI – SF = Brief Pain Inventory Scale-Short Form; AKET = Active knee extension test; SE = Standard error.

Univariate analysis of covariates did not detect any influential outliers (z-score > 3.29; *p* < 0.001, two-tailed test), therefore no logarithmic transformation was performed (Table 5.18) (Tabachnick & Fidell, 2012). Similarly, Mahalanobis distance to access multivariate outliers using critical value of $\chi^2 = 24.33$ (degrees of freedom (df) = 7 at $\alpha = 0.001$) did not detect any outliers (Mahalanobis distance ranges: 3.41 to 16.43).

Table 5.18 : Identification of univariate outliers.

Covariates	Minimum	Maximum	N
Z-score: Age (year)	- 0.653	2.888	28
Z-score: Length of injured area (cm)	- 1.984	1.687	28
Z-score: Duration of injury before enrolment (day)	- 1.946	1.110	28
Z-score: Pain intensity (BPI-SF)	- 2.255	1.459	28
Z-score: Pain interference (BPI-SF)	- 1.660	1.927	28
Z-score: AKE angle injured side at enrolment (°)	- 1.332	2.506	28
Z-score: Distance of injured site from ischial tuberosity (cm)	- 1.750	2.531	28

Note: Z-score > 3.29 (*p* < 0.001, two tailed test) = potential outliers; IQR = Interquartile range; BPI – SF = Brief Pain Inventory Scale-Short Form; AKE = Active knee extension.

Descriptive statistics were displayed in a Kaplan-Meier survival table (Table 5.19) and a survival curve was plotted to show survival functions over time for all participants. Participants in the PRP group reach full recovery sooner than control (Figure 5.15). Half of the participants in the PRP group achieved full recovery after 21 days of follow-up in contrast to only 4 (33.3 %) of the control. The medians DRP were $34.0 \pm \text{IQR } 37.3$ and $21.0 \pm \text{IQR } 13.0$ days for control and PRP respectively and were compared using the Log Rank (Mantel-Cox) test. There was a significant difference in survival function between the two groups $\chi^2(1, N = 14) = 6.189, p = 0.013$.

Table 5.19 : Kaplan-Meier survival table.

Study group	DRP (day)	Status	Cumulative proportion injured at the time		N of cumulative events	N of remaining cases
			Estimate	SE		
Control						
1	10.00	Injured	-	-	0	13
2	13.00	Recovered	0.923	0.074	1	12
3	14.00	Injured	-	-	1	11
4	20.00	Recovered	0.839	0.104	2	10
5	21.00	Recovered	-	-	3	9
6	21.00	Recovered	0.671	0.135	4	8
7	22.00	Recovered	0.587	0.142	5	7
8	34.00	Recovered	-	-	6	6
9	34.00	Recovered	0.420	0.143	7	5
10	35.00	Recovered	0.336	0.137	8	4
11	41.00	Recovered	0.252	0.126	9	3
12	64.00	Recovered	0.168	0.108	10	2
13	71.00	Recovered	-	-	11	1
14	71.00	Recovered	0	0	12	0
PRP						
1	6.00	Injured	-	-	0	13
2	12.00	Recovered	0.923	0.074	1	12
3	14.00	Injured	-	-	1	11
4	16.00	Recovered	-	-	2	10
5	16.00	Recovered	-	-	3	9
6	16.00	Recovered	0.671	0.135	4	8
7	19.00	Recovered	0.587	0.142	5	7
8	21.00	Recovered	-	-	6	6
9	21.00	Recovered	0.420	0.143	7	5
10	26.00	Recovered	0.336	0.137	8	4
11	29.00	Recovered	-	-	9	3
12	29.00	Recovered	-	-	10	2
13	29.00	Recovered	0.084	0.080	11	1
14	34.00	Recovered	0	0	12	0

Note: DRP = duration to return-to-play; SE = standard error.

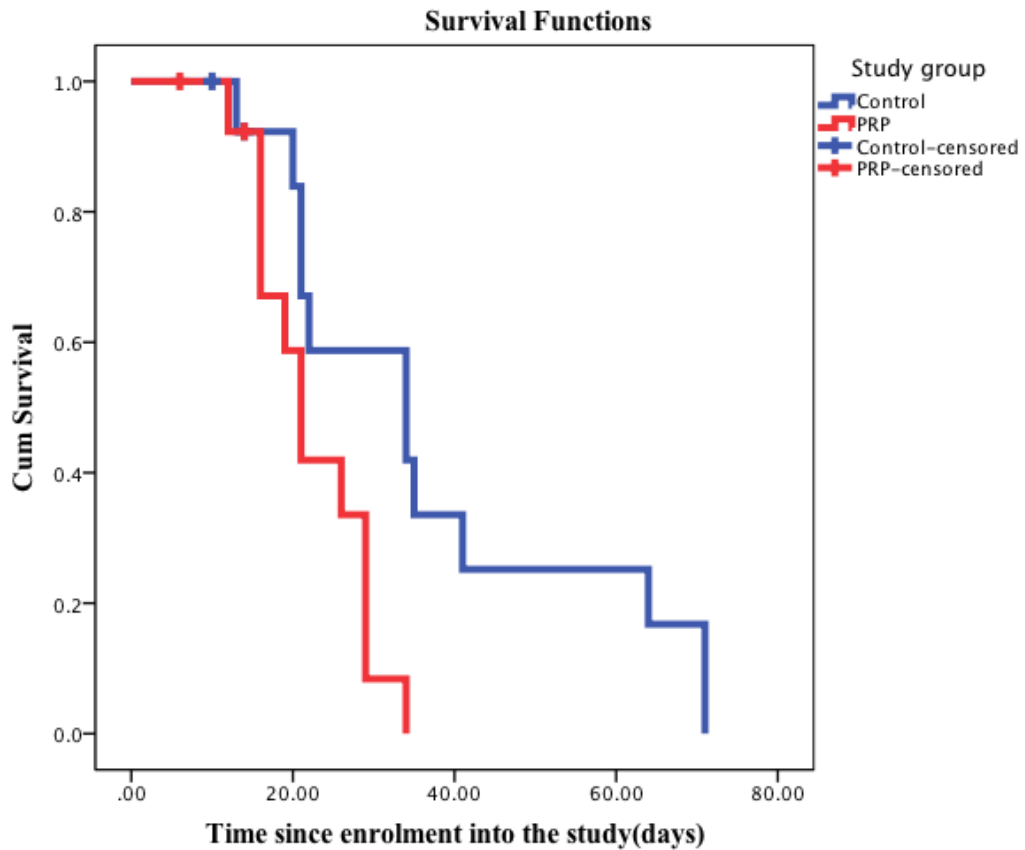


Figure 5.15. Survival functions of the control and PRP groups.

Regression analysis was conducted with status used to form dichotomous dependent variables (DV). Participants who withdrew from the study were given a value of 1 while all other cases score 0. Nine covariates including treatment group served as the independent variables (IV) for the regression analysis. Eight of these covariates were reported by previous study as significant predictors of muscle recovery (Dedrick & Clarkson, 1990; Verrall *et al.*, 2003; Connell *et al.*, 2004; Askling *et al.*, 2006; Schneider-Kolsky, 2006; Askling *et al.*, 2008; Malliaropoulos *et al.*, 2010; Warren *et al.*, 2010; Shariff *et al.*, 2013).

A Cox regression analysis was used to evaluate the effects of treatment and other covariates on DRP. Covariates other than treatments were entered first, followed

by treatment as this allow a likelihood-ratio test of the effect of treatment, after statistical adjustment for the other covariates (Tabachnick & Fidell, 2012). The PRP therapy has a significant effect on the DRP of hamstring injury after considering the other covariates, $G^2(1) = 8.517, p = 0.004$. None of the other covariates significantly predicted hamstring recovery DRP. Table 5.20 shows regression coefficients, degrees of freedom, p value and hazard ratios for each covariate.

Table 5.20 : Cox-regression analysis of other covariates on hamstring recovery.

Covariate	<i>B</i>	df	<i>P</i> value	Odds ratio
Age (year)	0.049	1	0.159	1.050
Length of injured area (cm)	-0.509	1	0.131	0.601
Duration of injury before enrolment (day)	0.114	1	0.351	1.121
Pain severity (BPI-SF)	0.498	1	0.051	1.646
Pain interference (BPI-SF)	-0.500	1	0.114	0.607
AKE angle injured side at enrolment (°)	-0.021	1	0.332	0.979
Distance of injured site from ischial tuberosity (cm)	-0.013	1	0.724	0.987
History of previous injury	-0.227	1	0.764	0.797
PRP therapy	2.387	1	0.005*	10.882

Note: * $P \geq 0.05$ = Significant predictors; *B* = Regression coefficient; df = Degrees of freedom; IQR = Interquartile range; BPI – SF = Brief Pain Inventory Scale - Short Form; AKE = Active knee extension.

The chance for participants in the PRP group to reach earlier DRP was 10.8 times higher compared with the control group. The DRP was well predicted by PRP therapy, $R^2 = 0.262$ with a small to moderate effect size (Cohen, 1992). Therefore, hypothesis 1 that participants received PRP injection combined with hamstring rehabilitation programme would show significantly shorter DRP (faster recovery) compared with group that received hamstring rehabilitation programme alone is accepted.

5.3.13 Effects of intervention on changes in pain severity, pain interference and AKE angle

5.3.13 (a) Changes in pain intensity score

The secondary objectives of this study were to explore the effects of PRP on changes in pain severity and pain interference scores over time. A linear mixed model using intention-to-treat analysis was applied to determine the effectiveness of interventions across the different groups over time. The following assumptions were checked and fulfilled: 1) linear relationship between residuals of different levels, 2) residuals are normally distributed, 3) equal variances of residuals at each level, 4) absence of multicollinearity, and 5) absence of outliers.

Both groups showed gradual improvement (lower score) in pain severity score over time (Figure 5.16). The pain severity mean score as assessed by BPI - SF, (Q2 - 6) was significantly different between control and PRP group at all time points, $F(1, 115.47) = 7.50, p = 0.007, \eta^2 = 0.771$) with a large effect size. Participants in PRP group had significantly lower pain severity score ($M = 1.14 \pm SE 0.19$) than controls ($M = 2.31 \pm SE 0.23$) (mean difference $= -1.17 \pm SE 0.30, p < 0.001$) across time. Therefore the hypothesis 2 that participant in the PRP group will display significantly faster improvement in pain severity score (BPI - SF) over time is accepted. The adjusted R^2 was 0.212 suggested small to moderate effect size (Cohen, 1992) and the final model $\chi^2(3) = 71.82, p < 0.001$. All assumptions were checked and fulfilled. The final equation for mean BPI severity score (control group as reference) $= 3.84 + (-0.52)(\text{Visits}) + (-0.39)(\text{PRP group} * \text{visits}) + 1.59$. The mean BPI severity score reduces over the period of this study and the PRP group showed greater drop during the study compared with controls.

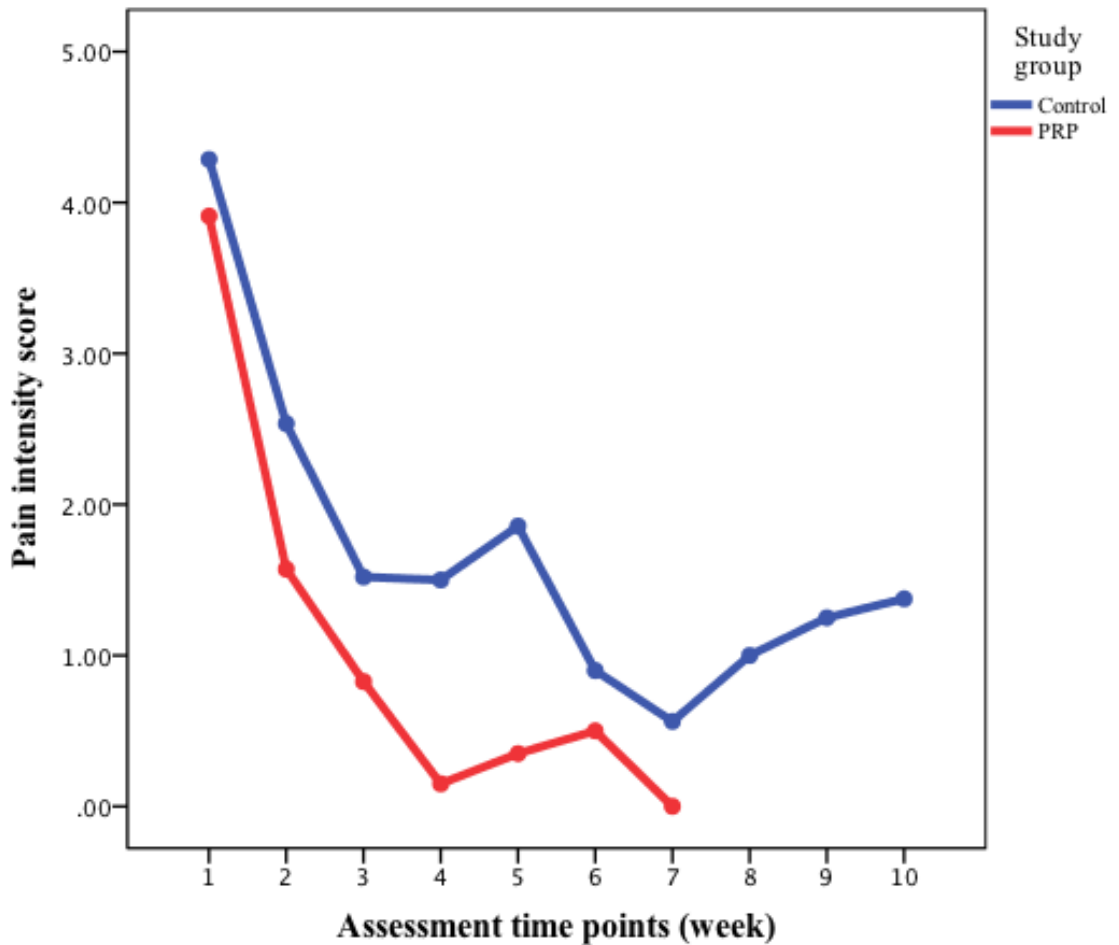


Figure 5.16. Comparisons of the mean pain severity scores between intervention groups across study period.

5.3.13 (b) Changes in pain interference score

Gradual decreases in pain interference scores were observed in both groups. Even though the PRP groups showed lower pain interference mean score across time (Figure 5.17), the difference was not statistically significantly, $F(1, 108.61) = 2.030, p = 0.157$. Therefore hypothesis 3 that participant in the PRP group will display significantly faster improvement in pain interference score (BPI - SF) over time is rejected.

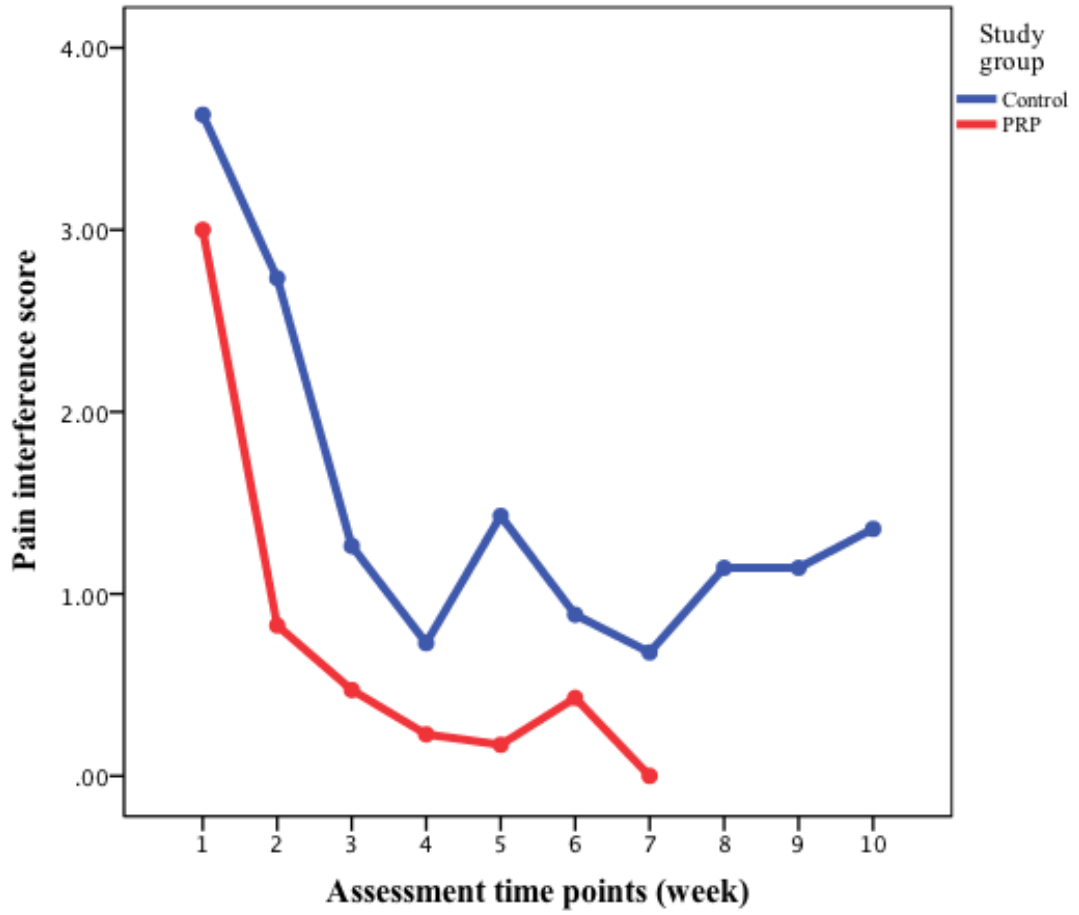


Figure 5.17. Comparisons of mean pain interference scores between groups across study period.

5.3.13 (c) Changes in active knee extension angle

A higher mean AKE angle corresponds to greater limitation of knee extension. Both groups showed decrease in AKE knee angle suggesting full range of movement of the knee has been regained (Figure 5.18). The mean AKE angle in the first 4 weeks was comparable. Greater differences between groups were observed beyond the 4th week of follow up period. The difference in the AKE angle of the affected leg (as assessed by active knee extension test) throughout the study period was not statistically significant $F(1, 111.78) = 1.350, p = 0.248$). Therefore the hypothesis 4 that participant in the PRP

group will show significantly faster improvement in the injured leg active knee extension angle over time is rejected.

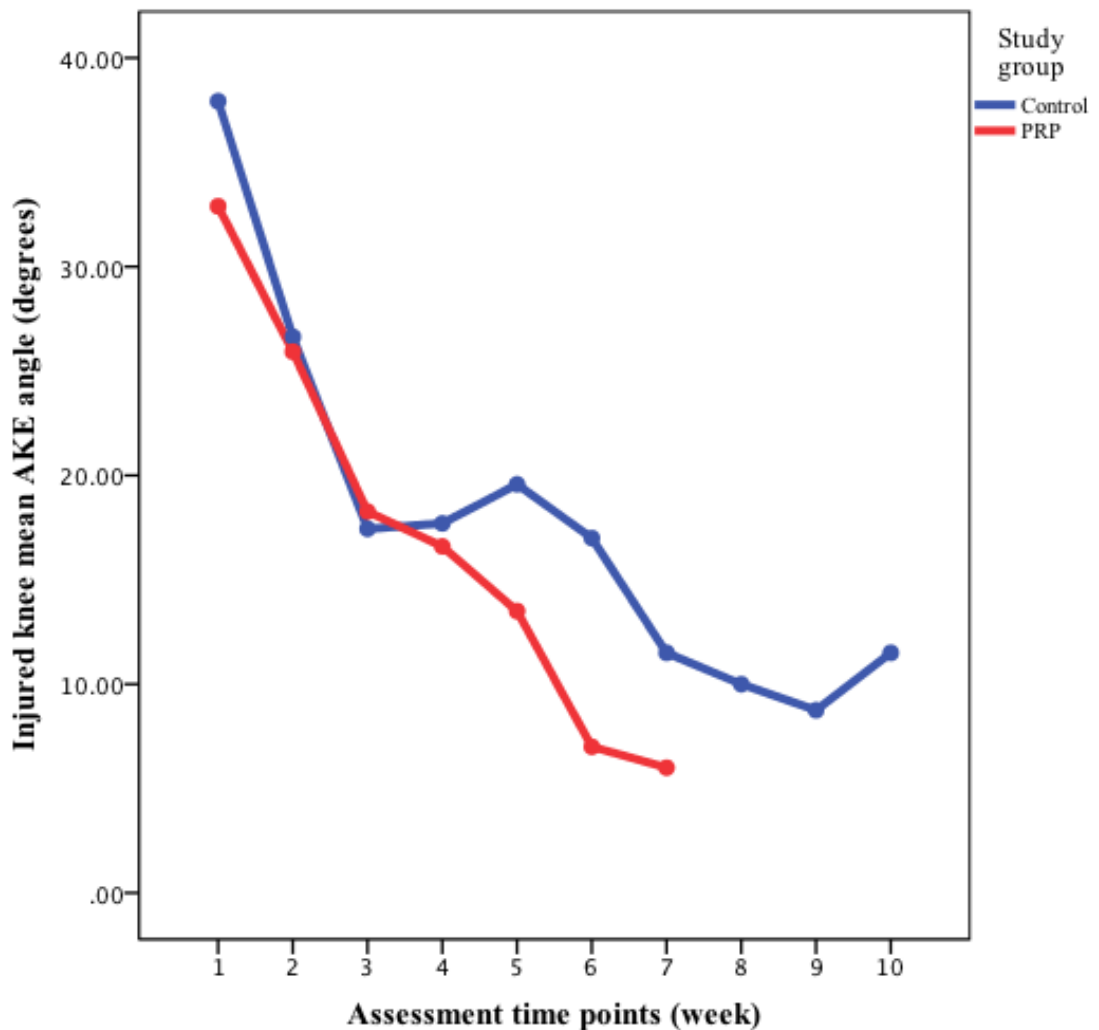


Figure 5.18. Comparisons of mean AKE angle between groups across study period.

5.3.14 Platelet-rich plasma (PRP) characteristic

The PRP produced in this study was characterised using the PAW classification systems (DeLong *et al.*, 2012). The PAW classification is one of the simplest methods in describing PRP characteristic. This system was based on several parameters including 1) the absolute number of platelets (P), the presence of activating agent during

PRP administration (A) and 3) the presence or absence of white blood cells (W) (Figure 5.19).

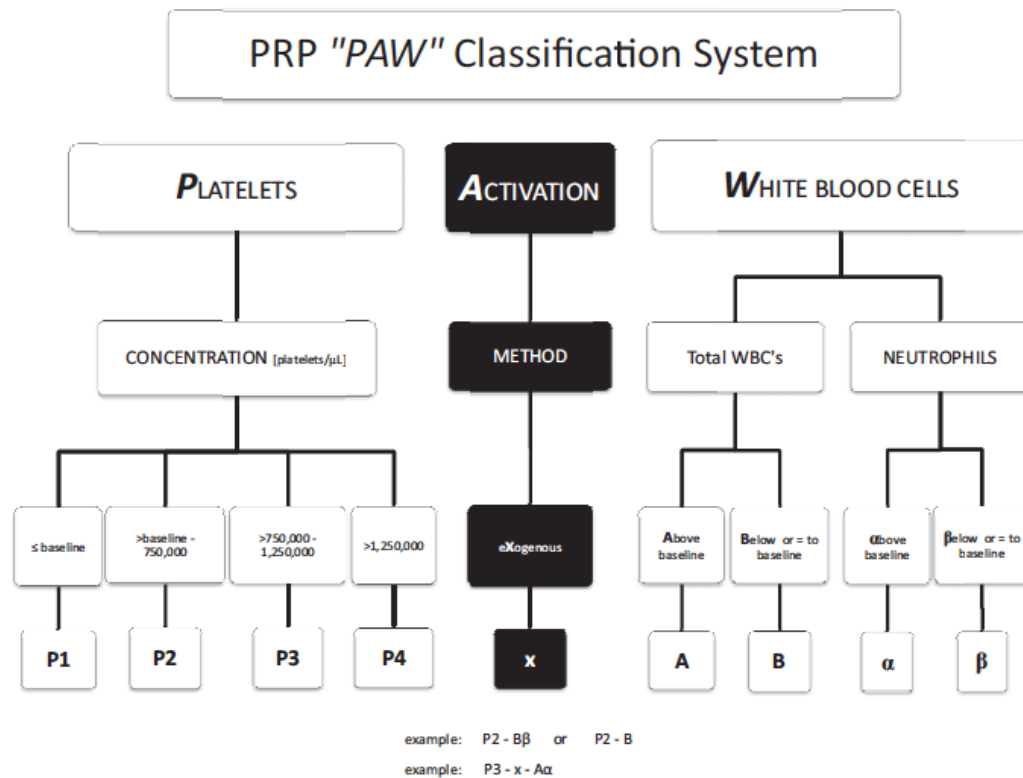


Figure 5.19. The PAW Classification System. Source: DeLong *et al.*, 2012 (p.1006).

