

Conservative management of MRI-confirmed knee osteoarthritis with instrument-assisted soft-tissue mobilization, joint manipulation, and platelet-rich plasma

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Objective: To describe the successful conservative management of a patient with chronic degenerative knee pain.

Clinical Features: An active 47 year-old female office-worker with intermittent right knee pain inferolateral to the right patella for eight months described difficulty ascending stairs, sitting on a chair for one hour, and squatting. A physical exam led to the diagnosis of a suspected chronic degenerative tear of the posterior horn of the right medial meniscus and chronic right subpatellar chondrosis, in addition to extensive cartilaginous degeneration revealed by an MRI study conducted prior to initial presentation.

Intervention and Outcome: A conservative chiropractic treatment plan was implemented in addition to two successive intra-articular and subpatellar

Objectif : Présenter le traitement conservateur et efficace de la douleur chronique associée à la dégénérescence articulaire du genou.

Caractéristiques cliniques : Une employée de bureau de 47 ans, menant une vie active, se plaint de douleurs antérolatérales au genou droit depuis huit mois; elle a de la difficulté à monter les escaliers, à rester assise sur une chaise pendant une heure et à s'accroupir. L'examen physique a permis de confirmer les soupçons d'une déchirure dégénérative chronique de la corne postérieure du ménisque interne droit, d'une chondropathie sous-rotulienne chronique droite et d'une dégénérescence cartilagineuse étendue, laquelle a été attestée par un examen par IRM pratiqué avant le premier tableau clinique.

Intervention et résultats : On a établi un plan de traitement chiropratique conservateur comprenant l'administration de deux injections intra-articulaires et sous-rotuliennes de plasma riche en plaquettes. Au

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platelet-rich plasma injections. The patient reported no pain after sixteen weeks and 93.75% functionality six months after the second injection.

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KEY WORDS: knee, chondral, fissuring, chiropractic, platelet rich plasma, instrument-assisted soft tissue mobilization, osteoarthritis

bout de seize semaines, la patiente n'avait plus aucune douleur et avait récupéré 93,75 % de sa capacité fonctionnelle après la seconde injection.

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MOTS CLÉS : genou, chondral, fissure, chiropratique, plasma riche en plaquettes, mobilisation des tissus mous avec instrument, arthrose

Introduction

Chiropractors frequently see patients with lower extremity pathology that includes the knee. Respondents to the 2015 National Board of Chiropractic Examiners¹ Practice Analysis indicated that on average 8.8% of patients present with a chief complaint involving the lower extremity. Osteoarthritis (OA) is the most common joint disease in the world and a major cause of pain and disability in older adults.²⁻⁶ In fact, there is a higher incidence of knee OA than hip or hand OA and a large increase in newly diagnosed cases of knee OA in the past decade, particularly in younger adults.²⁻⁶ The knee joint is the largest and most superficial joint and primarily operates as a hinge type of synovial joint permitting flexion and extension.⁷ The hinge movements are combined with gliding, rolling, and rotation about a vertical axis.⁷ Despite being well constructed, the knee joint is relatively vulnerable mechanically because of the incongruence of its articular surfaces, which has been likened to two balls laying on a warped tabletop.⁷

This vulnerability coupled with its weight-bearing function makes the knee a common site for degenerative joint changes.⁸ Several factors have been identified in the predisposition of the knee to arthritis, including altered knee extension mechanisms⁹ and the use of wide-heeled shoes¹⁰. With specific regard to diagnostic imaging, radiographic examination in the early stages of degeneration is often futile, however, MRI shows degeneration well before it becomes apparent on plain film.¹¹ The purpose of this case presentation is to highlight the successful conservative management of a patient with chronic degenerative knee pain.