5.3.14 (a) Platelet concentration.

The amount of platelets in the venous whole blood and in the PRP produced from the GPS IIITM system ranges from 187.0 to 347.0 x 10³/ μ L and 936.0 to 1586.0 x 10⁹/ μ L respectively (Table 5.21). A Wilcoxon signed-rank test showed the Biomet GPS III kit could produce significantly higher number of platelets in PRP (Median = 1297 x 10³/ μ L, IQR = 51.32 x 10³/ μ L) compared with whole blood (Median = 234 x 10³/ μ L, IQR = 491.75 x 10³/ μ L); (Z = - 3.2, p = 0.001). The method used in the current study

could produce PRP with mean platelets content of 5.37 ± 0.92 with ranges between 3.73 to 6.93 times higher than the number present in whole blood (baseline).

Table 5.21 : Amount of platelets in participant's whole blood and PRP.

Participants No.	Platelet count		
	Whole blood x $10^3/\mu\text{L}$	PRP x $10^3/\mu\text{L}$	PRP : Whole blood
1	234.0	1276.0	5.45
4	335.0	1252.0	3.73
6	347.0	2021.0	5.82
8	220.0	1002.0	4.55
11	195.0	837.0	4.29
12	229.0	1242.0	5.42
13	234.0	936.0	4.00
16	237.0	1454.0	6.14
19	323.0	1873.0	5.80
20	245.0	1380.0	5.63
21	220.0	1318.0	5.99
23	201.0	1397.0	6.95
24	256.0	1586.0	6.19
26	187.0	975.0	5.21

PRP = Platelet-rich plasma.

5.3.14 (b) Activating agent

No exogenous platelets activator was used before administration of the PRP in this study. The interaction between platelets and tissue collagen may provide the appropriate endogenous clotting factors needed for activation. Further, endogenous activation resulted in slower platelet aggregation and more sustained release of growth factors (Harrison *et al.*, 2011).

5.3.14 (c) White blood cells (WBC)

The complete result on the number of WBC present in venous whole blood and in PRP was available only in six participants in the intervention group (Table 5.22). The

mean absolute number of WBC in the whole blood and in the PRP was $7.3 \times 10^3/\mu\text{L} \pm 1.3 \times 10^3/\mu\text{L}$ and $38.3 \times 10^3/\mu\text{L} \pm 11.9 \times 10^3/\mu\text{L}$ respectively. A paired-samples *t*-test was conducted to compare the number of platelets present in PRP and the whole blood. Significantly higher number of WBC present in PRP (Mean = $7.3 \times 10^3/\mu\text{L}$, = $1.31 \times 10^3/\mu\text{L}$) compared with whole blood (Mean = $38.3 \times 10^3/\mu\text{L}$ = $11.87 \times 10^3/\mu\text{L}$); $t(6) = -7.05$, $p = 0.001$. Based on this finding the current method of PRP produced in this study contained total WBC above the level present in the peripheral blood. Therefore the PRP produced was classified according to PAW classification as below (Table 5.23).

Table 5.22 : Amount of white blood cells (WBC) in participant's whole blood and PRP.

Participants No.	WBC count		
	Whole blood $\times 10^3/\mu\text{L}$	PRP $\times 10^3/\mu\text{L}$	PRP : Whole blood
1	7.80	45.60	5.85
4	7.90	43.10	5.46
6	7.40	29.80	4.03
8	9.20	55.70	6.05
11	6.10	24.00	3.93
12	5.60	31.30	5.59

WBC = white blood cells; PRP = platelet-rich plasma.

Table 5.23 : PAW classification of PRP used in the current study.

Platelet Concentration	Activation method	WBC's		PAW Classification
		Total WBC Content	Neutrophil Content	Endogenous
P4	Endogenous (x)	A	Not available	P4-x-A

WBC = white blood cells; P4 = Platelet level $> 1250 \times 10^3/\mu\text{L}$; A = WBC content above baseline.

5.3.14 (d) Growth factors.

Despite carefully following the manufacturer's guideline on performing the IGF-1 test. We could not obtain a reliable reading, as the coefficient of determination was very low ($R^2 = 0.012$). Therefore, the IGF-1 level is not reported. The coefficient of determinations for TGF- β 1 and bFGF were $R^2 = 0.964$ and 0.943 respectively. The TGF- β 1 and bFGF levels from 11 participants are displayed in Table 5.24. The median (IQR) level TGF- β 1 and bFGF were $50.34 \pm \text{IQR } 54.00$ ng/ ml and $42.73 \pm \text{IQR } 25.51$ pg/ ml respectively.

Table 5.24 : Amount of TGF- β 1 and bFGF present in PRP.

Participant No.	Growth factors	
	TGF- β 1 (ng/ ml)	bFGF (pg/ ml)
1	34.00	29.83
4	36.34	27.62
6	31.09	39.54
8	42.21	32.58
11	38.59	42.73
12	80.46	55.23
13	111.47	58.09
16	224.71	46.28
19	81.21	32.98
20	50.34	91.42
21	90.34	75.12

TGF - β 1 = transforming growth factor beta - 1; bFGF = basic fibroblast growth factor.

5.3.15 Blinding success

The blinding index corresponds to percent incorrect guesses + percent undecided guesses (James *et al.*, 1996). Blinding indices of > 50 % suggest successful blinding. The blinding index for assessor in this study ranged from 78.6 to 85.7 %, suggesting successful blinding (Table 5.25).

Table 5.25 : Assessment of blinding

Assessor's guess	Actual treatment		
	PRP	Control	Total
PRP	2 (14.3 %)	3 (21.4 %)	5
Control	0 (0 %)	0 (0 %)	0
Undecided	12 (85.7 %)	11 (78.6 %)	23
Total	14	14	28

Assessor was asked upon study completion to identify which treatment arm the patient was assigned. Blinding index equals (percent [incorrect] + percent [undecided]). When blinding exceeds 50 %, subjects have been successfully blinded (Hertzberg *et al.*, 2008).

5.4 Discussion

Over the last decade the use of PRP has received increasing attention for its potential favourable effects on soft tissue healing. Platelet-rich plasma (PRP) is currently used to treat several soft tissue conditions including lateral epicondylitis (tennis elbow)(Gosens *et al.*, 2011; Peerbooms *et al.*, 2010; Thanasas *et al.*, 2011), tendinopathies (Achilles, patellar and rotator cuffs)(Ark *et al.*, 2011; Barber *et al.*, 2011, Spang *et al.*, 2011), osteoarthritis (Sampson *et al.*, 2010; Wang-Saegusa *et al.*, 2011), and acute muscle injuries (Wright-Carpenter *et al.*, 2004a; Wright-Carpenter *et al.*, 2004b; Hamilton *et al.*, 2011). With the exception of lateral epicondylitis (tennis elbow) the clinical evidence to support PRP use in other conditions are limited to experimental and Grade III – IV human studies. Also some studies showed contrasting effects of PRP for certain soft tissue injuries (Kon *et al.*, 2009; Filardo *et al.*, 2009; Gaweda *et al.*, 2010; deVos *et al.*, 2010; deAlmeida *et al.*, 2012). Lack of uniformity in method of PRP preparation, administration procedures and rehabilitation exercises programme post PRP injection have been implicated as reasons for differences between studies (Andia *et al.*, 2011; Hamilton *et al.*, 2011; Redler *et al.*, 2011; Sheth *et al.*, 2012). While the theoretical science seems to support PRP use for muscle injury (Ross *et al.*, 1979; Allen

& Boxhorn, 1989; Husmann *et al.*, 1996), clinical evidence to prove such effects is limited. Most data were based on experimental studies and sporadic cases reports (Wright - Carpenter *et al.*, 2004a; Wright - Carpenter *et al.*, 2004b; Creaney *et al.*, 2008; Loo *et al.*, 2009; Gigante *et al.*, 2012). Therefore this RCT was conducted to bridge the gap in knowledge on PRP effects on acute muscle injury.

5.4.1 PRP preparation and administration protocols

Currently there are many commercially available kits to produce PRP. Some used the buffy coat methods (Biomet GPS III, Harvest SmartPRep 2 and Artericyte/Medtronic) while others rely on the plasma-based methods (Arthrex/ACP, Cascade/MTF Fibrinet and BTIPRGF). Investigators vary in the methods of PRP preparation. Accordingly the PRP quality produced by the various methods differs in the amount of platelets, white blood cells and even the level of growth factors (DeLong *et al.*, 2012). These differences might affect the clinical outcome of PRP intervention (Mazzocca *et al.*, 2012). Developing a standardised PRP classification system is much needed as it allows standardised comparisons between studies (Dohan *et al.*, 2009; Engebretsen *et al.*, 2010; DeLong *et al.*, 2012).

In this study, PRP was produced using a commercially available kit that used the buffy coat methods. Following the manufacturer guideline on the use of these kits, each 60 ml kit was able to produce about 6 ml of PRP. The amount of platelet contained in the PRP ranges from $837 \times 10^3 / \mu\text{L}$ to $2021 \times 10^3 / \mu\text{L}$. The mean platelet level was $1325 \times 10^3 / \mu\text{L} \pm \text{SD}341 \times 10^3 / \mu\text{L}$. The PRP produced had $5.4 \pm \text{SD} 0.92$ times higher number of platelets than the peripheral venous blood. The level of platelet concentration

in this study corresponds to levels reported in the literatures (Woodell-May *et al.*, 2005; Castillo *et al.*, 2011; Schippinger *et al.*, 2011; DeLong *et al.*, 2012).

The ideal amount of platelet necessary to demonstrate its potential useful effects is yet to be determined. Lack of standardisation of study protocols, platelet-separation techniques and outcome variables of the limited available literatures further complicates comparison between studies (Andia *et al.*, 2011; Hamilton *et al.*, 2011; Sheth *et al.*, 2012). Several *in vivo*, *in vitro* and clinical studies have showed beneficial effects of PRP when platelet concentration was 2 to 3 times higher than baseline (peripheral blood) (Anitua *et al.*, 2005; Anitua *et al.*, 2006; Sanchez *et al.*, 2007; Anitua *et al.*, 2008; Mazzocca *et al.*, 2012).

Though it is tempted to suggest there is a linear relationship between amount of platelet and rate of tissues healing. Platelet concentration greater than 6 times may have paradoxical negative effect on tissue healing. Weibrich *et al.*, (2004) found highly concentrated platelets (6 to 11 times higher than peripheral blood; 1852 to 3200×10^3 platelets/ μL) had an inhibitory effect on osteoblast activity when compared with lower concentration (Weibrich *et al.*, 2004).

The mean platelet concentration of $5.4 \pm \text{SD } 0.92$ times higher than peripheral blood ($1325 \times 10^3 / \mu\text{L} \pm \text{SD } 341 \times 10^3$ platelets/ μL) produced in this study was well within the therapeutic level as described by previous authors (Weibrich *et al.*, 2004; Anitua *et al.*, 2006; Anitua *et al.*, 2008; Andia *et al.*, 2011; Hamilton *et al.*, 2011; DeLong 2012).

As there were reports that levels of platelet growth factors not necessarily proportionate to the platelet count (Borzini *et al.*, 2005; Weibrich *et al.*, 2005) two types of growth factors; human transforming growth factor - β 1 (TGF - β 1) and basic fibroblasts growth factor (bFGF) levels were determined in the current study. Both growth factors were measured with enzyme-linked immunosorbent assay (ELISA) kits. The TGF - β 1 and bFGF levels were analysed using SSB - E04725h and CSB - E08000h kits respectively (Cusabio, Wuhan, China). The plasma TGF- β 1 and bFGF levels in healthy adults ranged between 1.9 ± 5.2 ng/ml and 1.89 ± 1.20 pg/ml respectively (Kropf *et al.*, 1999; Larsson *et al.*, 2002; Wakefield *et al.*, 2005). The median level of TGF - β 1 in the current study was $50.34 \pm$ IQR 54.09 ng/ml representing a 90 to 260 % increase than previously reported level in plasma. Meanwhile the median bFGF level was $42.73 \pm$ IQR 25.51 pg/ml, representing 22 fold increases than previous study.

The effect of highly concentrated WBC within PRP preparations is not fully understood (Schneider *et al.*, 2007; Ehrenfest *et al.*, 2009). Although normal levels of WBCs have positive immunodulatory effect, higher level may contribute to harmful impact on soft tissue healing (Toumi *et al.*, 2003; Tidball *et al.*, 2004; Smith *et al.*, 2009). Others however suggested the large amount vascular endothelial growth factors (VEGF) produced by WBC is crucial for promotion of angiogenesis and healing (Werther *et al.*, 2002; Peterson *et al.*, 2010). Further WBC rich PRP has been successfully used for the treatment of tendonitis and delayed long bone healing (Mishra *et al.*, 2006; Schnabel *et al.*, 2007). The mean amount of WBC present in the PRP produced in the current study was $38.3 \times 10^3 / \mu\text{L} \pm$ SD $11.9 \times 10^3 / \mu\text{L}$ which was 5 times higher than the WBC level in the peripheral blood ($7.3 \times 10^3 / \mu\text{L} \pm$ SD $1.3 \times 10^3 / \mu\text{L}$).

In summary, the PRP produced and used in the current study has a platelet and WBC level that was 5.4 and 5 times higher than peripheral venous blood respectively. Therefore the PRP in the study was classified as leucocyte rich-PRP (L-PRP) or Type 4 PRP according to the classification described by Ehrenfest *et al.*, (2009) or Mishra *et al.*, (2012) respectively. Alternatively the PRP in the study could also be classified as P4 – x - B according to the more recent PAW Classification System (DeLong *et al.*, 2012).

5.4.2 Hamstring injuries

In this study hamstring injury mostly occurred among practitioner of sports that require rapid acceleration and changes in direction including soccer, track and field's, hockey, netball, rugby, basketball and tennis. Similar observations reported by previous authors (Verrall *et al.*, 2001; Seward *et al.*, 2003; Gabbe *et al.*, 2006; Liu *et al.*, 2012). Most injuries (60.7 %) in the current study were classified as new injury. Hamstring recurrence injury was observed only in 39.3 % of athletes. Similarly 12 to 48 % recurrent hamstring injuries were reported among English professional soccer and Australian football players (Orchard *et al.*, 2002a; Orchard *et al.*, 2002b; Dadebo *et al.*, 2004; Askling *et al.*, 2004; Woods *et al.*, 2004; Ekstrand *et al.*, 2011). The current study also noted that majority of hamstring injury involved the biceps femoris muscle (67.9 %). This observation is similar to those documented by previous studies (Kalimo *et al.*, 1997; Hawkins *et al.*, 2001; Henderson *et al.*, 2010; Comin *et al.*, 2012).

All athletes diagnosed with grade-2 hamstring injury in this study complained of pain (BPI - SF, Q2 - 6), which also interfered with daily activities (BPI - SF, Q9A -

9G). Interestingly, only 11 (39.3 %) participants displayed positive active knee extension (AKE) test (angle difference of $> 10^\circ$) between the injured and non-injured sides. This finding was in agreement with earlier studies (Best *et al.*, 1995; Kalimo *et al.*, 1997; Tiidus, 2008). This finding suggests hamstring-grading systems that based solely on physical assessment (including AKE test) might underestimate injury severity. Therefore, a more comprehensive method of hamstring injury classification systems that incorporated physical assessment and radiological imaging (ultrasound and MRI) is recommended (Peetrons, 2002; Hurme *et al.*, 2006; Järvinen *et al.*, 2007; Cohen *et al.*, 2011; Chan *et al.*, 2012; Meuller-Wohlfahrt *et al.*, 2013).

5.4.3 PRP effect on duration to return-to-play (DRP) after muscle injury

The main outcome of this study was to investigate the effect of PRP therapy on the duration to return-to-play (DRP). The DRP was defined as duration (in days) from injury onset until participants' reached full recovery. A set of criteria was used in deciding participant's readiness to recommence preinjury activities. A significant difference in participants' median DRP between the two intervention groups was found. Participants in the PRP group achieved DRP significantly earlier compared with controls (21.0 vs. 34.0 days). Unfortunately, no other RCT that explore the effect of PRP on DRP was available to allow comparison with the present finding.

A significantly shorter recovery time was reported among athletes diagnosed with moderate hamstring strain (second-degree) treated with autologous conditioned serum (ACS) by Wright *et al.* (2004). The author reported athletes who received ACS injection every second day from study enrolment took 16.6 days to achieve full

recovery. Meanwhile participants in the control group took 22.3 days to recover (Wright-Carpenter *et al.*, 2004a). The ACS was prepared using a technique that was clearly described by the researcher. While the authors did report higher content of several growth factors (including TGF - β 1, FGF - 2 and IGF - 1), other ACS characteristics such as platelets and WBC levels was not stated (Wright - Carpenter *et al.*, 2004a).

Higher level of growth factors noted in the previous study could be attributed to the different methods of PRP preparation. The ACS preparation involved a 24-hour incubation period of the collected bloods on to glass beads. This incubation period initiated monocyte activation and cytokines release (Wehling *et al.*, 2007). In contrast, the method used in the current study involved no incubation period; also no platelet activation was used before PRP administration.

In addition, concurrent use of oral medication in the previous study may also have affected symptoms of pain allowing earlier DRP. Participants in the ACS study were allowed to take antiphlogistics; bromelain during the study. Bromelain is a natural supplement with documented anti-inflammatory and analgesic actions (Brien *et al.*, 2004). Further, Bromelain has demonstrated clinical effectiveness in reducing symptoms of pain among patients with osteoarthritis (Singer *et al.*, 2001; Tilwe *et al.*, 2001; Walker *et al.*, 2002).

Furthermore the criteria used by previous and the current study to determine recovery differs. Determination of muscle recovery was based entirely on the participant's subjective readiness to resume activities at competitive level in the previous study. Such method is vulnerable to a range of individual response biases

(Furnham *et al.*, 1982). Contrary the current study combined participant's subjective assessment of pain (BPI - SF), standardised clinical assessment and objective hamstring strength (Biodex isokinetic machines) to determine participant's readiness to return to preinjury activities. The criteria used in the current study were based on recent clinical sports medicine recommendations (Croisier *et al.*, 2002; Drezner *et al.*, 2003; Croisier *et al.*, 2004; Brukner & Khan, 2010; Mendiguchia *et al.*, 2010). In addition, to reduce risk of biasness, the physiotherapist who performed all the standardised clinical assessment was blinded to participant's treatment group.

In an abstract presented at the 2nd World Congress on Rehabilitation Medicine, Sanchez *et al.*, (2005) reported full recovery after hamstring and adductor muscles injury was two-times faster in 20 professional athletes treated with preparation rich in growth factors (PRGF). Also the author noted smaller tears progressed well even after a single injection of PRGF. While medium to large size tears however needed two to three applications of PRGF at one-week intervals (Sanchez *et al.*, 2005).

The potential effects of PRGF to hasten muscle healing were also reported in several case reports (Loo *et al.*, 2009; Hamilton *et al.*, 2010; Borrione *et al.*, 2011). Borrione *et al.*, (2011) noted athletes with Grade III muscle strain treated with PRP injection therapy showed faster functional improvement and more complete recovery than those treated conservatively. Similarly, Hamilton *et al.* (2010) successfully treated an athlete with grade-2 semimembranosus muscle injury with a single 3 ml infiltration of platelet-enriched plasma (PEP) under ultrasound guidance. A repeated MRI approximately one week after PEP administration showed mild resolution of oedema. Further, 17 days after injection, the athlete was pain free and had attained full range of movement and could trained at his preinjury level one week later.

The potential effect of PRGF as adjunct treatment to speed up muscle recovery was also reported by Loo *et al.* (2009) in a 35-year-old professional bodybuilder diagnosed with right adductor longus rupture. The athlete was treated with serial PRGF injection once a week for 3 weeks combined with analgesia and physiotherapy. The athlete reported good pain relief and a repeat ultrasound revealed haematoma reorganisation and muscle healing. He returned to competitive training within one week after the third PRGF injections (Loo *et al.*, 2009).

Contrary, Rettig *et al.* (2013) reported no significant difference in the time to return-to-play (recovery) following hamstring injury (grades-1 and -2) between athletes treated either with combined single PRP injection and rehabilitation programme or rehabilitation programme alone. Several differences were identified between the study by Rettig *et al.* (2013) and the current study. First, the design of the study, Rettig *et al.* (2013) conducted a retrospective case control study of ten professional National Football League (NFL) players. Further, they included both grades of (MRI grades-1 and -2) hamstring injuries. Whereas the severity of injury in the current study was more homogeneous as only grade-2 hamstring injury (US grading) was included. Even though both studies used similar commercial kit for PRP preparation, higher amount (0.5 ml for every 1 ml of PRP) of bicarbonate was added to the PRP prior to administration in the previous study. The main function of sodium bicarbonate addition was to buffer the effect of the anticoagulant citrate dextrose solution (ACD - A) used in earlier steps of PRP preparation. Higher amount of sodium bicarbonate might have affected the final pH of the prepared PRP, and influence platelets functions (Han *et al.*, 1974). Also, the author did not classify the PRP used in their study and concurrent use of PRP activating substance was not reported (Rettig *et al.*, 2013).

Another difference between the two studies was the rehabilitation programme prescribed to participants. The rehabilitation programme used by Rettig *et al.*, (2013) focussed on hamstring stretching and strengthening. In addition participants also received other forms of therapy including electrotherapeutic therapy, soft tissue massage, trigger point release and contrast bath types throughout the study. In contrast all participants (PRP and control groups) in the current study received a standard rehabilitation programme. This rehabilitation programme focussed on progressive agility and trunk stabilisation (PATS) exercises. The PATS rehabilitation programme has been shown to be more effective in accelerating hamstring recovery than the program that emphasise on stretching and strengthening. Further the PATS programme also reduces the risk of recurrent hamstring injuries (Sherry & Best, 2004). Also participants in the current study received no other treatment modalities.

Finally, the definition of full return to play (DRP) in the current study was clearly stated. The DRP was based on athlete's having fulfilled several clinical criteria including isokinetic strength assessment. More importantly the assessor was blinded to treatment received by athletes. While the RTP criteria used by Rettig *et al.*, (2013) study was not clearly defined.

5.4.4 PRP effect on symptoms of pain severity and pain interference after hamstring injury

The symptom of pain was assessed using the self-administered brief pain inventory – short form (BPI - SF) questionnaires. The BPI - SF assesses on the severity of pain (Q2 - 6) and effect of pain on daily function (interference) (Q9A - 9G).

Over time participants in both groups showed gradual decline in pain scores. Participants in the PRP group however, displayed significantly lower mean pain severity scores than the control group throughout the study. Unfortunately previous studies on muscle injury only focussed on functional improvement and radiological changes. Often changes in symptoms of pain severity and effect of pain on daily function (interference) were not assessed (Wright - Carpenter *et al.*, 2004a; Rettig *et al.*, 2013). Therefore comparisons can only be made with PRP intervention studies on other chronic conditions such as lateral epicondylitis, Achilles and patellar tendionpathies.

A prospective RCT to study the effect of PRP injection on rotator cuff healing was studied by Randelli *et al.*, (2011). Patients were followed-up regularly until 24 months post-operative. They found patients who received intraoperative PRP injection during arthroscopic rotator cuff repair had lower visual analogue score (VAS) for pain at 3, 7, 14, and 30 days after surgery than controls. Also, patients in PRP group showed significantly higher clinical (functional) outcomes assessed with Constant, strength in external rotation (SER), modified University of California (UCLA), simple shoulder test (SST) at 3 months after surgery. However, the difference between groups was not statistically significant at 6 and 12 months post-operatively.

The effect of PRP was also studied in a double-blind, prospective, multicentre controlled trial of 230 patients with chronic tennis elbow (Mishra *et al.*, 2013). The effect of PRP was assessed using VAS for pain and the Patient-Rated Tennis Elbow Evaluation (PRTEE). The PRTEE is a 15 - item questionnaire designed to measure forearm pain and disability (MacDermid, 2007). Patients randomised into the PRP group reported more improvement in pain scores compared with controls throughout the study. The differences were statistically significant at 8 and 24 weeks of follow-up.

Patients in both groups also showed improvement in functional scores (PRTEE) with time. The PRP group reported more improvement over baseline at 8-, 12-, and 24-week follow-up. The differences in PRTEE scores between groups however were not statistically significant. The findings observed by Mishra *et al.*, (2013) were consistent with those reported by other clinical studies (Mishra & Pavelko, 2006; Peerbooms *et al.*, 2010; Creaney *et al.*, 2011; Thanasas *et al.*, 2011). Similarly, Creaney *et al.*, (2011) demonstrated clinically significant improvement in the mean PRTEE scores among patients with resistant elbow tendinopathies treated with either PRP or autologous blood (ABI) injections. The author further recommended PRP or ABI injections as a second line therapy for patients who are resistant to first-line therapy such as eccentric loading (Creaney *et al.*, 2011).

In a different study de Almeida *et al.* (2012) examine the effect of PRP on patellar tendon healing. Patients were randomised using computer-generated sequence to receive (PRP group) or not (control) PRP in the harvest site during ACL reconstruction. The study found patellar tendon gap was significantly smaller in the PRP group than control. Further, patients in the PRP group had lower VAS pain scores in the immediate post-operative period. Both groups demonstrated clinically significant improvement in knee function scores at 6 months follow-up. The difference between groups however was not statistically significant.

The effect of PRP in improving pain associated with hamstring injury was obvious in the current study. This finding is consistent with those reported by previous studies (Mishra & Pavelko, 2006; MacDermid, 2007; Peerbooms *et al.*, 2010; de Almeida *et al.*, 2012; Randelli *et al.*, 2011; Thanasas *et al.*, 2011). The mean pain severity scores improved with time. Patient in the PRP group had significantly lower

pain severity scores throughout the study. In addition, participants in the PRP groups also showed lower pain interference scores at each time points. The group differences in pain interference scores however were not statistically significant ($p = 0.248$).

The mechanisms upon how PRP influence the symptoms of pain associated with soft tissue injury is uncertain (Peerbooms *et al.*, 2010; Mishra *et al.*, 2013). It has been postulated the bioactive substances within PRP responsible for enhancing soft tissue healing also positively improved function and reduces pain (Mishra *et al.*, 2013). It is also suggested the accelerated haemostasis effect of PRP play a significant role in pain reduction (Gardner *et al.*, 2007).

5.4.5 PRP safety

Autologous PRP used was described as safe treatment alternative for muscle injury (Aspenberg *et al.*, 2004; Chan *et al.*, 2005; Sanchez *et al.*, 2007; deMos *et al.*, 2008; Majewski *et al.*, 2009). Participants in the current study reported no severe adverse effect. Most participants only describe slight pain associated with blood taking (venepuncture) and during PRP injection. This finding is in agreement with those reported by other studies (Wright-Carpenter *et al.*, 2004a; Loo *et al.*, 2009; Hamilton *et al.*, 2010; Mishra *et al.*, 2013).

5.5 Study strength and limitation

One of the strength of the current study is the randomised controlled trial (RCT) design. The RCT design enables vigorous method for assessing cause-effect relation between treatment and outcome (Silverman *et al.*, 1992). In addition the random allocation in RCT ensures no systematic differences exist between intervention groups (Schulz *et al.*, 1995). The present study is the first RCT that evaluated the effectiveness of single PRP injection for treating acute grade-2 hamstring injury.

Homogeneity of the injury severity was achieved by only including participants with grade-2 hamstring injury. Further the severity of hamstring injury was based on combination of physical assessment and ultrasonography appearance of injured hamstring. This is important as grading of muscle injury based solely on physical assessment may underestimate injury severity (Best *et al.*, 1995; Kalimo *et al.*, 1997; Tiidus, 2008).

Currently there are several methods in preparing PRP (plasma based versus buffy-coat based) available in the market. The PRP prepared from the different kits varies in the contents (amount) of platelets, WBC, and growth factors levels (Dohan *et al.*, 2009; Mazzucco *et al.*, 2009; Zumstein *et al.*, 2011; DeLong *et al.*, 2012). The components of PRP produced in the current study was analysed and later classified according to the PAW classification system. Classification of PRP would allow comparisons of PRP efficacy between systems. In addition PRP classification is also useful for replication of study in the future.

Few limitations require to be mentioned in interpreting the findings from this study. First, the current study included only 28 participants with grade-2 hamstring injury. While a larger sample size may provide higher statistical power, shorter study period and limited financial support were the main constraints faced by the researchers. Since annual incidence of hamstring muscle injury in this centre was 35 cases and as the study recruitment period was only a year (later extended another 6 months), the target number of 28 participants was practical (Shariff *et al.*, 2013). The high cost of the PRP commercial kit around RM 3000.00 each, further limits the number of participants included in the study.

Second, while it may appear that all participants were homogeneous in term of injury severity (only grade-2 injury was recruited). The type of muscle involvement varies; most injuries affected the biceps femoris muscles, followed by semimembranosus and semitendinosus. The anatomical, functional and histological (percentages muscle fibres types) difference between these muscles might affect the recovery (Tiidus, 2008).

Another limitation worth mentioning is compliance to the unsupervised daily home rehabilitation exercises. Despite incorporating a simple diary (to record daily exercise session performed) into the PATS rehabilitation booklet provided to each participants. Less than 10 % of participants managed to complete the diary. Most participants stated that they performed their rehabilitation exercise at least once a day on verbal clarification.

Finally, even though the adverse effects of PRP experienced by the participants in the study was considered minor. Shorter duration of study follow-up did not allow for

any long-term potential adverse effects to be assessed. In addition the long-term effects of PRP therapy on hamstring injury recurrence cannot be evaluated.

5.6 Summary of the overall findings

In this study, participants in the PRP group achieved full recovery (DRP) significantly earlier compared with participants in the control group even after adjusting for the other potential covariates. Further PRP therapy was the only significant predictors of DRP. Participants who received PRP injection had a 10.88 times higher chances of reaching earlier DRP compared with control.

Participants in both groups demonstrated gradual improvement in the mean pain severity and mean pain interference scores over time. Statistically significant difference between groups was only demonstrated in the mean pain severity score. Participants in the PRP group had significantly lower mean pain severity score at all time points compared with control. Even though participants in the PRP demonstrated lower mean pain interference score and AKE angle across time, the difference observed did not reached significance level.

In this study the PRP was produced using the buffy-coat methods. The PRP produced contained amount of platelet $> 1250 \times 10^3/\mu\text{L}$ and WBC level above baseline. The current method of PRP injection used no exogenous PRP activation and relies totally on endogenous activation. Therefore the PRP in this study can be classified as P4-x-A based on the PRP PAW classification system.

Chapter 6 General Discussion and Conclusion

6.1 Introduction

The overall purpose of this thesis was to study the effect of autologous platelet-rich plasma (PRP) injection on muscle recovery following injury. The current research comprises of four studies to address the objectives of this thesis.

First, a systematic review to explore the existing literatures on the role of PRP for muscle injury was performed. The results of which among others clearly showed lack of clinical evidence to support PRP use for muscle injury. Hence a randomised controlled trial (RCT) to investigate the effect of PRP for muscle injury among Malaysian athlete was planned.

In planning the RCT it was noted that several key information such as muscle injury prevalence, pattern of muscle injury including injury location and severity, treatment approaches and average recovery time among Malaysian athletes were not available. Thus, a cross-sectional study to explore the patterns of muscle injury and injury management among Malaysian athletes was conducted in the second study. In addition factors that predict early muscle injury recovery among local athletes were also explored.

Hamstring flexibility test is a clinical test commonly used in diagnosing and prognosticating hamstring injury. In this RCT the active knee extension (AKE) test was chosen as an objective assessment of hamstring flexibility. However several existing AKE tests to measure knee range of motion were complicated and require more than a

single person to perform. Therefore, a simpler and reliable tool for hamstring flexibility assessment was designed and constructed for the RCT. The reliability of this active knee extension (AKE) test was the primary objective in the third study.

Even though PRP have gained a lot of attention for treatment of soft tissue over the last two decades, controversies still exist. More conclusive evidence from clinical studies is needed to guide sports medicine practitioners on the role PRP in sports injury. Information on pattern of muscle injury among Malaysian athletes in Study 1 was used to develop the RCT. Furthermore the excellent reliability of the newly designed AKE test supported the test inclusion in the RCT.

The primary objective of the RCT was to evaluate the effect of PRP therapy on hamstring muscle recovery after grade-2 injury.

6.2 Systematic reviews on platelet-rich plasma therapy

A computerized literature search, citation tracking and hand searching for original studies assessing the effect of PRP on skeletal muscle injury were conducted. This systematic review was conducted according to the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) guideline using OvidMEDLINE, PubMed, EMBASE, SPORTDiscus and CINAHL databases. We could not identify any randomised controlled trial (RCT) on platelet-rich plasma (PRP) for acute muscle injury. As such the search criteria was extended to include case control and laboratory studies. Even so only four relevant literatures were identified and reviewed. Few laboratory and animal studies showing positive effects of PRP for acute muscle healing.

The effect of PRP in humans however is not yet known. We concluded that currently there is not enough evidence to support or refute PRP use for acute muscle injury. More studies using robust clinical design were needed. This review further supports our plan to conduct a randomised controlled trial on PRP for acute muscle injury (Study 4).

6.3 Muscle injury patterns and characteristics among Malaysia athletes

From the literature search performed we noticed that several important information on muscle injury in the local setting were not available. The pattern on muscle injury including injury location and severity among Malaysian athletes had not been reported. Furthermore common treatment approaches and average recovery time from muscle injury had not been documented. Hence, a cross-sectional study to explore pattern of muscle injury among Malaysian athletes was performed. It was found that the pattern of muscle injury among Malaysian athletes was comparable to existing literatures. Grade-1 and -2 muscle injuries were the two most common types of injury sustained by athletes. Injury often affects the hamstring muscle group particularly the biceps femoris muscle. Most athletes responded well with conservative treatment that includes short term (less than 5 days) non-steroidal anti-inflammatories combined with electrotherapeutic modality. The median time to recovery among Malaysian athletes is considerably longer than those reported by other studies. Early consultation on injury onset, recurrent muscle injury and female gender were identified as significant predictors of recovery time.

Key information including type of muscle injury, location of injury, length of follow up period, treatment of injury and predictors of DRP gathered from this preliminary study were used to develop the final study (RCT).

6.4 Reliability of the active knee extension (AKE) test

One of the test regularly used to assess hamstring recovery from injury is the hamstring flexibility test. Hamstring flexibility is often used to diagnose and prognosticate hamstring injury. The active knee extension (AKE) test developed by Davies *et al.* (2008) was considered as the gold standard for assessing hamstring flexibility. However, previous AKE tests often require more than one person to perform. Moreover most of the existing AKE tests employ complicated apparatus to help stabilise the pelvis during the test. Therefore a standard method of AKE assessment using a simple and portable pelvic stabilising apparatus was constructed. The reliability of this AKE test was determined in the third study.

Our study showed the AKE test used in this study has excellent interater ($ICC_{2,1}$ values ranges from 0.81 to 0.87 [0.58 – 0.97; 95 % CI]) and test-retest ($ICC_{3,1}$ values ranges from 0.78 to 0.92 [0.32 – 0.97; 95 % CI]) reliabilities. Subsequently, this test was included as one of the assessment method in the final study (RCT).

6.5 Effectiveness of platelet-rich plasma for the treatment of grade-2 hamstring injury (RCT)

Despite receiving much attention for its potential benefits on soft tissue healing, evidence to show PRP effectiveness is scarce. Except for lateral epicondylitis, the clinical evidence to support PRP use in the other conditions was limited to experimental and Grades III – IV human studies. Realising this insufficiency, we conducted a randomised clinical trial to investigate the effect of PRP on hamstring injury.

Twenty-eight athletes with acute (within seven days of injury onset) grade-2 hamstring injury took part in this RCT. Participants were randomised to receive either a hamstring rehabilitation exercise program alone or combined with a single injection of PRP into the injured area.

The effect of PRP on the time taken for the participants to achieved full recovery (duration to return-to-play [DRP]) was evaluated.

The following conclusion could be drawn from this study based on the stated hypothesis;

H1: Patient group receiving PRP combine with hamstring rehabilitation program will demonstrate significantly shorter DRP (faster recovery) compared with group receiving hamstring rehabilitation program alone.

This hypothesis was **accepted**. Participants in the PRP group achieved full recovery significantly ($p = 0.013$) earlier compared with controls (median DRP

21 days versus 34 days). Further PRP therapy was shown to significantly ($p = 0.004$) predict DRP after acute hamstring injury.

H2: Patient group receiving PRP combine with hamstring rehabilitation program will demonstrate significantly faster improvement in pain severity score (BPI - SF) compared with group receiving hamstring rehabilitation program alone.

This hypothesis was **accepted**. Participants in both groups showed gradual improvement in pain severity score over time. Participants in the PRP group had significantly ($p \leq 0.001$) lower (better) pain severity score (as assessed by BPI - SF, Q2 - Q6) at all time points compared with controls.

H3: Patient group receiving PRP combine with hamstring rehabilitation program will demonstrate significantly faster improvement in pain interference (BPI - SF)

This hypothesis was **rejected**. Both groups showed gradual improvement in pain interference score (as assessed by BPI - SF, Q9A - Q9G). Despite the PRP group demonstrated lower pain interference score at all time points, the difference was not statistically significant.

H4: Patient group receiving PRP combine with hamstring rehabilitation program will demonstrate significantly faster improvement in active knee extension (AKE) test compared with group receiving hamstring rehabilitation program alone.

This hypothesis was **rejected**. Both groups showed gradual improvement in hamstring flexibility (assessed using active knee extension [AKE] test). However no significant difference between groups was detected.

6.6 Limitations

1. In our systematic review (Study 1), only English language peer-reviewed articles published until 2012 were included in the data extraction, as such the possibility of selection bias might occur.
2. In the cross sectional survey on pattern of muscle injury among Malaysian athletes (Study 2), 53 % of the injured athletes were lost to follow-up. High loss to follow-up rate might have been responsible for the longer DRP compared with other studies.
3. Small samples size could have explained the wide range of confidence intervals (CI) for point estimates in our active knee extension (AKE) test reliability study (Study 3). Therefore a study with a larger samples size is recommended for future study.
4. Small sample sizes may have limit the statistical power of our randomised controlled trial (Study 4). However, small sample sizes have always been a challenge in RCT. Besides the number of participants in this RCT was calculated based on previous study.

5. To ensure homogeneity of injury severity only acute grade-2 hamstring injury was included in this study. However this may not be fool proof as the hamstring muscle group consists of four individual muscles that differ functionally and physiologically which might affect rate of recovery.
6. For logistical reasons we were unable to design a study with longer follow-up period. Hence any long-term adverse effects related to PRP therapy could not be assessed.

6.7 Clinical implication

Several clinical implications can be drawn from studies performed in this thesis, in relation to PRP used for sports related muscle injuries. First, muscle injury is one of the most common sports related injuries, which mainly affects muscle of the lower limb especially the hamstring muscle groups. Majority of muscle injuries can be classified as moderately severe or grade-2 injury. Further, it is comforting to know that most muscle injuries responded well to conservative (non-surgical) treatment.

Second, muscle injury classification system that is based entirely on physical assessment may misclassify injury severity leading to false negative impression and affect injury management. A comprehensive method of muscle injury classification systems that combined physical assessment and radiological (ultrasound or MRI) is therefore recommended.

Third, active knee extension (AKE) test proposed demonstrated excellent interater and intrarater (test-retest) reliability for assessing hamstring flexibility. Further, with the aid of a simple, portable and inexpensive stabilising apparatus a single person can performed AKE test easily. So AKE test should be incorporated in the clinical assessment of hamstring flexibility.

Fourth, delaying medical consultation by more than one-week places an athlete at higher risk of taking longer time to full recovery. Hence, athlete with suspected muscle injuries must be assessed immediately and treatment commenced within one week if not as soon as possible after injury onset.

Fifth, progressive agility and trunk stabilisation (PATS) exercise program is effective for treating grade-2 hamstring muscle injury. All participants in the control group (PATS program alone) achieved full recovery by 71 days of follow-up period (median = 34.0 ± IQR 37.3 days). Therefore PATS is recommended to be included as part of hamstring muscle injury rehabilitation.

Finally, PRP therapy combined with PATS rehabilitation program was effective for treating acute hamstring injury. The DRP of participants with acute grade-2 hamstring injury was significantly shorter compared with controls. Therefore PRP can be used as a treatment modality for acute muscle injury.

6.8 Recommendations for future research

Based on the encouraging findings of this study, a single PRP intralesional injection has a positive effect on grade-2 hamstring muscle recovery. Multicentre RCT is recommended in future as more participants can be recruited which increases the statistical power of the findings.

This study proved that PRP therapy is safe as participants reported only minor adverse effect (pain during venesection and during PRP administration). However, future study of longer follow-up is much recommended to establish more comprehensive assessments of both short- and long-term effects of PRP therapy. Further, study with longer follow-up period would also allow risk assessments of muscle injuries recurrence to be conducted. This is important, as the risk of recurrence hamstring muscle injury is great.

The protocol for PRP production and the method of delivery including the dose and the frequency of delivery was clearly described. It is reminded that the best dose and frequency of PRP therapy is yet to be determined. Future study using different dosages and frequency of PRP injection/s is needed to find out the best methods of PRP therapy for muscle injury.

6.9 Conclusion

In accordance with the primary and secondary objectives, the following overall conclusions were drawn:

1. Grade-2 hamstring muscle injury is one of the commonest types of injury among Malaysian athletes.
2. Single 3 ml injection of PRP that contained 5-fold increase in platelets (median platelet count = $1297 \times 10^3/\mu\text{L}$, IQR = $51.32 \times 10^3/\mu\text{L}$) combined with a standard rehabilitation program was safe and effective treatment for grade-2 hamstring muscle injury. Participants treated with PRP achieved full recovery (DRP) at a significantly earlier duration than controls.
3. Platelet-rich plasma therapy (PRP) is significant predictor of the DRP of hamstring injuries.
4. Platelet-rich plasma therapy (PRP) significantly lowers participant's pain severity score following hamstring injury.
5. Both PRP therapy and PATS rehabilitation program were equally effective in lowering the pain interference score after hamstring injury.

6.10 Summary

Although muscle injury occurs frequently in sports, the best treatment is not yet known. Muscle injury frequently affected muscle of the lower limb. Hamstring muscle injury was the most frequent type of injury diagnosed among Malaysian athletes. Majority of this injury responded well with conservative treatment, however the DRP among Malaysian athletes was relatively longer compared to other studies.

Sports physician and researchers used several approaches to try and hasten muscle recovery following injury. Unfortunately evidence to support the various treatment approaches is lacking. Over the past few years' researches are turning towards autologous biological substances including platelet-rich plasma (PRP). It is believed that the myriad growth factors and cytokines released from platelets could positively influence muscle cells differentiation and regeneration and enhances recovery. Our systematic review however found limited clinical evidence to support PRP use for muscle injury. Lack of standardisation in PRP extraction techniques and variation in definition of recovery complicate comparisons between studies.

A new set of criteria to determine athlete's readiness to return-to-play based on current guideline was proposed. Athletes required demonstrating improvement in clinical and questionnaire-based assessments before been allowed to resume to pre-injury activities level. Clinical assessments of recovery proposed include return of hamstring flexibility and strength (isokinetic strength test). Hamstring flexibility was assessed using the active knee extension (AKE) test. This test showed excellent interater and intrarater reliability in healthy adults.

The effect of PRP on duration to return-to-play after hamstring injury was studied using a RCT design. Participants randomised in the PRP group attained full recovery at significantly shorter period compared with controls. Further the PRP groups also demonstrated significantly lower pain scores compared with controls at all time points. The PRP therapy significantly predicts DRP even after considering ten other potential predictors.

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SUPPLEMENTARY

LIST OF PUBLICATIONS AND PAPERS PRESENTED

Published articles

1. A Hamid, M.S., Mohamed Ali, M.R., Yusof, A., & George, J. (2012). Platelet-rich plasma (PRP): An adjuvant to hasten muscle recovery. A randomized controlled trial protocol (ISCRTN66528592). *BMC Musculoskeletal Disorders*, 13(1), 138. Doi:10.1186/1471-2474-13-138.
2. Hamid, M. S. A., Ali, M.R.M., & Yusof, A. (2013). Interrater and Intrarater Reliability of the Active Knee Extension (AKE) test among healthy adults. *Journal of Physical Therapy Science*, 25(8), 957-961. Doi:10.1589/jpts.25.957.
3. Shariff, A.H., Ashril, Y., & Razif, M.A. (2013). Pattern of muscle injuries and predictors of return-to-play duration among Malaysian athletes. *Singapore Medical Journal*, 54(10), 587-591. Doi:10.11622/smedj.2013204.
4. Shariff, A.H., Ashril, Y., & Razif, M.A. (2014). Platelet-Rich Plasma (PRP) for Acute Muscle Injury: A Systematic Review. *PLoS ONE* 9(2): e90538. Doi:10.1371/journal.pone.0090538.

Articles in press

1. Shariff, A.H., Ashril, Y., Razif, M.A., George, J., & Lee L.P.C. Platelet-rich plasma injection for the treatment of hamstring injury. A randomized controlled trial. Submitted to *American Journal of Sports Medicine*.

Presentation and conferences

1. Shariff, A.H., Ashril, Y., & Razif, M.A. Skeletal muscle injuries among athletes. *4th AFC Conference on Science and Football Medicine*, Kuala Lumpur, Malaysia 18 – 20 March 2011.
2. Shariff, A.H. PRP therapy: PRP: How does it works? PRP: Does it work? 42nd Malaysian Orthopaedic Association, Pahang, Malaysia, 14 – 17 June 2012.
3. Shariff, A.H., Ashril, Y., Razif, M.A., George, J., & Lee L.P.C. Can the use of platelet-rich plasma (PRP) accelerate muscle recovery? A randomized controlled trial (ISCRTN66528592). *XXXII World Congress of Sports Medicine*, Roma, Italy, 27 -30 September 2012.
4. Shariff, A.H. PRP therapy: Facts and fallacies. *13th Asian Federation of Sports Medicine Congress*, Kuala Lumpur, Malaysia 25 – 28 September 2013.

Appendix A - OvidMEDLINE Search Strategy

Search Journals Books My Workspace Primal Pictures EBP Tools

Search History saved as "SR PRP 11072013"

Basic Search | Find Citation | Search Tools | Search Fields | Advanced Search | Multi-Field Search

1 Resource selected | [Hide](#) | [Change](#)
 Ovid MEDLINE(R) 1946 to Present with Daily Update

Enter keyword or phrase (* or \$ for truncation) Keyword Author Title Journal Search

Limits (Click to close) Map Term to Subject Heading

Abstracts Structured Abstracts English Language
 Article Reviews (ACP Journal Club) Review Articles Article Reviews (DARE)
 Humans Topic Reviews (Cochrane) Core Clinical Journals (AIM)
 Latest Update Pharmacologic Actions

Publication Year - -

[Additional Limits](#) [Edit Limits](#)

Search History (14 searches) (Click to close) [View Saved](#)

<input type="checkbox"/>	#	Searches	Results	Search Type	Actions
<input type="checkbox"/>	14	12 and 13	5	Advanced	Display More >>
<input type="checkbox"/>	13	Muscle injury.mp.	1738	Advanced	Display More >>
<input type="checkbox"/>	12	1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11	2417	Advanced	Display More >>
<input type="checkbox"/>	11	Platelet leucocyte rich plasma.mp.	0	Advanced	Delete More >>
<input type="checkbox"/>	10	Leucocyte platelet rich plasma.mp.	1	Advanced	Display More >>
<input type="checkbox"/>	9	Platelet lysate.mp.	281	Advanced	Display More >>
<input type="checkbox"/>	8	Platelet releasate.mp.	101	Advanced	Display More >>
<input type="checkbox"/>	7	Preparation rich in growth factors.mp.	14	Advanced	Display More >>
<input type="checkbox"/>	6	Autologous growth factors.mp.	52	Advanced	Display More >>
<input type="checkbox"/>	5	Platelet gel.mp.	173	Advanced	Display More >>
<input type="checkbox"/>	4	Platelet concentrate.mp.	602	Advanced	Display More >>
<input type="checkbox"/>	3	Autologous conditioned serum.mp.	22	Advanced	Display More >>
<input type="checkbox"/>	2	Platelet rich fibrin matrix.mp.	16	Advanced	Display More >>
<input type="checkbox"/>	1	exp Platelet-Rich Plasma/	1338	Advanced	Display More >>

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Appendix B - PubMed Search Strategy

Search Details

Query Translation:

```

((((((((("platelet-rich plasma"[MeSH Terms] OR
("platelet-rich"[All Fields] AND "plasma"[All Fields]) OR
"platelet-rich plasma"[All Fields] OR ("platelet"[All
Fields] AND "rich"[All Fields] AND "plasma"[All Fields])
OR "platelet rich plasma"[All Fields]) OR (("blood
platelets"[MeSH Terms] OR ("blood"[All Fields] AND
"platelets"[All Fields]) OR "blood platelets"[All Fields]
OR "platelet"[All Fields]) AND rich[All Fields] AND
("fibrin"[MeSH Terms] OR "fibrin"[All Fields]) AND
("Matrix"[Journal] OR "Matrix Suppl"[Journal] OR "matrix"

```

Search URL

Result:

[176](#)

Translations:

Platelet rich plasma	"platelet-rich plasma"[MeSH Terms] OR ("platelet-rich"[All Fields] AND "plasma"[All Fields]) OR "platelet-rich plasma"[All Fields] OR ("platelet"[All Fields] AND "rich"[All Fields] AND "plasma"[All Fields]) OR "platelet rich plasma"[All Fields]
Platelet	"blood platelets"[MeSH Terms] OR ("blood"[All Fields] AND "platelets"[All Fields]) OR "blood platelets"[All Fields] OR "platelet"[All Fields]
fibrin	"fibrin"[MeSH Terms] OR "fibrin"[All Fields]
matrix	"Matrix"[Journal] OR "Matrix Suppl"[Journal] OR "matrix"[All Fields]
conditioned	"conditioning (psychology)"[MeSH Terms] OR ("conditioning"[All Fields] AND " (psychology)"[All Fields]) OR "conditioning (psychology)"[All Fields] OR "conditioned"[All Fields]
serum	"serum"[MeSH Terms] OR "serum"[All Fields]
growth factors	"intercellular signaling peptides and proteins"[MeSH Terms] OR ("intercellular"[All Fields] AND "signaling"[All Fields] AND "peptides"[All Fields] AND "proteins"[All Fields]) OR "intercellular signaling peptides and proteins"[All Fields] OR ("growth"[All Fields] AND "factors"[All Fields]) OR "growth factors"[All Fields]
rich in	rich in[Author]
Leucocyte	"leukocytes"[MeSH Terms] OR "leukocytes"[All Fields] OR "leucocyte"[All Fields]
plasma	"plasma"[MeSH Terms] OR "plasma"[All Fields]
Muscle	"muscles"[MeSH Terms] OR "muscles"[All Fields] OR "muscle"[All Fields]
injury	"wounds and injuries"[MeSH Terms] OR ("wounds"[All Fields] AND "injuries"[All Fields]) OR "wounds and injuries"[All Fields] OR "injury"[All Fields]

Database:
PubMed
User query:
((((((((((((Platelet rich plasma) OR Platelet rich fibrin matrix) OR Autologous conditioned serum) OR Platelet concentrate) OR Platelet gel) OR Autologous growth factors) OR Preparation rich in growth factors) OR Platelet releasate) OR Platelet lysate) OR Leucocyte platelet rich plasma) OR Platelet leucocyte rich plasma)) AND Muscle injury AND ("0001/01/01"[PDat] : "2012/12/31"[PDat]))

Appendix C - EMBASE Search Strategy



Embase Session Results

No.	Query	Results
#15	#12 AND #13 AND ([article]/lim OR [review]/lim) AND [english]/lim AND [<1966-2012]/py	487
#14	#12 AND #13	661
#13	'muscle'/exp OR muscle AND ('injury'/exp OR injury) AND [<1966-2012]/py	199,141
#12	#1 OR #2 OR #3 OR #4 OR #5 OR #6 OR #7 OR #8 OR #9 OR #10 OR #11	32,692
#11	'platelet'/exp OR platelet AND ('leucocyte'/exp OR leucocyte) AND rich AND ('plasma'/exp OR plasma) AND [<1966-2012]/py	533
#10	'leucocyte'/exp OR leucocyte AND ('platelet'/exp OR platelet) AND rich AND ('plasma'/exp OR plasma) AND [<1966-2012]/py	533
#9	'platelet'/exp OR platelet AND ('lysate'/exp OR lysate) AND [<1966-2012]/py	870
#8	'platelet'/exp OR platelet AND releasate AND [<1966-2012]/py	165
#7	preparation AND rich AND in AND ('growth'/exp OR growth) AND factors AND [<1966-2012]/py	1,015
#6	autologous AND ('growth'/exp OR growth) AND factors AND [<1966-2012]/py	14,041
#5	'platelet'/exp OR platelet AND ('gel'/exp OR gel) AND [<1966-2012]/py	5,511
#4	'platelet'/exp OR platelet AND concentrate AND [<1966-2012]/py	4,311
#3	autologous AND conditioned AND ('serum'/exp OR serum) AND [<1966-2012]/py	109
#2	'platelet'/exp OR platelet AND rich AND ('fibrin'/exp OR fibrin) AND matrix AND [<1966-2012]/py	137
#1	'platelet'/exp OR platelet AND rich AND ('plasma'/exp OR plasma) AND [<1966-2012]/py	9,164



ELSEVIER

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Appendix D - SPORTDiscus Search Strategy



Wednesday, July 10, 2013 10:24:58 PM

#	Query	Limiters/Expanders	Last Run Via	Results
S18	(Muscle injury) AND (S15 AND S16)	Limiters - Published Date: -20121231; Language: English; Publication Type: Journal Article Search modes - Boolean/Phrase	Interface - EBSCOhost Search Screen - Advanced Search Database - SPORTDiscus with Full Text	345
S17	(Muscle injury) AND (S15 AND S16)	Search modes - Boolean/Phrase	Interface - EBSCOhost Search Screen - Basic Search Database - SPORTDiscus with Full Text	412
S16	Muscle injury	Search modes - Boolean/Phrase	Interface - EBSCOhost Search Screen - Basic Search Database - SPORTDiscus with Full Text	2,460
S15	S1 OR S2 OR S3 OR S4 OR S5 OR S6 OR S7 OR S8 OR S9 OR S10 OR S11 OR S12 OR S13 OR S14	Search modes - Boolean/Phrase	Interface - EBSCOhost Search Screen - Basic Search Database - SPORTDiscus with Full Text	112,254
S14	Platelet leucocyte rich plasma	Search modes - SmartText Searching	Interface - EBSCOhost Search Screen - Basic Search Database - SPORTDiscus with Full Text	49
S13	Platelet leucocyte rich plasma	Search modes - Boolean/Phrase	Interface - EBSCOhost Search Screen - Basic Search Database - SPORTDiscus with Full Text	0
S12	Leucocyte platelet rich plasma	Search modes - SmartText Searching	Interface - EBSCOhost Search Screen - Basic	49

			Search Database - SPORTDiscus with Full Text	
S11	Leucocyte platelet rich plasma	Search modes - Boolean/Phrase	Interface - EBSCOhost Search Screen - Basic Search Database - SPORTDiscus with Full Text	0
S10	Platelet lysate	Search modes - Boolean/Phrase	Interface - EBSCOhost Search Screen - Basic Search Database - SPORTDiscus with Full Text	1
S9	Platelet releasate	Search modes - Boolean/Phrase	Interface - EBSCOhost Search Screen - Basic Search Database - SPORTDiscus with Full Text	5
S8	Preparation rich in growth factors	Search modes - SmartText Searching	Interface - EBSCOhost Search Screen - Basic Search Database - SPORTDiscus with Full Text	575
S7	Preparation rich in growth factors	Search modes - Boolean/Phrase	Interface - EBSCOhost Search Screen - Basic Search Database - SPORTDiscus with Full Text	0
S6	Autologous growth factors	Search modes - Boolean/Phrase	Interface - EBSCOhost Search Screen - Basic Search Database - SPORTDiscus with Full Text	13
S5	Platelet gel	Search modes - Boolean/Phrase	Interface - EBSCOhost Search Screen - Basic Search Database - SPORTDiscus with Full Text	14
S4	Platelet concentrate	Search modes - Boolean/Phrase	Interface - EBSCOhost Search Screen - Basic	17

			Search Database - SPORTDiscus with Full Text	
S3	Autologous conditioned serum	Search modes - Boolean/Phrase	Interface - EBSCOhost Search Screen - Basic Search Database - SPORTDiscus with Full Text	5
S2	Platelet rich fibrin matrix	Search modes - Boolean/Phrase	Interface - EBSCOhost Search Screen - Basic Search Database - SPORTDiscus with Full Text	6
S1	Platelet rich plasma	Search modes - Boolean/Phrase	Interface - EBSCOhost Search Screen - Basic Search Database - SPORTDiscus with Full Text	228

Appendix E - CINAHL Search Strategy



Wednesday, July 10, 2013 9:17:08 PM

#	Query	Limiters/Expanders	Last Run Via	Results
S14	S12 AND S13	Search modes - Boolean/Phrase	Interface - EBSCOhost Search Screen - Advanced Search Database - CINAHL Plus	3
S13	S1 OR S2 OR S3 OR S4 OR S5 OR S6 OR S7 OR S8 OR S9 OR S10 OR S11	Search modes - Boolean/Phrase	Interface - EBSCOhost Search Screen - Advanced Search Database - CINAHL Plus	209
S12	"Muscle injury"	Search modes - Boolean/Phrase	Interface - EBSCOhost Search Screen - Advanced Search Database - CINAHL Plus	319
S11	"Platelet leucocyte rich plasma"	Search modes - Boolean/Phrase	Interface - EBSCOhost Search Screen - Advanced Search Database - CINAHL Plus	0
S10	"Leucocyte platelet rich plasma"	Search modes - Boolean/Phrase	Interface - EBSCOhost Search Screen - Advanced Search Database - CINAHL Plus	0
S9	"Platelet lysate"	Search modes - Boolean/Phrase	Interface - EBSCOhost Search Screen - Advanced Search Database - CINAHL Plus	6
S8	"Platelet releasate"	Search modes - Boolean/Phrase	Interface - EBSCOhost Search Screen - Advanced Search Database - CINAHL Plus	9
S7	"Preparation rich in growth factors"	Search modes - Boolean/Phrase	Interface - EBSCOhost Search Screen - Advanced Search Database - CINAHL Plus	4
S6	"Autologous growth	Search modes -	Interface - EBSCOhost	16

Pattern of muscle injuries and predictors of return-to-play duration among Malaysian athletes

Hamid A Mohamad Shariff¹, MBBS, MMed, Yusof Ashril², MSES, PhD, Mohamed Ali Mohamed Razif³, MBBCh BAO, FRCSE

INTRODUCTION The purpose of this study was to investigate the pattern of muscle injuries and the factors that predict the return-to-play duration among Malaysian athletes.

METHODS This is a retrospective review of the case notes of athletes who attended the National Sports Institute Clinic in Malaysia. The medical records of athletes with muscle injury, diagnosed on clinical assessment and confirmed by diagnostic ultrasonography, were included for final analysis.

RESULTS From June 2006 to December 2009, 397 cases of muscle injury were diagnosed among 360 athletes. The median age of the athletes with muscle injuries was 20.0 years. Muscle injuries were mostly diagnosed among national-level athletes and frequently involved the lower limb, specifically the hamstring muscle group. Nearly all of the athletes (99.2%) were treated conservatively. The median return-to-play duration was 7.4 weeks. Athletes who waited more than one week before seeking medical attention, those with recurrent muscle injuries and female athletes were significantly more likely ($p < 0.05$) to take more than six weeks before returning to the sport.

CONCLUSION Grade 2 lower limb muscle injury was commonly diagnosed among national-level athletes in this study. The frequency of weekly physiotherapy sessions did not affect the return-to-play duration. Factors such as initial consultation at more than one week post injury, recurrent muscle injuries and female gender were significant predictors of return-to-play duration among Malaysian athletes. These predictive factors should be kept in mind during clinical assessment so as to aid in prognosticating recovery after muscle injury.

Keywords: athletes, Malaysia, muscle injury, musculoskeletal, return to play

INTRODUCTION

Muscle injury is one of the most common injuries affecting athletes.⁽¹⁾ It accounts for up to 28% of injuries in sports events.⁽²⁾ Contusion and strain are two common causes of muscle injuries. Muscle strain often occurs during sprinting or jumping, when the muscle is under tension while lengthening (eccentric contraction).⁽³⁾ Earlier studies have identified several factors that predispose one to muscle injury, including a history of muscle strain, increasing age and leg dominance.⁽⁴⁻⁶⁾ Muscle injuries often occur at the muscle-tendon (myotendinous) junction of muscles that span across two joints, such as the rectus femoris, semitendinosus and gastrocnemius. Diagnosis and grading of muscle injuries are usually done through clinical assessments.⁽²⁾ Ultrasonography (US) is recommended for localising injury and characterising severity of injury.⁽⁷⁾

In professional sports, muscle injuries can lead to significant pain and disability, resulting in time away from participation (training and competition) and high medical costs.⁽⁸⁾ Athletes and coaches are often concerned about the time to full recovery and return-to-play (RTP). Unfortunately, issues on duration to return-to-play (DRP) are often not directly discussed during consultation with the medical team.⁽⁹⁾ Predicting DRP is not only important for planning the rehabilitation programme, but also for enabling the coaching staff to restructure the team for competitions.

Recent studies have identified several factors that may help in estimating DRP.^(6,10,11) An observational study of 59 players

from ten Victoria-based Australian Football League clubs showed that the time taken for an athlete to walk pain-free after a hamstring injury was a significant predictor of time to RTP.⁽¹²⁾ That study, however, did not discuss the severity of the muscle injury sustained and give details of the rehabilitation programme. In addition, a prospective study among athletes with grade 1-4 hamstring injuries suggested that active knee range of motion deficit was an objective and accurate measurement in predicting DRP.⁽¹¹⁾

Information on the pattern of muscle injuries among Malaysian athletes is limited.⁽¹³⁾ Differences in physical build, climate, dietary intake and training regimen between Malaysian and foreign athletes may affect muscle injury pattern. Identifying the pattern of muscle injuries, including the magnitude of the problem, is an important initial step in injury prevention programmes.⁽¹⁴⁾ However, there is no information on the current management of muscle injuries and the effectiveness of treatment (e.g. DRP) among Malaysian athletes. Hence, the aim of this study was to examine the pattern of muscle injuries and explore the predictors of DRP among Malaysian athletes.

METHODS

A retrospective study using data extracted from athletes' medical records was conducted at the National Sports Institute Clinic, Kuala Lumpur, Malaysia. A structured form was prepared to record the sociodemographic background of the athletes and clinical information of their injury. All of the athletes were

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under the care of sports medicine specialists. A visiting musculoskeletal radiologist with 14 years of experience performed all of the US assessments. US was conducted using an ACUSON Antares™ Ultrasound System (Siemens AG, Erlangen, Germany) with a 4-cm linear transducer set at 10 MHz. Severity of muscle injury was graded based on the US classification described by Peetrans.⁽¹⁵⁾ The University of Malaya Medical Centre Ethics Committee approved the study.

The US registration records from June 2006 to December 2009 were reviewed. The medical records of athletes diagnosed with muscle injuries on US were evaluated. Information on the athlete's age, gender, playing level (school, club, state or national) and type of sport was collected. Information on injuries, including date of injury, date of first consultation, event leading to injury (training session or competition), injury severity and date of RTP, was also recorded. Pattern of muscle injuries including injury severity, region of injury and event leading to injury, was reviewed. DRP following muscle injury was recorded. DRP was defined as the difference (in weeks) between the date on which the attending doctor allowed full participation in sports and the date of onset of injury.

Data was analysed using the Statistical Package for the Social Sciences version 19.0 (SPSS Inc, Chicago, IL, USA). Data was described descriptively and a normality test was performed using the Shapiro-Wilk test. DRP < 6 weeks was used as the cutoff value for adequacy of DRP – this definition was based on a recent systematic review of muscle injury by Prior et al.⁽¹⁶⁾ and supported by the results of another study, where athletes whose DRP was > 6 weeks after muscle injury were found to have a significantly lower chance (3.1%) of sustaining repeat injury compared to those who resumed sports at 2 weeks (8.1%) or 3 weeks (6.8%) post injury.⁽¹⁷⁾

The associations between DRP and gender; age group (≥ 18 vs. < 18 years); and duration before first consultation (≤ 1 vs. > 1 week) were assessed using Mann-Whitney *U* test. Kruskal-Wallis test was used to determine the association between DRP and type of sport; frequency of weekly physiotherapy sessions; playing level (school, state, national or others); new vs. recurrent injury; region of injury (upper limb lower limb or truncal muscles); and US grading of injury (grade 0–3). Stepwise logistic regression analysis was conducted to identify the predictors of DRP. Variables < 0.25 on univariate testing were included in the multivariate logistic regression model, as recommended by previous researchers.^(18,19) Adjusted odds ratios (ORs) and 95% confidence intervals (CIs) of ORs were calculated, with the significance level set at $p < 0.05$.

RESULTS

A total of 562 medical records of athletes with suspected muscle injuries were screened. Of these, 202 medical records were excluded from analysis for the following reasons: incomplete medical information ($n = 25$); missing US report ($n = 75$); and injuries involving structures other than muscles

(i.e. ligaments and tendons) ($n = 102$). Only 360 medical records (237 men and 123 women) were eventually analysed. Among these 360 athletes, 397 muscle injuries were diagnosed. The majority (60.6%) of muscle injuries were classified as a new injury. The median age of the athletes at the time of injury was 20.0 (interquartile range [IQR] 6.0) years.

Most injuries (90.0%) occurred among national-level athletes participating in various sports – track and field (30.3%), field hockey (17.8%), racket sports (11.4%), martial arts (6.7%), soccer (5.3%), weightlifting (5.0%), gymnastics (4.7%), swimming (4.2%) and others (14.4%). Injuries were frequently diagnosed in muscles of the lower limb, especially the hamstring and adductors muscle groups (Table I). Athletes with a primary complaint of lower back pain ($n = 29$) were clinically assessed, and plain radiography of the lumbosacral region was performed to rule out any bony pathology. Magnetic resonance (MR) imaging was performed in three cases, as the clinical assessments led to suspicions of neurological involvement; this was in accordance with the clinical practice guidelines by the American College of Physicians and American Pain Society.⁽²⁰⁾ MR imaging was unremarkable in two athletes, while a sacrospinalis tear was demonstrated in the third. All athletes subsequently underwent US assessment of the lumbosacral region using a simple grading system for severity.^(15,21)

The median time to first consultation was 7.0 (IQR 12.0) days after injury, and the median time before US evaluation was 17.0 (IQR 29.0) days. Out of a total of 397 muscle injuries, grade 2 muscle injury was diagnosed in 368 (92.7%) athletes (grade 1 in 26 (6.5%) and grade 3 in 3 (0.8%)). Most (93.9%) injuries occurred while the athletes were performing sports-related activities, with the majority (82.5%) occurring during training or practice sessions. A large number of track and field athletes (69.7%) sustained muscle injuries during sprinting; the injuries occurred less frequently during jumping (13.8%) and weight training (5.5%). Similar results were observed among the field hockey athletes, whose muscle injuries occurred primarily during sprinting (75.0%). In contrast, approximately 40% of the racket sport athletes sustained injury during jumping activities (e.g. jumping smash).

Nearly all athletes (99.2%) were treated conservatively (i.e. nonsurgical intervention). Most (66.4%) received a short course (< 1 week) of analgesia (e.g. nonsteroidal anti-inflammatory drugs), combined with at least one form of electrotherapeutic modality. Only three athletes with complete muscle rupture underwent surgical intervention. Documented dates of RTP were available for only 168 athletes, while that for the remaining 192 athletes were unavailable as they were lost to follow-up. Approximately 40% ($n = 67$) of athletes were allowed full RTP within six weeks after injury. DRP ranged from 1 to 72 weeks, with a median of 7.4 (IQR 8.5) weeks. No significant differences in DRP across the type of sport ($H(26) = 25.32$, $p = 0.50$) and frequency of weekly physiotherapy session

Table I. Muscle injuries (n = 397) according to body region.

Body region/muscle group	No. (%)
Lower limb	
Hamstring	145 (36.5)
Adductor	43 (10.8)
Calf	49 (12.3)
Quadriceps	31 (7.8)
Others*	11 (2.8)
Upper limb	
Deltoid	15 (3.8)
Biceps	6 (1.5)
Triceps	4 (1.0)
Rotator cuff	15 (3.8)
Others†	35 (8.8)
Abdomen	
Rectus abdominis	12 (3.0)
Others‡	2 (0.5)
Back	
Muscles of the back§	29 (7.3)

*Anterior tibialis, posterior tibialis, peroneal muscles; †Pectoralis, rhomboids, small muscles of the hand; ‡External obliques, transversus abdominis; §Erector spinae, quadratus lumborum

($H(3) = 0.44, p = 0.93$) were found. In most cases, a physiotherapy session typically started with range of motion exercises (stretching), followed by progressive muscle strengthening activities and cryotherapy at the end of the session. In addition, the treating physiotherapists of ten incorporated various electrotherapeutic modalities during these sessions. Further analysis revealed that athletes who were lost to follow-up were significantly older ($U = 13197, z = -3, p = 0.03$).

A moderate, significantly positive relationship was found between time to first consultation and DRP ($U = 2023, p < 0.001$). A significant relationship between DRP and muscle region (limb versus trunkal) was also demonstrated ($\chi^2 = 6.8, p = 0.04$) (Table II).

Gender, time to first consultation, injury type (new vs recurrent), injury severity, number of injured muscles and side of injury were factors that met the criteria for inclusion in the multivariate model. Delay in first consultation of more than one week, recurrent muscle injuries and female gender were identified as predictors of DRP of > six weeks (Table III). No interactions were noted between the predictors.

All other variables were eliminated by the stepwise procedure.

DISCUSSION

In this study, grade 2 muscle injury was the most common form of injury diagnosed among national-level athletes. We also found that the muscle injuries often affected the lower limb, especially the hamstring muscle groups. Similar findings were also noted in a study conducted among intercollegiate hockey players.⁽²²⁾ Furthermore, lower extremity muscle strain was the most frequent injury diagnosed at the 2007

International Association of Athletics Federations World Athletics Championships.⁽²³⁾ Excessive tensile force on muscle fibres during fast bursts of speed has been suggested to be the main cause of muscle injury. Such an injury predominantly affects muscles that span two joints, such as the biceps

Table II. Factors associated with duration to return-to-play among patients with documented date of return-to-play (n = 168).

Factor	No. (%)	U*/ χ^2 †	p-value
Gender*			
Male	107 (63.7)	2,898	0.23
Female	61 (36.3)		
Age group (yrs)*			
< 18	52 (31.0)	2,730	0.32
≥ 18	116 (69.0)		
Time to first consultation (wk)*			
≤ 1	91 (54.2)	2,023	< 0.001
> 1	77 (45.8)		
Injury type*			
New	104 (61.9)	2,908	0.17
Recurrent	64 (38.1)		
Injurious event*			
Traumatic	10 (6.0)	646	0.34
Nontraumatic	158 (94.0)		
Injury grade (via US)*			
Grade 1	12 (7.1)	681	0.12
Grade 2	156 (92.9)		
No. of muscles injured*			
1	147 (87.5)	1,144	0.07
2	21 (12.5)		
Activity leading to injury†			
Training	140 (83.3)	0.69	0.71
Competition	26 (15.5)		
Others	2 (1.2)		
Affected side†			
Right	73 (43.5)	3.50	0.18
Left	84 (50.0)		
Bilateral	11 (6.5)		
Affected region†			
Upper limb	31 (18.5)	6.8	0.04
Lower limb	121 (72.0)		
Truncal	16 (9.5)		
Level of play†			
National	155 (92.3)	0.24	0.97
State	3 (1.8)		
School	7 (4.2)		
Others	3 (1.8)		
Physiotherapy session†			
Daily	119 (70.8)	0.60	0.90
Weekly	22 (13.1)		
Twice a week	21 (12.5)		
Thrice a week	6 (3.6)		

*Mann-Whitney U test †Kruskal-Wallis test
US: ultrasonography

femoris, semimembranosus, semitendinosus, gastrocnemius and rectus femoris.⁽²⁴⁾

The pattern of muscle injuries among Malaysian athletes is comparable to that reported in other studies.^(22,23) However, the median DRP of 7.4 (IQR 8.5) weeks among the athletes in this study is longer than that in earlier studies.^(6,25) A study conducted by Malliaropoulos et al in Greece reported a mean time loss from training and competition of 14.7 days among elite-level track-and-field athletes.⁽²⁵⁾ This shorter DRP could be explained by the higher proportion (64.5%) of low-grade muscle injury (grade 1) in Malliaropoulos et al's study.⁽²⁵⁾ Another study on hamstring injury among Australian footballers reported a

Table III. Predictors of duration to full return-to-play of more than six weeks after muscle injury.

Determinant	B (SE)	p-value	Adjusted OR	95% CI
Time to consultation > 1 wk	1.29 (0.32)	< 0.001	3.63	1.80–7.30
Recurrent injuries	0.76 (0.37)	0.038	2.14	1.04–4.38
Female gender	0.74 (0.37)	0.048	2.09	1.01–4.34

SE: standard error; OR: odds ratio; CI: confidence interval

median time of 26 days before the injured athletes returned to competition.⁽⁶⁾ However, the authors of that study did not describe the severity of muscle injury suffered by the athletes.

The present study found that athletes who delayed medical consultation by more than one week (after the onset of injury) had a significantly higher likelihood of taking more than six weeks to recover compared to those who sought treatment earlier. In a study by Askling et al, a median DRP of 31 weeks was reported among 30 elite-level Swedish athletes who presented 12 weeks after sustaining hamstring injuries, with 47% of the athletes making the decision to retire after a follow-up period of 63 weeks.⁽²⁶⁾ Early management of muscle injuries was shown to affect the extent of injury and the amount of scar tissue formed, which influences the duration of muscle healing.^(1,27,28) Early immobilisation (less than one week) has been shown to limit the size of connective tissue (scar) formed within the site of injury in rat gastrocnemius muscle.⁽²⁷⁾ In addition, early use of cryotherapy hastens regeneration and has been associated with significantly smaller haematomas, less inflammation and less tissue necrosis.^(1,29) Educating athletes on the importance of early medical consultation following injury and improving medical accessibility (e.g. having readily available onsite medical team support) may help to shorten the duration between the time of injury and the first consultation, which may in turn positively affect DRP.

History of previous muscle injury is one of the most important risk factors for subsequent muscle injury. Athletes with a history of muscle strain are two to six times more likely to experience recurrent strains.^(5,12) Some possible explanations for this observation include reduced tensile strength of scar tissue, decreased muscle strength, diminished muscle flexibility, as well as possible adaptive changes in the biomechanics and motor patterns of movements after injury.⁽²²⁾ Moreover, the current study found that athletes with a history of muscle injury were more likely to take more than six weeks to return to play than those with a new injury. A significantly longer recovery time was observed among National Football League athletes with hamstring re-injuries (56 days) compared to those with first-time hamstring injury (16.5 days).⁽³⁰⁾ In a laboratory study, the lack of activated myogenic satellite cells within the fibrotic discontinuity area (scar tissue) was suggested to be the phenomenon responsible for the delay in healing of recurrent muscle injuries.⁽³¹⁾

Female athletes with muscle injuries in the present study took a longer time (more than six weeks) to recover compared

to male athletes. While the reason for this is unclear, it could be due to the difference in the circulating sex hormones between males and females. It has been found that there are significantly fewer inflammatory cells (neutrophils and granulocytes) infiltrating the vastus lateralis muscle of female university students after a standardised pain-inducing eccentric exercise compared to males.⁽³²⁾ Infiltration of the muscle with leucocytes and macrophages is important for satellite cell activation and initiation of muscle regeneration. Therefore, the oestrogen-attenuating effects on leucocyte infiltration may delay important stages in muscle recovery.^(33–35) Bell et al demonstrated the presence of significant hamstring muscle extensibility changes throughout the different phases of the menstrual cycle,⁽³⁶⁾ which may increase the likelihood of sustaining acute hamstring injury, as was demonstrated in Watsford et al's study.⁽³⁷⁾

Interestingly, the frequency of physiotherapy sessions did not affect the DRP in our study. Contrary to our findings, Malliaropoulos et al demonstrated that athletes diagnosed with hamstring injury who underwent a more intensive stretching programme had a statistically significant shorter time of recovery.⁽³⁸⁾ It should be noted that an optimal method for the treatment of muscle injury has yet to be identified.⁽³⁹⁾ Consequently, the treating physiotherapists in our study used different treatment protocols based on anecdotal reports and personal experience. The treatment protocols differed with respect to the type and sequence of activities prescribed, duration of the treatment session and the use of electrotherapeutic modalities, further complicating comparisons among the different regimens.

The high loss to follow-up rate of about 53% is of major concern, especially when it involves national-level athletes. It is, however, possible that the athletes who defaulted had recovered from their injuries, retired or sought treatment elsewhere. A prospective study to explore the factors associated with loss to follow-up is currently underway. It should also be noted that the reliability and accuracy of US in diagnosing acute back muscle strain is still not documented.⁽⁴⁰⁾ Hence, it is possible that other conditions such as abnormalities of the intervertebral discs and facet joints were missed or overlooked in these athletes. This study has demonstrated that the timing of first consultation, past history or recurrence of muscle injury and female gender were useful factors in predicting the DRP among Malaysian athletes.

In conclusion, grade 2 lower limb muscle injury was the most common type of injury diagnosed among the national-

level athletes in our study. The athletes with muscle injuries were conservatively treated, with a median DRP of 7.4 weeks. This study has identified several predictors of DRP of more than six weeks post muscle injury – time to first consultation of more than one week, recurrent muscle injury and female gender. These factors are important and should therefore be considered during early assessments of muscle injuries. Strategic steps need to be taken to ensure early consultation and treatment as soon as an injury occurs. It is important to increase awareness of the factors associated with extended DRP among athletes, coaches and practitioners involved in the care of athletes. A prospective study with a larger sample size could better show the associations between clinical assessments and outcomes, including potential variables with small to moderate effects. Such a study is being planned for the near future.

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Appendix G - Clinical Research Form

PATTERN OF MUSCLE INJURIES AMONG ATHLETES

No:

Date: _____

1 RN _____

2 DOB _____

3 Sex: Male
Female

4 Sports: _____

5 Level National
State
School
Others

Specify: _____

6 Injury date: _____

7 Injury New
Recurrent

8 Region: Upper limb
Lower limb
Abdomen
Back
Others

Go to 9
Go to 10
Go to 11
Go to 12

Specify: _____

9 Muscle groups
Shoulder
Elbow flexor
Elbow extensor
Wrist flexor
Wrist extensor
Fingers
Others

Specify: _____

10 Muscle groups
Hip flexor
Hip extensor
Knee flexor
Knee extensor
Calf
Foot
Others

Specify: _____

11 Muscle groups
Upper abdomen
Lower abdomen
Others

Specify: _____

PATTERN OF MUSCLE INJURIES AMONG ATHLETES

12	Muscle group	Upper back	<input type="checkbox"/>	Specify: _____	<input type="checkbox"/>
		Lower back	<input type="checkbox"/>		
		Others	<input type="checkbox"/>		
13	Injurious event	Traumatic	<input type="checkbox"/>	Specify: _____	<input type="checkbox"/>
		Activity induced	<input type="checkbox"/>		
		Others	<input type="checkbox"/>		
14	Injury sustained	Training	<input type="checkbox"/>	Specify: _____	<input type="checkbox"/>
		Competition	<input type="checkbox"/>		
		Others	<input type="checkbox"/>		
15	Grading of injury	Clinical	Grade 1	<input type="checkbox"/>	<input type="checkbox"/>
			Grade 2	<input type="checkbox"/>	
			Grade 3	<input type="checkbox"/>	
			Unrecorded	<input type="checkbox"/>	
		Ultrasound	Grade 1	<input type="checkbox"/>	<input type="checkbox"/>
			Grade 2	<input type="checkbox"/>	
			Grade 3	<input type="checkbox"/>	
			Unrecorded	<input type="checkbox"/>	
16	Management	Conservative	<input type="checkbox"/>	Go to 17	<input type="checkbox"/>
		Surgical	<input type="checkbox"/>		
17	Conservative management	Physio.type	US	<input type="checkbox"/>	<input type="checkbox"/>
			TENS	<input type="checkbox"/>	
			IT	<input type="checkbox"/>	
			Others	<input type="checkbox"/> Specify _____	
		Frequency/week	Once	<input type="checkbox"/>	<input type="checkbox"/>
			Twice	<input type="checkbox"/>	
			Thrice	<input type="checkbox"/>	
			Daily	<input type="checkbox"/>	
		Medication	Yes	<input type="checkbox"/>	Go to 18
			No	<input type="checkbox"/>	
18	Medication type	NSAIDs	<input type="checkbox"/>	Specify _____	<input type="checkbox"/>
		Narcotic	<input type="checkbox"/>		
		Acetaminophen	<input type="checkbox"/>		
		Others	<input type="checkbox"/>		

PATTERN OF MUSCLE INJURIES AMONG ATHLETES

Duration
< 1 week
1 - 2 weeks
> 2 weeks
Unrecorded

--

19 Duration of activity modification/return to play
< 1 week
1 - 2 weeks
2 - 3 weeks
3 - 4 weeks
4 - 5 weeks
5 - 6 weeks
> 6 weeks
Unrecorded

--

20 Date RTP _____

THE END

Appendix H - UMMC Ethics Committee Approval



يونيڤرسيتي مالايا

No. Rujukan:- PPUM/MDU/300/04/03

13 Ramadhan 1431H
23 Ogos 2010

Dr. Mohammad Shariff bin A. Hamid

Unit Perubatan Sukan
Pusat Perubatan Universiti Malaya

Tuan,

SURAT PEMAKLUMAN KEPUTUSAN PERMOHONAN MENJALANKAN PROJEK PENYELIDIKAN

Skeletal muscle injuries among Malaysian athletes

Protocol No :

MEC Ref. No : 806.10

Dengan hormatnya saya merujuk kepada perkara di atas.

Bersama-sama ini dilampirkan surat pemakluman keputusan Jawatankuasa Etika Perubatan yang bermesyuarat pada 18 Ogos 2010 untuk makluman dan tindakan tuan selanjutnya.

2. Sila maklumkan kepada Jawatankuasa Etika Perubatan mengenai butiran kajian samada telah tamat atau diteruskan mengikut jangka masa kajian tersebut.

Sekian, terima kasih.

Yang benar,

Norashikin Mahmood
Setiausaha
Jawatankuasa Etika Perubatan
Pusat Perubatan Universiti Malaya


s.k Ketua
Unit Perubatan Sukan

MEDICAL ETHICS COMMITTEE COMPOSITION, UNIVERSITY MALAYA MEDICAL CENTRE
Date: 18th AUGUST 2010

Member (Title and Name)	Occupation (Designation)	Male/Female (M/F)	Tick (✓) if present when above items were reviewed
Chairperson: Prof. Looi Lai Meng	Senior Consultant Department of Pathology	Female	✓
Deputy Chairperson: Prof. Kulenthran Arumugam	Senior Consultant Medical Education Research and Development Unit (MERDU)	Male	✓
Secretary (non-voting): Cik Norashikin Mahmood	Scientific Officer Medical Development Unit	Female	✓
Members: 1. Y. Bhg. Prof. Dato' Patrick Tan Seow Koon	Deputy Director (Professional)	Male	
2. Prof. Nor Zuraida Zainal	Head Department of Psychological Medicine	Female	
3. Ascco. Prof. Mohamed Ibrahim Noordin	Head Department of Pharmacy Faculty of Medicine	Male	
4. Ascco. Prof. Tan Chong Tin	Representative of Head Department of Medicine	Male	
5. Ascco. Prof. Alizan Abdul Khalil	Representative of Head Department of Surgery	Male	✓
6. Tuan Haji Amrahi Buang	Senior Principal Assistant Manager Pharmacy Centre University Malaya Medical Centre	Male	✓
7. Y. Bhg. Prof. Madya Datin Grace Xavier	Representative of Dean Faculty of Law University Malaya	Female	✓
8. Y. Bhg. Datin Aminah Pit Abdul Rahman	Public Representative	Female	✓
9. Madam Ong Eng Lee	Public Representative	Female	✓

Comments: The MEC of University Malaya Medical Centre is operating according to ICH-GCP guidelines and the Declaration of Helsinki. Member's no. 7, 8 & 9 are representatives from Faculty of Law in the University Malaya and the public, respectively. They are independent of the hospital or trial site.


.....
PROF. LOOI LAI MENG
Chairman
Medical Ethics Committee



institut sukan negara
National Sports Institute of Malaysia
ISNRP:13/2011
Bil ISNM:
Bil Tuan: 20 Sept. 2011
Tarikh:

Dr. Mohamad Shariff A. Hamid
Unit Perubatan Sukan
Universiti Malaya
Lembah Pantai
59100 Kuala Lumpur
Tel: 03 – 7967 4968

Tuan,

**MEMOHON KEBENARAN MENJALANKAN PENYELIDIKAN UNTUK PENULISAN
ILMIAH TAJUK PENYELIDIKAN:
ISNRP: 13/2011: PLATELET-RICH PLASMA INJECTION IN THE TREATMENT OF
GRADE 2 MUSCLE INJURY**

Dengan hormatnya perkara tersebut di atas adalah dirujuk.


2. Permohonan tuan telah didaftarkan sebagai ISNRP:13/2011 dan kertas kerja tersebut telah diteliti dan dikaji oleh Jawatankuasa Penyelidikan ISN.

3. Sukacitanya dimaklumkan bahawa pihak Institut tiada halangan dengan permohonan tersebut. Namun demikian, tuan adalah diminta untuk menyediakan satu salinan ringkasan eksekutif dan satu salinan tesis terperinci yang dihasilkan kepada Bahagian Perubatan Sukan, ISN.

4. Tuan juga dikehendaki menghantar laporan perkembangan penyelidikan (*progress report*) kepada Jawatankuasa Penyelidikan ISN dan membentangkan hasil penyelidikannya di ISN apabila kajian tersebut telah selesai.



Bersama – sama ini disertakan borang laporan perkembangan penyelidikan untuk tindakan pihak tuan.

...2/



KEMENTERIAN BELIA DAN SUKAN

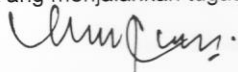
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Sekian, terima kasih.

**'Pemangkin Kecemerlangan Sukan'
BERKHIDMAT UNTUK NEGARA**

Yang menjalankan tugas,



(DR. YEO WEE KIAN)

Pengerusi Jawatankuasa Penyelidikan ISN /
Pengarah Bahagian Penyelidikan dan Inovasi

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'1 BELIA 1 MALAYSIA'

Interrater and Intrarater Reliability of the Active Knee Extension (AKE) Test among Healthy Adults

J. Phys. Ther. Sci.
25: 957–961, 2013

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Abstract. [Purpose] The purpose of this study was to determine the reliability of the active knee extension (AKE) test among healthy adults. [Subjects] Fourteen healthy participants (10 men and 4 women) volunteered and gave informed consent. [Methods] Two raters conducted AKE tests independently with the aid of a simple and inexpensive stabilizing apparatus. Each knee was measured twice, and the AKE test was repeated one week later. [Results] The interrater reliability intraclass correlation coefficients (ICC_{2,1}) were 0.87 for the dominant knee and 0.81 for the nondominant knee. In addition, the intrarater (test-retest) reliability ICC_{3,1} values range between 0.78–0.97 and 0.75–0.84 for raters 1 and 2 respectively. The percentages of agreement within 10° for AKE measurements were 93% for the dominant knee and 79% for the nondominant knee. [Conclusion] The finding suggests the current AKE test showed excellent interrater and intrarater reliability for assessing hamstring flexibility in healthy adults.

Key words: Hamstring, Flexibility, Range of movement

(This article was submitted Feb. 13, 2013, and was accepted Mar. 29, 2013)

INTRODUCTION

Hamstring muscle injury is one of the commonest sports injuries among athletes^{1, 2)}. Based on the literature, risks of hamstring muscle injury include previous injury, strength imbalance, older age, inadequate warm-up, poor quadriceps flexibility and muscle fatigue^{1–5)}. Another possible cause of hamstring injury is muscle tightness. This factor remains inconclusive, as studies have shown conflicting results^{6–10)}. Despite this, hamstring flexibility is recommended during routine pre-participation examinations and in deciding athlete readiness to return-to-play following injury^{11–13)}. Therefore, a simple and reliable method of hamstring flexibility assessment is relevant.

Methods to assess hamstring flexibility include the straight-leg-raising (SLR) test, sit and reach (SR) test, and active knee extension (AKE) test^{14–16)}. The SLR test specificity has been questioned, as it is also widely used as a neurological test¹⁷⁾. Further, cinematographic study showed that pelvic rotation may influence the validity of SLR angle measurements¹⁸⁾. Even though hamstring flexibility assessment is easy using the sit and reach (SR) test, the validity of this test is considered poor¹⁹⁾. The AKE test is an active test that involves movement at the knee joint, and most considers it safe, as the patient dictates the end point of movement. Further, the AKE test aided by a metal rig and straps to limit pelvic and leg motion (as a stabilizing apparatus) showed a high intrarater correlation coefficient when

conducted within 30 minutes^{16, 20)}. Others though, question the practicality of such method, as the apparatus used by researchers are complicated, rarely available in a clinical setting, and require more than one assessor to conduct the test^{21, 22)}. Therefore, a simple and reliable method of hamstring flexibility assessment that ensures pelvic and leg stability during measurement is needed.

We designed and made a simple, portable and easy-to-use stabilizing apparatus for use in performance of the AKE test. The apparatus was made using polyvinyl chloride (PVC) hollow tubes. PVC tubes were selected because they are light, easily cut, commonly available in hardware stores, and inexpensive. When used with a universal goniometer, this apparatus allows measurement in the AKE test to be conducted by a single assessor without assistance.

The objective of this study was to determine the interrater and intrarater reliability of the AKE test using a PVC stabilizing apparatus for hamstring flexibility assessment in healthy subjects.

SUBJECTS AND METHODS

The University of Malaya Medical Centre Ethics Committee approved the study (MEC Ref no. 835.11). A convenient sample of 16 healthy participants (10 men and 6 women) with ages ranging from 28 to 39 years was used in this study. Participants were Sports Medicine postgraduates and staff at the Faculty of Medicine, University of Malaya. All participants were free from any orthopaedic or neurological disorders.

We designed a simple, portable apparatus made up of PVC tubes (total cost of USD 6.00) to aid in performance

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Fig. 1. Flexion of the knee until the thigh touches the horizontal PVC bar

of the AKE test. The apparatus is based on those used by an earlier study¹⁶. It consisted of a single horizontal bar anchored (removable) by two vertical poles on either side of the plinth. Assessments were conducted at the Sports Medicine Clinic, University of Malaya Medical Centre. To ensure that all participants would be assessed in the standard manner, both assessors attended a half-day workshop on the AKE test using the PVC stabilizing apparatus. The AKE testing procedure was described to all participants, and each provided informed consent. The AKE measurements were taken for both knees. The dominant knee was determined based on the participant's preferred leg when kicking a ball or when performing a single-leg jump, while the other knee was considered the nondominant knee; a previous researcher used a similar definition²³.

A sports physician (SAH) and a physiotherapist (LPC) performed all the AKE tests independently. All assessments were conducted at the Sports Medicine Clinic of the University of Malaya Medical Centre between 9.00 and 11.00 am at room temperature. Participants were advised to avoid any sporting activities on the day of assessment. As previous study demonstrated changes in biomechanical characteristics of collagen and muscle viscoelastic properties, no warming up activities were allowed²⁴. Participants were assessed on a plinth in the supine position with both lower extremities extended. Both anterior superior iliac spines were positioned by aligning them with the vertical bars of the apparatus. The lower extremity not being measured was secured to the plinth using a strap across the lower third of the thigh. Each assessor marked the lateral knee joint line with washable ink. From there, two lines were drawn. The first was drawn to the greater trochanter, and another to other was drawn to the apex of the lateral malleolus. The participants were told to flex the hip until the thigh touched the horizontal PVC bar (Fig. 1). While maintaining the contact between the thigh and horizontal PVC bar, the participants were asked to extend the leg as much as possible while keeping their foot relaxed and to hold the position for about 5 seconds. A standard universal goniometer was placed over the previously marked joint axis, and the goniometer arms were aligned along the femur and fibula (Fig. 2).



Fig. 2. Knee position during the AKE test

The AKE measurement was defined as the degree of knee flexion from terminal knee extension. Each knee was measured twice, and the mean angle of the AKE test was used for analysis. All participants attended two testing sessions one week apart to allow for establishment of test-retest reliability of the method. The order in which the rater assessed the participants was randomly assigned in the first session and maintained thereafter.

All data were analyzed using the Statistical Package for the Social Sciences (SPSS) version 20.0 software. Data were described descriptively, and a normality test was performed using the Shapiro-Wilks test. Paired t-tests were performed to compare differences between tests and retest measurements within and between raters.

Two different types of measures of reproducibility were assessed: measures of agreement and measures of reliability. The interrater agreement was quantified by calculating the mean difference between the two raters (rater 1 – rater 2) and the standard deviation (SD) of this difference. Further, the 95% limits of agreement were calculated according to the method of Bland and Altman²⁵. These limits represent the range in which 95% of the differences between the two raters fall.

Plots of differences between raters against the corresponding mean of the two raters for each participant were produced to examine homoscedasticity as proposed by Bland-Altman²⁵. Further, the frequency of agreement of the raters within 5° and 10° was calculated. Differences exceeding 10° were determined as being unacceptable, as a previous study suggested they are likely to affect decisions on patient management²⁶.

The reliability was evaluated by computing intraclass correlation coefficients (ICCs), which analyzed the consistency of quantitative measures. An ICC based on a two-way random model was used to establish test-retest reliability, while an ICC based on a two-way mixed model was chosen for intrarater reliability²⁷. The ICC value of the standard error of measurement (SEM) was calculated to express the magnitude of disparity between measurements²⁸. SEM was calculated using the formula, $SEM = SD_{avg}(\sqrt{1-ICC})$, where SD corresponds to the pooled standard deviation and ICC is the reliability coefficient²⁹. A smaller SEM value

Table 1. Descriptive data of participants' physical characteristics

Variables	All participants		Male	Female
	Mean (SD*)	Range	Mean (SD)	Mean (SD)
Age (years)	31.5 (2.8)	28–39	32.3 (3.0)	30.5 (2.4)
Body weight (kg)	71.6 (13.5)	54–100	77.7 (12.9)	63.4 (10.3)
Height (cm)	168.4 (6.1)	159–181	170.9 (5.8)	165.0 (4.9)
Body mass index (BMI)	25.1 (3.5)	19.1–31.8	26.5 (3.4)	23.2 (3.0)
AKE† dominant knee (°)	24.9 (9.3)	10.0–45.0	26.6 (10.8)	22.8 (7.2)
AKE nondominant knee (°)	23.7 (8.1)	12.5–39.0	26.1 (9.3)	20.6 (5.3)

*SD, standard deviation

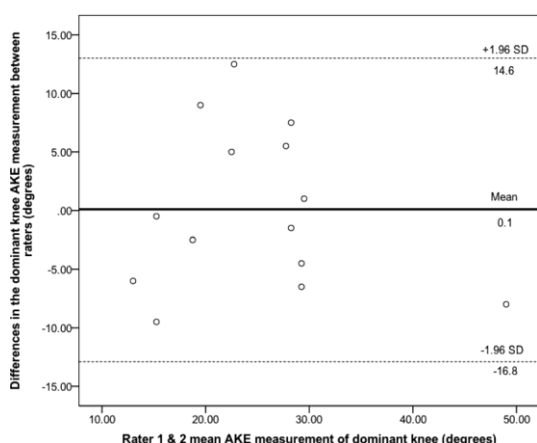
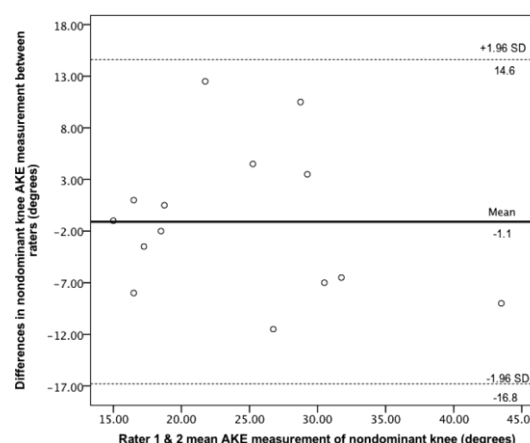
†AKE, active knee extension

Table 2. Mean, SD, and the frequency of agreement within 5 and 10 degrees

AKE* test	Rater 1 Mean (SD*)	Rater 2 Mean (SD)	Rater 1 – 2 Mean (SD)	Upper and lower limit of agreement	Rate of agreement (%)	
					5°	10°
Dominant knee (°)	24.9 (9.3)	24.8 (10.1)	0.1 (6.9)	–13.4 – 13.6	43	93
Nondominant knee (°)	23.7 (8.1)	24.9 (7.2)	–1.1 (7.2)	–15.2 – 13.0	43	79

*AKE, active knee extension

†SD, standard deviation

**Fig. 3.** Bland-Altman plot of the differences versus means of the dominant knee AKE measurements**Fig. 4.** Bland-Altman plot of the differences versus means of the nondominant knee AKE measurements

suggests greater agreement between measurements³⁰. The minimum detectable change (MDC) was calculated using the formula $MDC = 1.96 \times SEM \times \sqrt{2}$ ³¹.

RESULTS

Fourteen (8 men and 6 women) participants completed the test and retest sessions. Two participants were excluded from the final analysis, as one suffered a hamstring injury and the other was involved in a road traffic accident. A significant difference in body weight between male (mean=77.69, SD=12.86) and female (mean=63.42, SD=10.27) participants was found ($t=2.23$, $p=0.046$; Table 1). There was no significant difference in AKE measurements between the dominant and nondominant knees. In

addition, no significant difference was observed between test and retest sessions for both dominant ($t=0.77$, $p=0.46$) and nondominant ($t=-1.01$, $p=0.33$) knees.

A summary of the interrater agreement observed in this study is displayed in Table 2. Both raters had similar AKE measurements, with the limits of agreement being 0.1 ± 12.9 (SD) for the dominant knee and -1.1 ± 15.7 for the nondominant knee. The percentages of agreement within 10° for these measurements were 93% and 79% for the dominant and nondominant knees respectively.

The AKE measurement differences between raters were plotted against the mean value of both raters for both the dominant and nondominant knees (Fig. 3 and 4). Errors of measurement for both knees were independent of the magnitude of the range of measurements (homoscedasticity)

Table 3. Interrater reliability of the AKE test

AKE* measurements	Rater 1, mean (SD) [†]	Rater 2, mean (SD)	p value	ICC _{2,1} [‡]	95% CI [§] ICC	SEM	95% CI SEM
Dominant knee (°)	24.9 (9.3)	24.8 (10.1)	0.95	0.87	0.58 – 0.97	3.5	18.0 – 31.7
Nondominant knee (°)	23.7 (8.1)	24.9 (9.5)	0.56	0.81	0.41 – 0.94	3.8	16.9 – 31.7

*AKE, active knee extension test

[†]SD, standard deviation[‡]ICC, intraclass correlation coefficient[§]CI, confidence interval^{||}SEM, standard error measurement**Table 4.** AKE test-retest reliability of raters 1 and 2

AKE* test	Rater	Session 1, mean (SD) [†]	Session 2, mean (SD)	p value	ICC _{3,1} [‡]	95% CI [§] ICC	SEM	95% CI SEM
AKE dominant knee (°)	1	24.9 (9.3)	24.0 (6.8)	0.46	0.92	0.76 – 0.97	2.3	19.9 – 29.0
	2	24.8 (10.1)	22.3 (6.2)	0.21	0.78	0.32 – 0.92	3.9	16.0 – 31.2
AKE nondominant knee (°)	1	23.7 (8.1)	25.2 (8.6)	0.33	0.88	0.45 – 0.92	2.9	18.8 – 30.2
	2	24.9 (9.5)	23.1 (5.9)	0.31	0.82	0.46 – 0.94	3.3	17.5 – 30.5

*AKE, active knee extension test

[†]SD, standard deviation[‡]ICC, intraclass correlation coefficient[§]CI, confidence interval^{||}SEM, standard error measurement

The AKE measurements of raters 1 and 2 from the first testing session were compared (Table 3). No significant difference between raters was noted for both lower limbs. The AKE test interrater reliability was excellent, with ICC_{2,1} values of 0.87 (0.58–0.97; 95%CI) for the dominant knee and 0.81 (0.41–0.94; 95%CI) for the nondominant knee and standard error of measurement (SEM) values of 3.5° (18.0°–31.7°; 95%CI) and 3.8° (16.9°–31.7°; 95%CI) respectively. Minimal detectable change was between 9.7° and 10.5°. No significant difference in mean AKE measurements was noted between the first and second testing sessions for both raters (Table 4). The ICC_{3,1} values ranged from 0.78 to 0.92.

DISCUSSION

Despite conflicting findings concerning the association of poor hamstring flexibility and the risk of hamstring muscle injury, assessment of hamstring flexibility is routinely conducted. Hamstring flexibility assessment is useful, especially in deciding an athlete's readiness to return-to-play following injury^{11–13}. The AKE test is considered by some to be the gold standard for hamstring flexibility assessment³².

With the aid of a simple stabilizing apparatus and a universal goniometer, we showed that a single assessor can conduct the AKE test easily. Interrater reliability ICC_{2,1} values of 0.87 and 0.81 were found for the dominant and nondominant knees respectively. Further, the device also produced a standard error of measurement of 3.5° for the dominant knee and 3.8° for the nondominant knee. In addition, a good level of agreement between raters was established.

The test-retest reliability in this study was excellent,

with ICC_{3,1} values ranging from 0.78 to 0.92 for both raters. Our finding is in agreement with earlier studies. Gajdosik reported a Pearson product-moment correlation coefficient for the AKE test of 0.99 for both lower extremities³³. The higher reliability coefficient value could be explained by the different statistical analysis used. Further, the interval between the first AKE test and the retest session was very short. Both AKE tests were conducted on the same day 30 minutes apart, and this may have introduced systematic bias and affected reliability². We chose an interval of one week between the test and retest AKE assessment because it reflects our clinical practice, as majority of cases are reviewed a week apart.

Using the mean value of AKE measurements from both knees, Gabbe reported excellent test-retest reliability, with ICC_{3,1} values of 0.94–0.96²⁹. In contrast to Gabbe et al., the current study evaluated each knee separately to explore any potential differences between the dominant and nondominant knees, and such differences in method may explain the wider ICC_{3,1} values noted in this study. Further, the current study found no significant difference in AKE measurements between the dominant and nondominant knees of healthy individuals.

Similarly, a pilot study conducted by Davis reported excellent intrarater reliability of the knee extension angle (KEA) test, with an ICC_{3,1} of 0.94³². The method of hamstring flexibility assessment employed by the previous author differs from that in the current study. In the former study, measurements were taken from two gravity inclinometers placed immediately superior to patella, and another was placed on the distal anterior tibia of the patient's lower extremity. In addition, the examiners performed hip flexion and knee extension passively, whereas participants

actively performed all movements in the current study.

Our finding concerning interrater reliability was consistent with those reported by earlier studies^{29, 34}. Gabbe reported an interrater reliability ICC_{2,1} of 0.93 and 4° SEM for the AKE test in 15 healthy participants of comparable age group (mean age: 31.6 years)²⁹. Similarly, in a study to determine the effect of pelvic positioning and stretching method on hamstring flexibility, Sullivan reported an interrater reliability ICC_{1,1} and SEM of 0.93 and 4.81° respectively for the AKE test among 12 healthy subjects³⁴. On the other hand, Rakos et al. performed the AKE test with aid of an intricate stabilizing apparatus and demonstrated a good interrater reliability with an ICC_{2,1} of 0.79 among children age 10 to 13 years old²².

Despite demonstrating excellent interrater and test-retest reliability, a wide range of CIs was noted for some of the point estimates. Such findings, suggests that further research with larger samples may be necessary to determine the reliability estimates with greater precision³⁵. Second, the reliability displayed in this study was based on assessment in healthy and uninjured volunteers. Whether the AKE test would be as reliable in a population of injured athletes remains unanswered. The AKE test might still be useful, especially during the preseason assessment, as most athletes are free from injury at the beginning of the season.

The current study demonstrated that the AKE test can be performed easily by a single assessor with a simple, portable, and inexpensive stabilizing apparatus and have excellent interrater and intrarater reliability.

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Enhancing muscle healing using platelet-rich plasma (PRP) injection

ISRCTN	ISRCTN66528592
ClinicalTrials.gov identifier	
Public title	Enhancing muscle healing using platelet-rich plasma (PRP) injection
Scientific title	Platelet-rich plasma (PRP) injection for the treatment of grade-2 muscle injury: A randomised single blinded clinical trial
Acronym	PRP- RTP
Serial number at source	835.11
Study hypothesis	<ol style="list-style-type: none"> 1. There is a significant difference in the recovery time following acute muscle injury in patient receiving platelet-rich plasma (PRP) injection therapy compared with patient receiving rehabilitation therapy only. 2. The PRP intervention group will demonstrate a faster duration to return-to-play (RTP) than the control group.
Lay summary	Lay summary under review 1
Ethics approval	Medical Ethics Committee, University Malaya Medical Centre approved on 25 February 2011, MEC Ref. No: 835.11
Study design	Randomised single blinded trial
Countries of recruitment	Malaysia
Disease/condition/study domain	Muscle injury
Participants - inclusion criteria	<ol style="list-style-type: none"> 1. 18 years old and above 2. Acute muscle injury (less than 1 week) 3. Able to understand study protocol and informed consent
Participants - exclusion criteria	<ol style="list-style-type: none"> 1. Received any form of injection therapy 2. Using non-steroidal anti-inflammatory drugs (NSAIDs) within 1 week prior to randomisation 3. Unable to fulfill follow-up schedule 4. Significant cardiovascular, renal, hepatic disease, malignancy, history of anemia or previous muscle surgery
Anticipated start date	01/11/2011
Anticipated end date	01/01/2013
Status of trial	Completed
Patient information material	Not available in web format, please use the contact details below to request a patient information sheet
Target number of participants	30
Interventions	<p>Eligible injured athletes were randomised using computer block randomisation software into two groups.</p> <ol style="list-style-type: none"> 1. Control group (C) 2. PRP group (PRP) <p>Control group Participants in the control group will receive a standard physiotherapy treatment (attended by a trained sports physiotherapist). This consists of the use of cryotherapy, range of motion (ROM) and muscle strengthening exercises. In addition the use of non-steroidal anti-inflammatory drugs (NSAIDs) will not be allowed throughout the study. The use of oral paracetamol however is allowed.</p> <p>PRP group In addition to the standard physiotherapy treatment participants in this group will receive a single PRP (3 mls) injection into the site of injury. The PRP will be extracted from participant's own blood. The use of non-steroidal anti-inflammatory (NSAIDs) will not be allowed throughout the study. The use of oral paracetamol however is allowed.</p>

Primary outcome measure(s)	<p>1. Duration to full recovery (return-to-play) Criteria to RTP</p> <p>1.1. Muscle strength: At or near pre-injury levels or symmetrical with unaffected site 1.2. Range of motion: At or near pre-injury levels or symmetrical with unaffected site 1.3. Tenderness: Injury site should be non-tender 1.4. Inflammation or swelling: No swelling or inflammation</p> <p>Outcomes are measured on a weekly basis until full recovery of the end of week 16</p>
Secondary outcome measure(s)	<p>1. Level of platelets - blood and PRP 2. Level of growth factors in PRP</p> <p>2.1. Insulin-like growth factor (IGF-1) 2.2. Transforming growth factor (TGF)-beta 2.3. Basic (fibroblast growth factor) FGF 2.4. Platelet-derived growth factor (PDGF)</p> <p>3. Isokinetic muscle strength 4. Brief pain inventory - Short form (BPI-SF)</p> <p>Outcomes are measured on a weekly basis until full recovery of the end of week 16</p>
Sources of funding	University of Malaya (Malaysia)
Trial website	
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Platelet-rich plasma (PRP): an adjuvant to hasten hamstring muscle recovery. A randomized controlled trial protocol (ISCR TN66528592)

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Abstract

Background: Muscle injuries are one of the commonest injuries affecting athletes. It often leads to significant pain and disability causing loss of training and competition time. With current treatment, the duration to return-to-play ranges from six weeks to never, depending on injury severity. Recent researches have suggested that autologous platelet-rich plasma (PRP) injection into the injured site may hasten soft tissues healing. To-date, there has been no randomised clinical trials to evaluate the effects of PRP on muscle healing. The aim of this study is to examine the effects of autologous PRP on duration to return-to-play after muscle injury.

Methods and design: A randomised, single blind controlled trial will be conducted. Twenty-eight patients aged 18 years and above with a recent grade-2 hamstring injury will be invited to take part. Participants will be randomised to receive either autologous PRP injection with rehabilitation programme, or rehabilitation programme only. Participants will be followed up at day three of study and then weekly for 16 weeks. At each follow up visit, participants will be assessed on readiness to return-to-play using a set of criteria. The primary end-point is when participants have fulfilled the return-to-play criteria or end of 16 weeks.

The main outcome measure of this study is the duration to return-to-play after injury.

Conclusion: This study protocol proposes a rigorous and potential significant evaluation of PRP use for grade-2 hamstring injury. If proven effective such findings could be of great benefit for patients with similar injuries.

Trial registration: Current Controlled Trials ISCR TN66528592

Background

Muscle injuries are one of the commonest injuries affecting athletes [1]. They account for up to 30 – 50% of the injuries in sports events [2,3]. Majority of muscle injuries are results of excessive strain on muscle, which occurs during sprinting or jumping. Muscle injury may be the result of excessive eccentric contraction, when the muscle develops tension while lengthening [4]. This injury often affects the myotendinous junction of superficial muscles spanning across two joints, such as the rectus femoris semitendinosus, and gastrocnemius muscles [1].

The diagnosis and grading of muscle injury is usually made through a thorough clinical assessment. Diagnostic ultrasound examination is often recommended as the method of choice for confirming and grading the muscle injury [5]. Despite the high frequency of muscle injury, the best method of its treatment has not yet been clearly defined. Currently, many interventions are used, guided by limited randomised controlled trials and quality prospective studies [6]. In professional sports, muscle injury often leads to significant pain and disability causing loss of training and competition time. Despite many treatment options, the duration of the return-to-play (RTP) period ranges from six weeks to never, based on the severity of the strains [7]. Current treatment includes rest, ice, compression and elevation (RICE) with a short period of immobilization during the early phase. In addition, short-term use of nonsteroidal anti-inflammatory (NSAIDs),

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corticosteroid medications and rehabilitation programmes is recommended [6,8-14].

Basic science of muscle healing has directed attention towards the use of autologous biological products as a treatment alternative for muscle injury. Damaged muscle goes through the early phase of destruction (inflammatory phase), where affected cells including muscles, blood vessels, connective tissues and intramuscular nerve undergo necrosis [15]. This phase is followed by repair and remodelling phases, in which undifferentiated satellite cells, in response to various growth factors, proliferate and differentiate into mature myoblasts in an effort to replace the injured muscle fibers [1]. Many of the growth factors are stored in the alpha (α) granules within platelets [16].

Inflammation occurring after muscle injury usually leads to accumulation of inflammatory cells, neutrophils and macrophages. Activation of platelets also occurs early at the injured site. Activated platelets degranulate releasing various substances, including growth factors. In addition, platelets contain other metabolically active substances such as adhesive proteins (TSP-1), clotting factors and their inhibitors (TFPI), proteases (MMP-1, 2 & 9 and TIMP1-4), chemokines (SDF-1 α), cytokines and membrane glycoproteins (CD40L), involved in tissue repair and regeneration [16]. Platelet derived growth factors (PDGF), vascular endothelial growth factors (VEGF), epidermal growth factor (EGF), basic fibroblast growth factors (bFGF), insulin-like growth factor-1 (IGF-1) and transforming growth factor beta-1 (TGF- β 1) are some of the growth factors released by platelets [17]. IGF-1 and bFGF have the ability to accelerate healing following muscle and tendon injury [18]. A previous study from an animal model showed autologous PRP injection significantly hastens tibialis anterior muscle recovery from 21 days to 14 days [19]. Sanchez et al. at the 2nd World Congress on Regenerative Medicine 2005 presented a similar finding. They noted athletes receiving PRP injection under ultrasound guidance gain full recovery within half of the expected time [20]. In a study involving professional athletes, Wright-Carpenter et al. (2004) demonstrated autologous conditioned serum (ACS) injected into the injured muscle shortened the duration to full RTP by 30% (six days). They attributed the observed effects to the presence of increased levels of growth factors (FGF-2, HGF and TGF- β 1) demonstrable on ELISA [7]. In 2010, the International Olympic Committee (IOC) concluded that currently there is very limited scientific evidence of clinical efficacy and safety profile of PRP use in athletic injuries [21]. More recently, a systematic review article, reported there has been no randomised clinical trials of PRP effects on muscle healing. In addition, only four clinical reports (level of evidence 3 or 4) were available [22]. More work

on clinical science of PRP using robust clinical trials to demonstrate its efficacy has been recommended [21,22].

This paper describes the protocol of a randomised controlled trial to evaluate the clinical efficacy of a single injection of PRP combined with a rehabilitation programme on the duration to RTP after grade-2 hamstring injury. We hypothesized that distinct differences would be observed in the duration of RTP between those treated with combined PRP and rehabilitation programme versus rehabilitation programme alone. The presence of various growth factors in PRP could speed up muscle recovery.

Methods and design

Study design

This study involved a randomised, assessor-blinded controlled trial of 16-week duration. Participants were screened before enrolment. Measurements (described below) were taken upon study enrolment. On day three following the PRP injection, the participants were reviewed for any adverse reaction. Subsequently, all the participants were reassessed once a week until the end of the study period. The protocol conformed to the CONSORT guidelines for nonpharmacological interventions [23].

Participants

Patients with confirmed grade-2 hamstring muscle injury were invited to participate in this study. Study notice and invitation to take part were distributed to all sports physicians practicing within Klang Valley, Selangor, Malaysia. The eligibility criteria for inclusion were as follows:

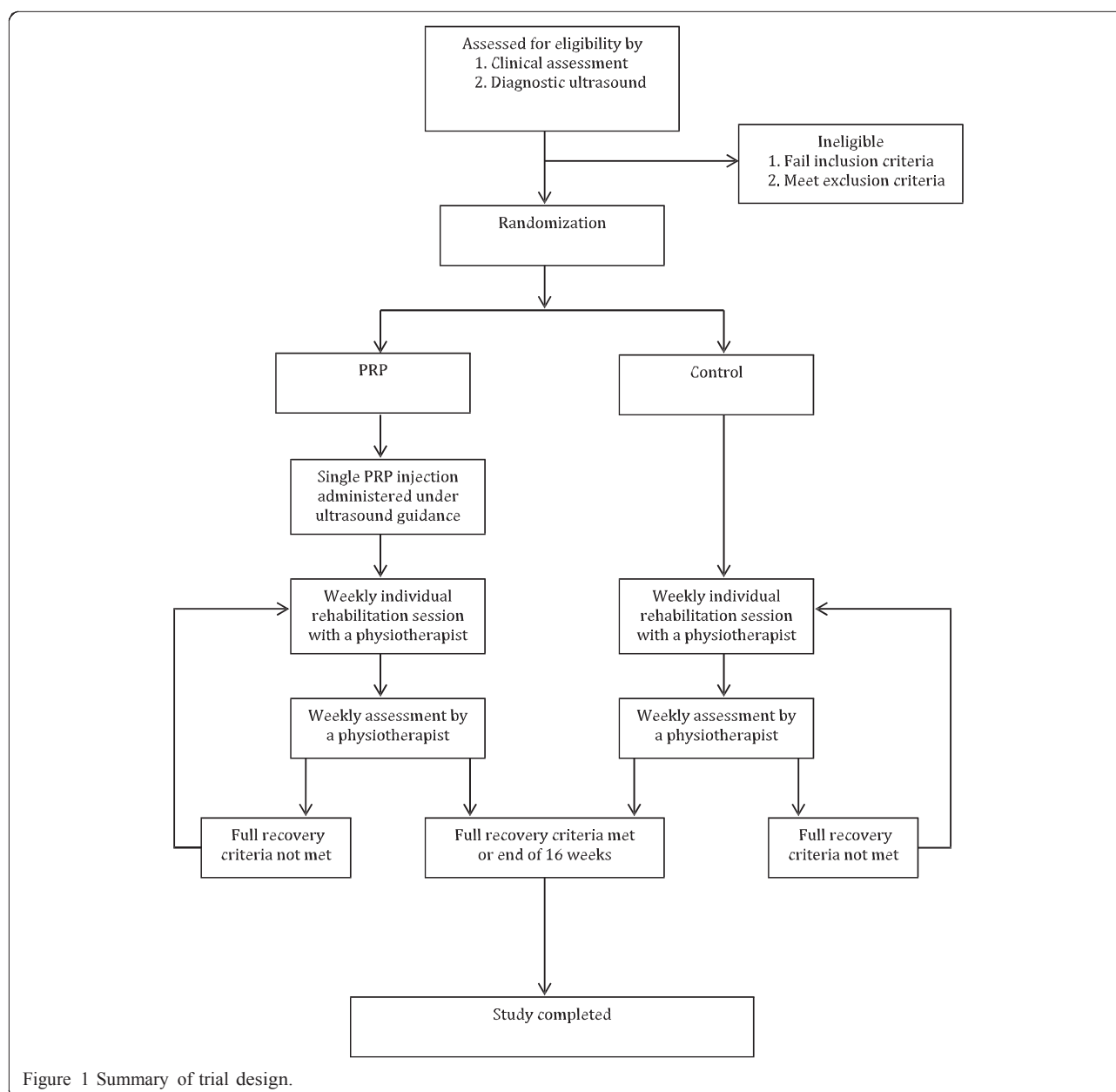
- (i) Aged ≥ 18 years;
- (ii) Acute hamstring muscle injury (\leq seven days);
- (iii) Able to understand study protocol and completing the written informed consent.

The exclusion criteria were:

- (i) Having received any form of injection therapy for current injury;
- (ii) Using nonsteroidal anti-inflammatory drugs (NSAIDs) within one week before randomisation;
- (iii) Unable to fulfil follow-up;
- (iv) Significant cardiovascular, renal, hepatic disease, malignancy, history of anaemia, and previous muscle surgery.

Procedure

The procedure is outlined in Figure 1. An initial screening was conducted at the Sports Medicine Clinic of the University of Malaya Medical Centre to determine injury severity. A sport physician and a physiotherapist conducted physical examination and grading of injury severity using



clinical grading as recommended by Jarvinen et al. and DeLee et al. [24,25].

Later, two experienced musculoskeletal radiologists conducted a diagnostic ultrasound (Philips IU 22 ultrasound with 17–5 MHz Probe) to confirm the diagnosis using the grading system used at our hospital (Table 1) and the grading suggested by Peetrons et al. [26]. Any disagreement between assessors was resolved through discussion. Diagnostic ultrasound assessment was conducted 24 to 48 hours after completion of physical examination. We also kept the record of those found to be ineligible. Patients with grade-2 hamstring muscle injury on clinical assessment and confirmed on diagnostic ultrasound examination were invited to participate.

Randomisation

Participants were randomly allocated into one of two groups: (i) autologous PRP group or (ii) control group. Randomisation was performed on those eligible after

Table 1 Grading of muscle strain injuries on ultrasound

Grade	Ultrasound findings
0	No ultrasound features seen
1	Muscle oedema only
2a	Partial tears of muscle fibres, disruption involving <33%
2b	Partial tears of muscle fibres, disruption involving ≥ 33 – 66%
2c	Partial tears of muscle fibres, disruption involving ≥ 66 - 99%
3	Complete tear of muscle

they had signed the written informed consent. A computer generated block randomisation of four was used to create a randomisation schedule. Treatment assignments were conducted by the trial manager MS.

Blinding

Three physiotherapists (PC, FJ, SR) acted as the outcome measure assessors. They were involved in providing standard rehabilitation programmes to all participants but were blinded about the participant group allocation. The participants were requested not to disclose details of their treatment. On trial completion, the assessor was asked to guess which treatment each participant received. The success of blinding was determined by calculating the 'blinding index' using the method demonstrated by James et al. [27].

Interventions

PRP intervention group

Participants in the PRP group received a single injection of autologous PRP under expert ultrasound guidance by a musculoskeletal radiologists trained in interventional musculoskeletal injections. The injection was administered once, following randomisation of the treatment group (day 1 of the study).

PRP preparation

Fifty-five millilitres (ml) of venous blood were collected from the participants' arm into a 60 ml syringe primed with ACD-A. In addition, 2 ml of venous blood were collected and sent to the hospital laboratory for determination of platelets and leucocytes count. The blood collected for PRP was prepared according to the GPSTM III Systems instruction for use (Biomet Biologics, Inc., Warsaw, Ind). Since an acidic anticoagulant was added during the collection of whole blood, PRP was buffered to increase the pH to normal physiological levels. This was accomplished by adding 8.4% sodium bicarbonate solution in a ratio 0.05 ml of sodium bicarbonate to 1 ml of PRP. No activating agent was added to the PRP. The time taken to prepare PRP was about 30 minutes. A standard 60 ml GPSTM III kit could produce approximately 6 ml of PRP.

In our study, 3 ml of extracted PRP were injected into the injured area under ultrasound guidance. One ml was sent to the hospital laboratory for platelets and leucocyte count, while the remaining 2 ml were stored in -20° Celsius for analysis of growth factors (basic fibroblast growth factor [bFGF]; insulin-like growth factor-1 [IGF-1]; transforming growth factor-β1 [TGF-β1]) which were done later.

Injection technique

As a recent study showed, a statistically significant decrease in tenocyte proliferation and cell viability, following PRP combined with the local anaesthetic agent (lidocaine and bupivacaine) [28], no local anaesthetic was given prior to PRP injection in the current study.

To the best of our knowledge the current existing guideline lacks information on the optimal timing, frequency of administration, clinical effective dose and volume, as well as post-injection rehabilitation technique following PRP injection for muscle injury [21,29]. Furthermore, no long-term clinical studies exist on potential adverse effects. Our decision to use a single injection of 3 ml of PRP in the intervention group was based on the findings of existing clinical studies. Sanchez et al reported ultrasound guided injection of autologous preparation rich in growth factors (PRGF) within the injured muscle enhances healing and functional recovery. Further, small tears indicated good progress with a single application of PRGF, while a medium to large size tears required two or three applications of PRGF [20]. Hamilton et al. reported single injection of PRP combined with daily physiotherapy programme was effective for grade II semimembranosus strain injury. They demonstrated 17 days following injection of 3 ml PRP, the athlete was pain free and able to achieve full range of motion. The athletes were back to their preinjury activities after 3 weeks [30].

Under ultrasound guidance, 3 ml of PRP were injected directly into the injured area via an 18 G needle using a peppering technique. All injections were done under aseptic technique. Each participant in the PRP combined rehabilitation programme group received a single injection of PRP throughout the study. Immediately after injection, the patient was kept in supine position for 10 to 15 minutes. Participants were advised to rest, limit their activities for the next 48 hours, and use only acetaminophen for pain. The use of non-steroidal medication was prohibited.

Participants were reassessed for any adverse reaction three days after receiving PRP. Later, weekly reassessment was conducted until the end of the study. All participants were asked to continue with an unsupervised daily home exercise programmes as prescribed and to keep a record of these sessions. The use of painkillers, other than nonsteroidal anti-inflammatory drugs, was allowed. All medication use was recorded.

Participants in both groups were required to attend a weekly rehabilitation session with a physiotherapist until full recovery or the end of 16 weeks. At each visit, outcome measures were assessed, and rehabilitation programmes were conducted under a physiotherapist's supervision. Each treatment session lasted for 45 – 60 minutes. Three-experienced physiotherapists (with at

least five years of clinical experience) practicing at University Malaya Medical Centre and National Institute of Sports were trained to assess outcome measures and deliver rehabilitation programmes. The training involved a half-day course delivered by the principal researcher and a treatment manual. The treatment manual contained a brief summary of the study, assessment methods and hamstring rehabilitation based on the programme used by Sherry et al. [31]. In addition, the participants were expected to independently track their exercise compliance by recording the days they performed the prescribed rehabilitation programme on a logbook and to report any difficulties at each follow-up visit. The rehabilitation programme used in the study focused on progressive agility and trunk stabilization exercises (Table 2). This programme was based on a set of exercises used in an earlier study [31]. Further, this programme was found to be more effective than a programme that only emphasized on hamstring stretching and strengthening in promoting RTP and preventing injury recurrence in athletes affected with an acute hamstring strain [31].

Primary outcome measures

In this study, primary outcome was the duration of RTP. Duration of RTP is defined as the duration (days) from the date of injury until the participants fulfil the criteria for RTP. The decision on determination of fitness for RTP is based on expert opinion [32]. As there were limited scientific studies done to examine the outcome of various RTP strategies [33], we decided to come up with our own criteria of RTP (Table 3) based on recent clinical sports medicine recommendations [8,34-37].

Direct hamstring palpation was conducted and pain elicited was recorded in the participants' clinical research form (CRF). Pain provocation test was evaluated by isometric contraction of the hamstring muscles when palpation did not elicit any tenderness. This test was performed in prone lying with the knee flexed at approximately 15° [38]. Hamstring range of movement (ROM) was assessed using the active knee extension (AKE) test. The AKE test involves movement of the knee joint but not the hip, unlike the straight-leg raise (SLR) test which involves movements of both hip and knee joints. AKE test is an active test and is considered safe as the participants dictate the end point. This test has been recommended and often used to measure hamstring tightness. AKE test normal values of knee motion were reported to be within 20° on full extension of the knee [39].

Hamstring muscle strength was assessed using an isokinetic dynamometer (System 4 Pro, Biodex Medical System, NY, USA). Assessment of hamstring and quadriceps muscles of both legs was also conducted during participants' weekly visit. Participants were allowed to

Table 2 Rehabilitation programme

Phase 1
1. Low to moderate-intensity sidestepping, 3 × 1 min
2. Low to moderate-intensity grapevine stepping (lateral stepping with the trail leg going over the lead leg and then under the leg), both directions, 3 × 1 min
3. Low to moderate-intensity steps forward and backward over a tape line while moving sideways, 2 × 1 min
4. Single-leg stand progressing from eyes open to eyes closed 4 × 20 sec
5. Prone abdominal body bridge (performed by using abdominal and hip muscle to hold the body face-down straight-plank position with the elbows and feet as the only point of contact), 4 × 20 sec
6. Supine extension bridge (performed by using abdominal and hip muscles to hold the body in a supine hook lying position with the head, upper back, arms, and feet as the points of contact), 4 × 20 sec
7. Side bridge, 4 × 20 sec on each side
8. Ice in long-sitting position for 20 min
Phase 2*
1. Moderate to high-intensity sidestepping, 3 × 1 min
2. Moderate to high-intensity grapevine stepping, 3 × 1 min
3. Moderate to high-intensity steps forward and backward while moving sideways, 2 × 1 min
4. Single-leg stand windmill touches, 4 × 20 sec of repetitive alternate hand touches
5. Push-up stabilization with trunk rotation (performed by starting at the top of a full push-up, then maintain this position with 1 hand while rotating the chest toward the side of the hand that is being lifted to point toward the ceiling, pause and return to the starting position), 2 × 15 reps on each side
6. Fast feet in place (performed by jogging in place with increasing velocity, picking the foot only a few inches off the ground), 4 × 20 sec
7. Proprioceptive neuromuscular facilitation trunk pull-downs with Thera-Band, 2 × 15 to the right and left
8. Symptom-free practice without high-speed manoeuvres
9. Ice for 20 min if any symptoms of local fatigue or discomfort present

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 allowed to progress from phase 1 to phase 2 when they could walk with a normal gait pattern and perform a high knee march in place without pain.

familiarise with the experimental protocol before testing. During the familiarization period, participants practiced with sub-maximal effort. The participants' knee joint centre was kept aligned with the axis of the dynamometer crank arm. The testing protocol included maximum voluntary strength of both legs, with the uninjured leg tested first. Muscle strength test was performed under concentric exertion at three angular speeds (60°, 180° and 240°/second). Each participant performed five maximum contractions at angular speeds of 60°/s, ten maximum contractions at angular speeds of 180°/s, and fifteen maximal contractions at angular speeds of 240°/s with a rest interval of about 60 seconds between each

Table 3 Criteria for return-to-play (RTP)

Sign	General Recommendation
Pain	Pain-free (on direct palpation) Pain free on hamstring contraction (resisted isometric hamstring muscle contraction)
Range of motion	Symmetrical with unaffected site
Strength	Isokinetic strength within 5% [29,30] to 10% [8] of contralateral side

speed. At each speed, quadriceps muscles were tested first followed by the hamstrings. The participants did not receive any visual feedback during the test; however, verbal encouragements were given.

The participants that failed to meet the RTP criteria at the end of week 16 were allowed to continue their treatment in the UMMC until full recovery.

Secondary outcome measures

The Brief Pain Inventory - Short Form (BPI-SF) questionnaire were used to assess the severity and impact of pain on the participants' daily functions. The BPI-SF is a self-reported questionnaire. It consists of four questions related to pain severity and seven questions related to pain interference on daily functions. The pain intensity items are presented as numeric rating scales, with a minimum score of 0 (indicating no pain) and a maximum score of 10 (when pain is as bad as one could imagine). Similar scales are used for the seven items of interference of participants' daily functions. The BPI-SF has been validated in several languages, including Malay [40] and demonstrated a Cronbach alpha reliability that ranges from 0.77 to 0.91 [41].

Platelet levels in participants' venous blood and PRF were determined. In addition, levels of insulin-like growth factor-1 (IGF-1), basic fibroblasts growth factor (bFGF) and transforming growth factor-beta 1 (TGF-β1) were determined using ELISA kits (Cusabio, USA).

The participants' attendances to the physiotherapy session were recorded to determine adherence. In addition, their daily logbook of self-home exercise was also evaluated.

Any adverse events occurring during the study were documented and proper measures were taken.

Sample size

Sample size was determined using the following formula [42]:

$$N = \frac{2 \times (Z_{\alpha/2})^2 \times \sigma^2}{(\mu_1 - \mu_2)^2}$$

Where:

N = the sample size in each of the groups

$Z_{\alpha/2}$ of .05 = 1.96 (percentage of the normal distribution for statistical significance level of .05)

$Z_{1-\beta}$ of 80% = .84 (percentage of the normal distribution for statistical power of 80%)

μ_1 = population mean in treatment Group 1

μ_2 = population mean in treatment Group 2

$\mu_1 - \mu_2$ = the mean difference

σ^2 = population [standard deviation (SD)]

Total sample size after estimation of 30% attrition rate = 11 + 3 = 14 participants in each intervention group giving a total of 28 participants altogether [7].

Data and statistical analysis

The primary analysis was done using the principle of intention-to-treat (ITT). ITT analysis includes participants with incomplete data, those who deviated from the study protocol and those who withdrew from the study. Missing data were handled through multiple imputation methods [43].

Socio-demographic, clinical characteristics and baseline information were presented to assess comparability between groups. Similar variables were also examined among the participants who withdrew from the study.

The primary endpoint of the study was the date when RTP was achieved or the end of week 16. Differences for categorical variables are tested with a chi-square test or Fischer's exact test. As clinical outcome variables were repeatedly measured over time, a multivariate analysis of variance (MANOVA) for repeated measures was performed to explore an overall time, general group, and the time by group interaction effect.

Signs and symptoms changes were explored using linear regression analysis to determine the rate of change. In addition, levels of the various growth factors (IGF-1, bFGF and TGF) were determined. Statistical analyses were carried out using SPSS (Version 19). For all analyses, a value of P < .05 was considered significant.

Timelines

The study was approved by the University Malaya Medical Centre (UMMC), Medical Ethics Committee in February 2011 (MEC Ref. No: 835.11). Recruitment and training of physiotherapists were conducted in September 2011. Patient recruitment started from February 2012. Expected completion date of the study is in December 2012.

Discussion

This is the first randomised controls study to examine the effect of PRP on duration of RTP after a grade-2 hamstrings injury. There are several major strengths of the intervention design in this study. The primary outcome of this study includes a combination of subjective and objective assessments of RTP criteria. The criteria used are based on several current recommendations from leading experts and reflect present clinical practice [8,34-37].

The rehabilitation programme for this study has been based on a contemporary programme that was effective for acute hamstring strain. The average (\pm SD) time needed to RTP in athletes under such a rehabilitation programme was 37.4 ± 27.6 days [31].

Grade-2 muscle injury is confirmed on ultrasound (US) assessment. US is a cheap, reproducible and well-tolerated imaging examination, which also provides a real-time functional assessment in multiplanar views [44]. US is suggested to have equal sensitivity to MRI for acute hamstring muscle complex injury, especially when performed within 2 weeks following injury [45]. US assessment of hamstring injury in our study would ensure uniformity of injury grading and allow comparison of treatment interventions between groups.

Infiltration of autologous PRP under ultrasound guidance allows accurate delivery of PRP contents to the site where it is to have the greatest effect [46]. Finally, levels of growth factors including IGF-1, bFGF and TGF- β 1 in the PRP are determined using ELISA kits. This would allow us to explore the potential individual effect of PRF constituents on muscle healing.

Conclusion

This is a randomised controlled trial exploring the effectiveness of a single injection of autologous PRP combined with hamstring rehabilitation programme on the duration of RTP after a grade-2 hamstring injury. The major strengths of this study include reproducibility and reflection of current clinical management of grade-2 hamstring injury. The findings enable recommendations of this treatment alternative for grade-2 hamstring injury.

Competing interest

None of the authors has any competing interest arising from this research.

Authors' contributions

MSAH, MRMA, AY and GJ were responsible for identifying the research question, and contributing to drafting of the study protocol. All authors provided comments on the drafts and have read and approved the final version.

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PATIENTS WITH HAMSTRING INJURY WANTED

A study using platelet-rich plasma (PRP) for the treatment of hamstring muscle injury will be conducted. We are inviting patients age 18 years and above to participate in this study. The study will be conducted at the Sports Medicine Clinic, University of Malaya Medical Centre and the cost for transportation will be compensated.

If you are interested, please discuss with your attending doctor.

If you have any questions please contact
Dr Mohamad Shariff Bin A Hamid at 012-2065452

Thank you for your kind attention.

PESAKIT KECEDERAAN OTOT HAMSTRING DI PERLUKAN

Satu kajian rawatan kecederaan otot hamstring dengan menggunakan kaedah suntikan *platelet-rich plasma* (PRP) akan dilaksanakan. Kami menjemput pesakit berumur 18 tahun ke atas untuk menyertai kajian ini. Kajian akan dilaksanakan di Klinik Perubatan Sukan, Pusat Perubatan Universiti Malaya dan kos pengangkutan akan dibiayai.

Jika anda berminat, sila bincangkan bersama doctor anda.

Jika anda mempunyai sebarang soalan, sila hubungi Dr Mohamad Shariff A Hamid di talian 012 206 5452.

Terima kasih atas perhatian anda.

Appendix N - RCT Invitation Letter



11th January 2012

Mr. S. Sivasvaran
Head of Department of Sport Science
Football Association Malaysia (FAM)
Wisma FAM, Jalan SS5A/9
Selangor 47301

Yg. Bhg.

Study on treatment for hamstring muscle injury

We are conducting a study to investigate the use of platelet-rich plasma (PRP) for the treatment of grade-2 muscle injury. We are inviting patients with acute hamstring muscle injury to participate in this study.

Participant inclusion and exclusion criteria are as follows:

Inclusion criteria

1. Age 18 years and above
2. Acute hamstring muscle injury (less than 5 days)
3. Able to understand study protocol and informed consent

Exclusion criteria

1. Any form of injection therapy
2. Using NSAIDs within 1 week prior to randomization
3. Unable to fulfill follow-up criteria
4. Significant cardiovascular, renal, hepatic disease, malignancy, history of anemia, and previous muscle surgery.

This study will be conducted at the Sports Medicine Clinic, University of Malaya Medical Centre. The cost for participant's transportation will be compensated. Attached is a copy of flowchart for this study.

We value your referral of patients fulfilling the study criteria.

Thank you for your kind assistance.

Yours sincerely,

(Dr. Mohamad Shariff A Hamid)
Principle Investigator

Sports Medicine Unit, 2nd Floor Pharmacy Building, Faculty of Medicine,
University of Malaya, 50603 Kuala Lumpur, Malaysia . Tel : (603) 79674968/7522
Faks: (603) 79677511 Email: razifali@um.edu.my * <http://fom.um.edu.my>

Appendix O - Structured Clinical Report Form

Platelet rich plasma in musculoskeletal injury

Assessment date:
Revised date:
Patient reference no:

HEALTH HISTORY QUESTIONNAIRE
 All questions contained in this questionnaire are strictly confidential
 And will become part of clinical research record.

A. PERSONAL HISTORY	
1. Name:	2. M ¹ <input type="checkbox"/> F ² <input type="checkbox"/> 3. Date of birth:
4. Sports:	5. Level: National ¹ <input type="checkbox"/> State ² <input type="checkbox"/> Club ³ <input type="checkbox"/> School/Others ⁴ <input type="checkbox"/>
6. Playing experience (years):	
7. Race: Malay ¹ <input type="checkbox"/> Chinese ² <input type="checkbox"/> Indian ³ <input type="checkbox"/> Others ⁴ <input type="checkbox"/>	
8. Dexterity (handedness): Right ¹ <input type="checkbox"/> Left ² <input type="checkbox"/> Ambidextrous (both) ³ <input type="checkbox"/>	
9. Smoking: Yes ¹ <input type="checkbox"/> No ² <input type="checkbox"/>	
B. INJURY HISTORY	
10. Date of injury:	11. First consultation date:
12. Current injury: <input type="checkbox"/> New injury ¹ <input type="checkbox"/> Exacerbated/aggravated ² <input type="checkbox"/> Recurrent injury ³	
13. Onset of injury <input type="checkbox"/> Sudden onset ¹ <input type="checkbox"/> Gradual onset over some time ²	
14. Pain score (at time of injury)	
<p>0 1 2 3 4 5 6 7 8 9 10 No pain Moderate pain Worst possible pain</p>	

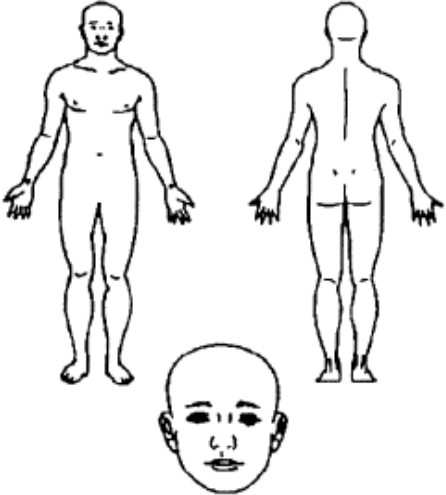
Platelet rich plasma in musculoskeletal injury

15. Injury circumstance		
<input type="checkbox"/> Training/practice ¹		
<input type="checkbox"/> Competition ²		
<input type="checkbox"/> Other ³ please specify: _____		
16. Injury mechanism		
<input type="checkbox"/> Running ¹		
<input type="checkbox"/> Stretching ²		
<input type="checkbox"/> Other ³ please specify: _____		
17. Injured region (area):	Right	Left
Upper limb ¹	<input type="checkbox"/>	<input type="checkbox"/>
Lower limb ²	<input type="checkbox"/>	<input type="checkbox"/>
Abdomen ³	<input type="checkbox"/>	<input type="checkbox"/>
Back ⁴	<input type="checkbox"/>	<input type="checkbox"/>
Others ⁵	<input type="checkbox"/> Please specify: _____	
18. Have you received any treatment? Yes ¹ <input type="checkbox"/> if yes, please proceed to Q16 No ² <input type="checkbox"/> if no, please proceed to Q17		
19. Are you currently taking any pain killer? Yes ¹ <input type="checkbox"/> Please specify _____ No ² <input type="checkbox"/>		
20. Have you received any injection treatment for the current injury? Yes ¹ <input type="checkbox"/> No ² <input type="checkbox"/>		
21. Have you been diagnosed with any of medical problems/surgery listed below?		
a. Heart (cardiovascular) disease	Yes ¹ <input type="checkbox"/>	No ² <input type="checkbox"/>
b. Kidney disease	Yes ¹ <input type="checkbox"/>	No ² <input type="checkbox"/>
c. Liver (hepatic) disease	Yes ¹ <input type="checkbox"/>	No ² <input type="checkbox"/>
d. Cancer	Yes ¹ <input type="checkbox"/>	No ² <input type="checkbox"/>
e. Low red blood cells level (anemia)	Yes ¹ <input type="checkbox"/>	No ² <input type="checkbox"/>

Athlete's signature: _____

Objective clinical assessment

Assessment date: _____

C. PHYSICAL EVALUATION															
<p>22. Inspection of affected area (mark on diagram):</p> <p>Abnormalities seen:</p> <p>a. Bruising Yes¹ <input type="checkbox"/> No² <input type="checkbox"/></p> <p>b. Dimension of bruising Length x Width (mm)</p> <p>_____ x _____</p> <p>c. Additional notes:</p> <p>_____</p> <p>_____</p> <p>_____</p>															
<p>23. Palpation (injured leg only)</p> <p>Tenderness :</p> <p style="margin-left: 100px;">Yes¹ <input type="checkbox"/></p> <p style="margin-left: 100px;">No² <input type="checkbox"/></p> <p>Distance of tender area measured from ischial tuberosity: M1: _____ cm</p> <p style="margin-left: 100px;">M2: _____ cm</p>															
<p>24. Active knee extension (AKE):</p> <table border="1" style="width: 100%; border-collapse: collapse; margin-bottom: 5px;"> <thead> <tr style="background-color: #f2f2f2;"> <th style="text-align: center;">Angle</th> <th style="text-align: center;">Right 1</th> <th style="text-align: center;">Right 2</th> <th style="text-align: center;">Average</th> <th style="text-align: center;">Left 1</th> <th style="text-align: center;">Left 2</th> <th style="text-align: center;">Average</th> </tr> </thead> <tbody> <tr> <td style="text-align: center;">AKE</td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> </tr> </tbody> </table> <p>Angle difference between injured vs uninjured side = _____</p> <p>AKE test: Positive¹ <input type="checkbox"/> (angle difference > 10°)</p> <p style="margin-left: 100px;">Negative² <input type="checkbox"/></p>		Angle	Right 1	Right 2	Average	Left 1	Left 2	Average	AKE						
Angle	Right 1	Right 2	Average	Left 1	Left 2	Average									
AKE															
<p>26. Pain provocation test: (Prone isometric contraction at 15° knee flexion)</p> <p style="margin-left: 100px;">Positive¹ <input type="checkbox"/></p> <p style="margin-left: 100px;">Negative² <input type="checkbox"/></p>															

Platelet rich plasma in musculoskeletal injury

27. Isokinetic assessment

Muscle	Contraction	Right (peak torque)	Left (peak torque)
Hamstring	Concentric 60°/sec		
	Concentric 180°/sec		
	Concentric 300°/sec		
Quadriceps	Concentric 60°/sec		
	Concentric 180°/sec		
	Eccentric 300°/sec		

28. Medication taken past 1 week: Yes / No

29. Comments:

D: RETURN TO PLAY CONSIDERATION

1. Tenderness over injured site on palpation: Positive¹ Negative²
2. Signs of inflammation or swelling: Positive¹ Negative²
3. Range of motion : Right : _____ Left : _____
4. Isokinetic assessment: Right : _____ Left : _____
5. Rehabilitation phase : 1 / 2

RTP Decision : Yes / No

Date : _____

Assessment made by: _____ Signature _____

Appendix P - Ultrasonography Report Form

**Platelet Rich Plasma Study
Radiological Assessment**

Assessment: Baseline / Follow-up

Date:

Patient:

Diagnostic criteria

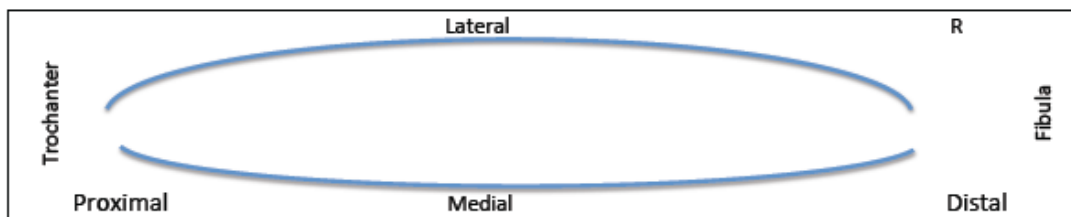
Side hamstring muscle injured (circle)

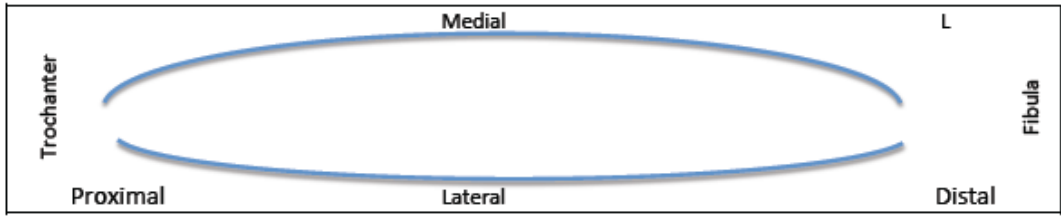
Longitudinal appearance

Abnormality seen

Description of abnormalities seen

Site of abnormality seen





Dimension of abnormality seen

Width (mm)
Length (mm)

L1	L2	Avg

Doppler flow within area of abnormality

None ¹	<input type="checkbox"/>
Less than 50% ²	<input type="checkbox"/>
More than 50% ³	<input type="checkbox"/>

Haematoma Yes¹

<input type="checkbox"/>	No ²	<input type="checkbox"/>
--------------------------	-----------------	--------------------------

Dimension of hematoma

Length (mm)
Width (mm)

L1	L2	Avg

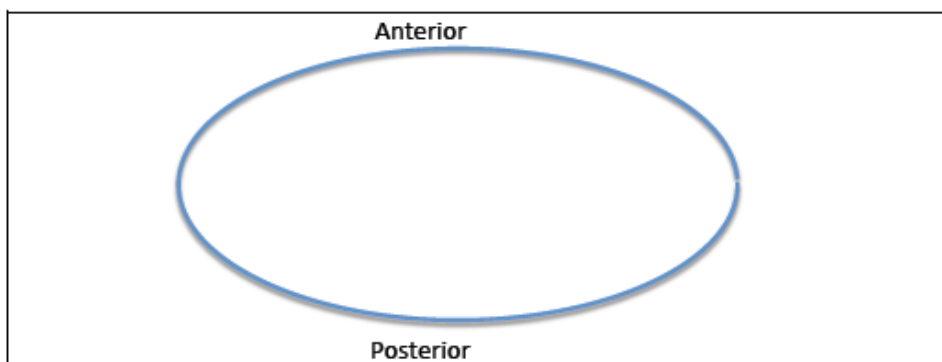
Transverse appearance

Abnormality seen Yes¹

<input type="checkbox"/>	No ²	<input type="checkbox"/>
--------------------------	-----------------	--------------------------

Description of abnormalities seen

Site of abnormality seen



Dimension of abnormality seen

Width (mm)

Depth (mm)

L1	L2	Avg

Doppler flow within area of abnormality

None¹

Less than 50%²

More than 50%³

Impression: Muscle injury grade

Grade 1¹

Grade 2²

Grade 3³

PT Type 1^a

PT Type 2^b

PT Type 3^c

Complete^d

	Muscle 1	Muscle 2	Muscle3
Grade 1 ¹			
Grade 2 ²			
Grade 3 ³			
PT Type 1 ^a			
PT Type 2 ^b			
PT Type 3 ^c			
Complete ^d			

Additional notes:

*Injection is made at the region of disruptions of the muscle fascicles
and not into the haematoma starting from the region with most disruption centrally
and then working outwards peripherally.*

Assessment made by:

PATIENT INFORMATION SHEET

Please read the following information carefully, do not hesitate to discuss any questions you may have with your Doctor.

Study Title

Platelet Rich Plasma Injection for the Treatment of Muscle Injury

Introduction

Muscle strains are one of the commonest injuries affecting athletes. It accounts for up to 30% of the injuries sustained in sports events. Majority of muscle injury are caused by contusion or excessive strain of the muscle especially in sports that require sprinting or jumping activities. The best treatment for muscle strains is still not clearly defined. Various interventions are being used, with limited evidence. Current approaches employed include rest, ice, compression and elevation (RICE) together with short period of immobilization during the acute phase, long rehabilitation protocols and injection therapies (prolotherapy, traumeel, autologous conditioned serum) Despite various approaches the duration to full recovery are still lengthy (6 weeks to never). The median duration to full return to play in Malaysian athletes was 45 days.

What is the purpose of this study?

The main purpose of this study is to determine the effectiveness of a single intralesional injection of platelet-rich plasma (PRP) for the treatment of muscle injury.

What are the procedures to be followed?

After given your written consent to participate in the study. You are required to fill in a set of questionnaire about yourself and your injury. A radiological assessment will be conducted to assist in diagnosing your injury. Patient diagnosed with second degree muscle injury will be randomly divided into Group 1 and Group 2. Patient in both groups will be required to follow a standard muscle injury rehabilitation programme under the supervision of a sports physiotherapist at the Sports Clinic, University Malaya Medical Centre.

FPU-DOF-BK-012-04-R00

In addition, 50 mls of blood will be withdrawn from patient in Group 2. The blood will be processed to obtain PRP. PRP obtained will be injected under ultrasound guidance into the injured muscle. A small amount of local anaesthetic will be given prior to PRP injection. Patient are allowed to take paracetamol when necessary. Patient from both groups will be monitored on a weekly basis until the week 16.

Who should not enter the study?

1. Patient who had received any form of injection therapy.
2. Patient with history of heart, kidney, liver disease, malignancy, history of anemia (hemoglobin < 5.0), and previous muscle surgery.

What will be benefits of the study:

(a) to you as the subject?

You may recover form your current injuries faster than with only muscle rehabilitation programme

(b) to the investigator?

The information gathered may be use as a guide on whether incorporate such treatment for any muscle injury.

What are the possible drawbacks?

Since the PRP is extracted from the patient and the process of injection is to be done under clean aseptic technique. We do not anticipate any significant drawbacks on any of the participants of this study

Can I refuse to take part in the study?

Yes.

Who should I contact if I have additional questions during the course of the study?

Doctor's Name: Dr Mohamad Shariff A Hamid Tel: 012-206 5452

Appendix R - Informed Consent Form

UNIVERSITY MALAYA MEDICAL CENTRE

CONSENT BY PATIENT FOR CLINICAL RESEARCH

I, Identity Card No.
 (Name of Patient)

of
 (Address)

hereby agree to take part in the clinical research (clinical study/questionnaire study/drug trial) specified below:

Title of Study: Platelet Rich Plasma (PRP) Injection for the treatment of muscle injury

.....

the nature and purpose of which has been explained to me by **Dr Mohamad Shariff A Hamid**
 (Name & Designation of Doctor)

..... and interpreted by
 (Name & Designation of Interpreter)

..... to the best of his/her ability in English language/dialect

I have been told about the nature of the clinical research in terms of methodology, possible adverse effects and complications (as per patient information sheet). After knowing and understanding all the possible advantages and disadvantages of this clinical research, I voluntarily consent of my own free will to participate in the clinical research specified above.

I understand that I can withdraw from this clinical research at any time without assigning any reason whatsoever and in such a situation shall not be denied the benefits of usual treatment by the attending doctors.

Date: Signature or Thumbprint
 (Patient)

IN THE PRESENCE OF

Name)
 Identity Card No.) Signature
 Designation) (Witness for Signature of Patient)

I confirm that I have explained to the patient the nature and purpose of the above-mentioned clinical research.

Date Signature
 (Attending Doctor)

CONSENT BY PATIENT
 FOR
 CLINICAL RESEARCH

R.N.
 Name
 Sex
 Age
 Unit

FPU-DOF-BK-012-08-R01

KEIZINAN OLEH PESAKIT UNTUK PENYELIDIKAN KLINIKAL

Saya..... No. Kad Pengenalan

(Nama Pesakit)

beralamat.....

(Alamat)

dengan ini bersetuju menyertai dalam penyelidikan klinikal (pengajian klinikal/pengajian soal-selidik/percubaan ubat-ubatan) disebut berikut:

Tajuk Penyelidikan: Suntikan Platelet Rich Plasma (PRP) bagi rawatan kecederaan otot

.....

yang mana sifat dan tujuannya telah diterangkan kepada saya oleh Dr. Mohamad Shariff Bin A Hamid
(Nama & Jawatan Doktor)

..... mengikut terjemahan

(Nama & Jawatan Penterjemah)

..... yang telah menterjemahkan kepada saya dengan sepenuh kemampuan dan kebolehannya di dalam Bahasa / toghat Malaysia

Saya telah diberitahu bahawa dasar penyelidikan klinikal dalam keadaan methodology, risiko dan komplikasi (mengikut kertas maklumat pesakit). Selepas mengetahui dan memahami semua kemungkinan kebaikan dan keburukan penyelidikan klinikal ini, saya merelakan/mengizinkan sendiri menyertai penyelidikan klinikal tersebut di atas.

Saya faham bahawa saya boleh menarik diri dari penyelidikan klinikal ini pada bila-bila masa tanpa memberi sebarang alasan dalam situasi ini dan tidak akan dikecualikan dari kemudahan rawatan dari doktor yang merawat.

Tarikh: Tandatangan/Cap Jari

(Pesakit)

DI HADAPAN

Nama)

)

No. K/P.....) Tandatangan

)

Jawatan) (Saksi untuk Tandatangan Pesakit)

Saya sahkan bahawa saya telah menerangkan kepada pesakit sifat dan tujuan penyelidikan klinikal tersebut di atas.

Tarikh: Tandatangan

(Doktor yang merawat)

**KEIZINAN OLEH PESAKIT
UNTUK
PENYELIDIKAN KLINIKAL**

No. Pend.
Nama
Jantina
Umur
Unit

A Randomization Plan
from
<http://www.randomization.com>

1. PRP _____
2. Control _____
3. Control _____
4. PRP _____
5. Control _____
6. PRP _____
7. Control _____
8. PRP _____
9. Control _____
10. Control _____
11. PRP _____
12. PRP _____
13. PRP _____
14. Control _____
15. Control _____
16. PRP _____
17. Control _____
18. Control _____
19. PRP _____
20. PRP _____
21. Control _____
22. PRP _____
23. Control _____
24. PRP _____
25. Control _____
26. PRP _____
27. PRP _____
28. Control _____

28 subjects randomized into 7 blocks
To reproduce this plan, use the seed 1161

Appendix T - PATS Rehabilitation Exercise Permission

Hello Shariff,

The STST protocol actually had a very high rate of re-injury, I would not suggest using that. You are welcome to use the PATS program --- actually you can use either, I just would not ethically recommend the STST after the results we saw. The paper has both programs outlined so I have attached it here for you.

take care,
Marc

Marc Sherry, PT, DPT, LAT, CSCS, PES
UW Sports Rehabilitation Dept.
UW Sports Medicine Center
621 Science Drive
Madison, WI 53711-1074
608.263.2215 (fax)
msherry@uwhealth.org
www.uwsportsmedicine.org

-----Original Message-----

From: Mohamad Shariff A Hamid [mailto:ayip@siswa.um.edu.my]
Sent: Monday, September 19, 2011 8:30 PM
To: Sherry Marc A
Subject: STST rehabilitation protocol for UM

Dear Mr Sherry

My name is Mohamad Shariff A Hamid from the University of Malaya (UM). We are conducting a research on muscle recovery following a grade 2 hamstring injury, and we are considering to use your STST rehabilitation program (that your team have published in the J of Orthopedic & Sports Physical Therapy 2004) in our control group.

I am writing to you to, firstly, to ask for your permission to used the published protocol. In addition we would be grateful if you could provide the details of phase 1 and phase 2 exercises used in your study.

Sincerely,
Shariff

Appendix U - Brief Pain Inventory - Short Form

BRIEF PAIN INVENTORY (SHORT FORM)

Study ID:
Hospital ID:

DO NOT WRITE ABOVE THIS LINE

Date : ___/___/___

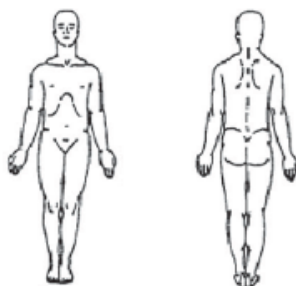
Time : _____

Name : _____

1. Throughout our lives, most of us have had pain from time to time (such as minor headaches, sprains, and toothaches). Have you had pain other than these everyday kinds of pain today

1. Yes 2. No

2. On the diagram, shade in the areas where you feel pain. Put an X on the area that hurts the most.



3. Please rate your pain by circling the one number that best describes your pain at its **worst** in the last 24 hours.

0 1 2 3 4 5 6 7 8 9 10
No pain Pain as bad as you can imagine

4. Please rate your pain by circling the one number that best describes your pain at its **least** in the last 24 hours.

0 1 2 3 4 5 6 7 8 9 10
No pain Pain as bad as you can imagine

5. Please rate your pain by circling the one number that best describes your pain on the **average**.

0 1 2 3 4 5 6 7 8 9 10
No pain Pain as bad as you can imagine

6. Please rate your pain by circling the one number that tells how much pain you have **right now**.

0 1 2 3 4 5 6 7 8 9 10
No pain Pain as bad as you can imagine

7. What treatments or medications are you receiving for your pain?

INVENTORI RINGKAS KESAKITAN – FORMAT PENDEK

No. Rujukan:

No. Kajian:

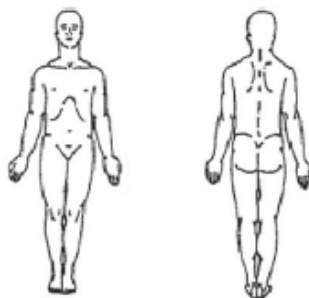
DILARANG MENULIS MELEPASI BAHAGIAN ATAS GARISAN INI

Tarikh: ___/___/___

Masa:

Nama Penuh:

1. Sepanjang hidup ini, kebanyakan kita pernah mengalami kesakitan dari semasa ke semasa (seperti sakit kepala, terseliuh/tergeliat, sakit gigi dan lain-lain). Adakah anda mengalami kesakitan selain kesakitan biasa seperti di atas pada hari ini?
 1. Ya
 2. Tidak
2. Pada gambarajah di bawah, hitamkan bahagian di mana anda merasa sakit. Tandakan X pada bahagian yang paling sakit.



3. Sila bulatkan satu nombor yang terbaik untuk menggambarkan tahap kesakitan yang paling teruk anda alami dalam masa 24 jam yang lalu.

0 1 2 3 4 5 6 7 8 9 10

Tiada

Sakit

Amat sakit

4. Sila bulatkan satu nombor yang terbaik untuk menggambarkan tahap kesakitan yang paling ringan anda alami dalam masa 24 jam yang lalu.

0 1 2 3 4 5 6 7 8 9 10

Tiada

Sakit

Amat sakit

5. Sila bulatkan satu nombor yang terbaik untuk menggambarkan kesakitan anda pada tahap sederhana.

0 1 2 3 4 5 6 7 8 9 10

Tiada

Sakit

Amat sakit

6. Sila bulatkan satu nombor yang terbaik untuk menggambarkan tahap kesakitan yang anda rasa sekarang.

0 1 2 3 4 5 6 7 8 9 10

Tiada

Sakit

Amat sakit

7. Apakah rawatan atau ubatan yang anda terima untuk kesakitan yang anda alami?

8. Dalam tempoh 24 jam yang lalu, berapa banyakkah kelegaan yang anda alami setelah menerima rawatan atau ubatan? Sila bulatkan satu peratusan yang menggambarkan kelegaan tersebut.

0%	10%	20%	30%	40%	50%	60%	70%	80%	90%	100%
Tiada Kelegaan										Kelegaan sepenuhnya

Sila bulatkan satu nombor yang menggambarkan bagaimana kesakitan dalam masa 24 jam yang lalu mengganggu keadaan berikut:

A. Aktiviti umum

0	1	2	3	4	5	6	7	8	9	10
Tidak Mengganggu										Mengganggu sepenuhnya

B. Mood / Keadaan perasaan

0	1	2	3	4	5	6	7	8	9	10
Tidak Mengganggu										Mengganggu sepenuhnya

C. Keupayaan untuk berjalan

0	1	2	3	4	5	6	7	8	9	10
Tidak Mengganggu										Mengganggu sepenuhnya

D. Kerja biasa (termasuk di dalam dan di luar rumah)

0	1	2	3	4	5	6	7	8	9	10
Tidak Mengganggu										Mengganggu sepenuhnya

E. Hubungan dengan orang lain

0	1	2	3	4	5	6	7	8	9	10
Tidak Mengganggu										Mengganggu sepenuhnya

F. Tidur

0	1	2	3	4	5	6	7	8	9	10
Tidak Mengganggu										Mengganggu sepenuhnya

G. Kenikmatan hidup

0	1	2	3	4	5	6	7	8	9	10
Tidak Mengganggu										Mengganggu sepenuhnya

Tandatangan Pesakit: _____

Terima Kasih Atas Kerjasama Anda

Appendix V - Brief Pain Inventory - Short Form Permission



Charles S. Cleeland, PhD
Professor and Chair
McCullough Professor of Cancer Research

Department of Symptom Research - Box 1450
Tel: 713-745-3470
Fax: 713-745-3475
E-mail: ccleeland@mdanderson.org

January 19, 2011

Dr. Mohamad Hamid
University of Malaya
Units of Sports Medicine, Faculty of Medicine
Kuala Lumpur, Malaysia

Re: Authorization to use the Brief Pain Inventory

Dear Dr. Hamid:

I am pleased that you have considered using the Brief Pain Inventory® (BPI) in your upcoming study. The study description you provided seems to be congruent with the intended use of the BPI. You are hereby granted permission to use it in your study. Please note that:

- Your use of the BPI is limited only to the study specified above; to use the BPI in additional studies, you must reapply online at www.mdanderson.org/departments/prg > Symptom Assessment Tools > The Brief Pain Inventory (BPI).
- You are permitted to reproduce the copy of the BPI that is included with this Letter of Authorization; however, you must not remove the copyright notice.
- The BPI may not be modified or translated into another language without the express written consent of the copyright holder, Charles S. Cleeland, PhD. Failure to comply may result in legal action. Permission to alter or translate the instrument may be obtained by contacting me at symptomresearch@mdanderson.org or by mail.

We would greatly appreciate your sending us a summary of your study results after the completion of your project, so that we can continue to evaluate the performance of our instrument.

Sincerely,

A handwritten signature in black ink, appearing to read "Charles S. Cleeland".

Charles S. Cleeland, PhD
McCullough Professor of Cancer Research and Chair
Department of Symptom Research

CARING • INTEGRITY • DISCOVERY
1515 HOLCOMBE BOULEVARD • HOUSTON, TEXAS 77030-4009
M. D. Anderson Switchboard (713) 792-2121 • www.mdanderson.org

A Comprehensive Cancer Center designated by the National Cancer Institute located in the Texas Medical Center



**JAWATANKUASA ETIKA PERUBATAN
PUSAT PERUBATAN UNIVERSITI MALAYA**

ALAMAT: LEMBAH PANTAI, 59100 KUALA LUMPUR, MALAYSIA
TELEFON: 03-79493209 FAKSIMILI: 03-79494638

No. Rujukan: PPUM/MDU/300/04/03

25 Februari 2011

Dr. Mohamad Shariff bin A. Hamid
Unit Perubatan Sukan
Universiti Malaya

Tuan,

SURAT PEMAKLUMAN KEPUTUSAN PERMOHONAN MENJALANKAN PROJEK PENYELIDIKAN
Platelet-rich plasma injection in the treatment of grade 2 muscle injury
Protocol No : -
MEC Ref. No : 835.11

Dengan hormatnya saya merujuk kepada perkara di atas.

Bersama-sama ini dilampirkan surat pemakluman keputusan Jawatankuasa Etika Perubatan yang bermesyuarat pada 23 Februari 2011 untuk makluman dan tindakan tuan selanjutnya.

2. Sila maklumkan kepada Jawatankuasa Etika Perubatan mengenai butiran kajian samada telah tamat atau diteruskan mengikut jangka masa kajian tersebut.

Sekian, terima kasih.

“BERKHIDMAT UNTUK NEGARA”

Saya yang menurut perintah,

Norashikin Mahmood
Setiasaha
Jawatankuasa Etika Perubatan
Pusat Perubatan Universiti Malaya

s.k Ketua
Unit Perubatan Sukan, UM

Unit Perkembangan Perubatan
PUSAT PERUBATAN UNIVERSITI MALAYA
(University Malaya Medical Centre)
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Leading Healthcare





**UNIVERSITY
OF MALAYA**
KUALA LUMPUR
UM MEDICAL CENTRE

**MEDICAL ETHICS COMMITTEE
UNIVERSITY MALAYA MEDICAL CENTRE**
ADDRESS: LEMBAH PANTAI, 59100 KUALA LUMPUR, MALAYSIA
TELEPHONE: 03-79493209 FAXIMILE: 03-79494638

NAME OF ETHICS COMMITTEE/IRB: Medical Ethics Committee, University Malaya Medical Centre	ETHICS COMMITTEE/IRB REFERENCE NUMBER: 835.11
ADDRESS: LEMBAH PANTAI 59100 KUALA LUMPUR	
PROTOCOL NO: TITLE: Platelet-rich plasma injection in the treatment of grade 2 muscle injury	
PRINCIPAL INVESTIGATOR: Dr. Mohamad Shariff bin A. Hamid	SPONSOR:
TELEPHONE: KOMTEL:	

The following item have been received and reviewed in connection with the above study to be conducted by the above investigator.

- | | |
|---|---------------------|
| <input checked="" type="checkbox"/> Borang Permohonan Penyelidikan | Ver date: 27 Jan 11 |
| <input checked="" type="checkbox"/> Study Protocol | Ver date: |
| <input type="checkbox"/> Investigator's Brochure | Ver date: |
| <input checked="" type="checkbox"/> Information and Consent Form | Ver date: |
| <input checked="" type="checkbox"/> Consent Form | Ver date: |
| <input type="checkbox"/> Questionnaire | |
| <input checked="" type="checkbox"/> Investigator(s) CV's (Dr. Mohamad Shariff bin A. Hamid) | |

and have been

- Approved
 Conditionally approved (identify item and specify modification below or in accompanying letter)
 Rejected (identify item and specify reasons below or in accompanying letter)

Comments:

Investigator are required to:

- 1) follow instructions, guidelines and requirements of the Medical Ethics Committee.
- 2) report any protocol deviations/violations to Medical Ethics Committee.
- 3) provide annual and closure report to the Medical Ethics Committee.
- 4) comply with International Conference on Harmonization – Guidelines for Good Clinical Practice (ICH-GCP) and Declaration of Helsinki.
- 5) note that Medical Ethics Committee may audit the approved study.

Date of approval: 23rd FEBRUARY 2011

c.c Head
Sports Medicine Unit, UM
Deputy Dean (Research)
Faculty of Medicine

Secretary
Medical Ethics Committee
University Malaya Medical Centre

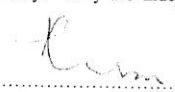
PROF. LOOI LAI MENG
Chairman
Medical Ethics Committee



MEDICAL ETHICS COMMITTEE COMPOSITION, UNIVERSITY MALAYA MEDICAL CENTRE
Date: 23rd FEBRUARY 2011

Member (Title and Name)	Occupation (Designation)	Male/Female (M/F)	Tick (✓) if present when above items were reviewed
Chairperson: Prof. Looi Lai Meng	Senior Consultant Department of Pathology	Female	✓
Deputy Chairperson: Prof. Kulenthran Arumugam	Senior Consultant Medical Education Research and Development Unit (MERDU)	Male	✓
Secretary (non-voting): Cik Norashikin Mahmood	Scientific Officer Medical Development Unit	Female	✓
Members: 1. Prof. Dato' Patrick Tan Seow Koon	Deputy Director (Professional)	Male	✓
2. Assoc. Prof. Jesjeet Singh Gill	Representative of Head Department of Psychological Medicine	Male	✓
3. Prof. Nor Azizan Abdullah	Representative of Head Department of Pharmacology	Female	✓
4. Assoc. Prof. Tan Chong Tin	Representative of Head Department of Medicine	Male	✓
5. Assoc. Prof. Alizan Abdul Khalil	Representative of Head Department of Surgery	Male	✓
6. Pn. Harbans Kaur A/P Harcharan Singh	Representative of Head Department of Pharmacy University Malaya Medical Centre	Female	✓
7. Prof. Madya Datin Grace Xavier	Representative of Dean Faculty of Law University Malaya	Female	✓
8. Y. Bhg. Datin Aminah bt. Pit Abdul Rahman	Public Representative	Female	
9. Madam Ong Eng Lee	Public Representative	Female	✓

Comments: The MEC of University Malaya Medical Centre is operating according to ICH-GCP guidelines and the Declaration of Helsinki. Member's no. 7, 8 & 9 are representatives from Faculty of Law in the University Malaya and the public, respectively. Member no. 10 is by invitation only. They are independent of the hospital or trial site.


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PROF. LOOI LAI MENG
Chairman
Medical Ethics Committee