Case Presentation

An active 47 year-old female office-worker presented with a chief complaint of intermittent right knee pain inferolateral to the right patella with no radiation. She rated her pain as a 5 out of 10 on a verbal pain-rating scale (VPRS) at rest and 7 out of 10 with certain activities, including ascending stairs, sitting on a chair for one hour, squatting, and is worse by the end of each day. She explained that she had struck her right knee in multiple slip-and-fall accidents while vacationing in Mexico eight months prior and her knee had not been doing well since then. She did not seek out treatment while on vacation. She further noted that her right knee had given out on her twice in the past five months and is no longer confident about the overall stability of her knee. She denied experiencing crepitus or any locking sensations. She was prescribed a series of medication including Ibuprofen, Naproxen, Arthrotec, Diclofenac, and Flexeril but found no relief. She scored 46 out of 80 on the Lower Extremity Functional Scale (LEFS), which translates to 57.5% maximal function. The LEFS is a self-report measure designed to assess the functional status of patients with any musculoskeletal condition related to the lower extremity and has demonstrated acceptable validity on outpatients.¹² She enjoys participating in long distance walks up to 10 kilometers and actively follows a resistance and aerobic training regimen. A systems review was unremarkable with respect to her chief complaint. An MRI study of her right knee ordered by her medical doctor was conducted seven weeks prior to presenting to our clinic and revealed focal severe chondral thinning with deep chondral fissuring and reactive subchondral cystic change over the patellar apex and medial patellar facet with associated full-thickness chondral fissuring over the medial trochlea;

moderate chondral thinning and chondral fissuring of articular cartilage in the medial femorotibial compartment; small to moderate-sized knee joint effusion with mild synovitis; no convincing meniscal tear.

Upon physical examination, the patient could heel-toe and tandem walk without any difficulty. She could perform ten toe pushups bilaterally, plantar responses were downgoing and there was no evidence of ankle clonus. All myelopathic lesion tests were unremarkable when performed throughout the lower extremities bilaterally. Focused orthopedic examination of her bilateral knees was performed with respect to the right knee. Double- and single-legged squat tests revealed 20 degrees of knee flexion with valgus deflection bilaterally, recreating the pain of chief complaint at the end range. Varus, valgus, anterior and posterior drawer, medial and lateral Slocum, Lachman's, and lateral pivot shift tests were negative bilaterally. The right medial joint line was tender and Thessaly's test was positive on the right and recreated the pain of chief complaint at the right posteromedial area. Clarke's test was also positive on the right and digital palpation of the medial and lateral patellar articular facets caused pain. Stressing the posterolateral corner with varus at zero and thirty degrees knee flexion, Dial, and reverse pivot shift tests were negative. Palpation of the right posterior horn revealed tenderness. The patient was diagnosed with a suspected chronic degenerative tear of the posterior horn of the right medial meniscus and chronic right subpatellar chondrosis, in addition to the extensive cartilaginous degeneration noted in the previously performed MRI study.

The patient underwent a course of treatment twice per week for three weeks. Her plan of management included instrument-assisted soft tissue mobilization (IASTM) of the right peripatellar capsule and anterior knee capsule mobilizations and manipulation¹³ (to patient tolerance). IASTM was performed over the right medial and lateral patellar retinaculum, as well as the right quadriceps and patellar tendons for approximately five minutes in total at a superficial depth. This was followed by two intra-articular and subpatellar leukocyte-rich platelet-rich plasma (L-PRP) injections six weeks apart. The patient responded very well to this plan of care, reporting a VPRS rating of 4 out of 10 at worst and 59 out of 80 on the LEFS (73.8% maximal function) during a subsequent visit three weeks after the initial treatment visit. The patient returned for follow-up visits five, eleven, and sixteen weeks after the

initial treatment visit. VPRS ratings reported by the patient were 3, 2, and 0 out of 10, respectively. The first PRP injection took place twelve weeks after the first treatment visit. Two days prior to receiving the first PRP injection (PRP1) the patient scored 60 out of 80 on the LEFS (75% maximal function). The second PRP injection (PRP2) took place six weeks later. At one, three, and six months post-PRP2 injection LEFS scores were reported by the patient and were recorded as 72 out of 80 (90% maximal function) and 73 out of 80 (91.3% maximal function), and 75 out of 80 (93.75% maximal function), respectively.

The successive ultrasound-guided L-PRP injections were performed by a naturopathic doctor using the following protocol: 8 cc citrate dextrose solution was drawn up in 60 cc syringe; 2 cc was placed in the side cup of the harvest preparation PRP kit; 54 cc blood was drawn from the right antecubital vein at a rate of 1 mL per second; the kit was placed in the harvest machine and run for 14 minutes; platelet-poor plasma (PPP) was drawn off and remaining the PRP/PPP mixture was mixed and placed in a syringe, as per standard harvest preparation protocol. 1 cc Traumeel was mixed with the PRP mixture; 15 cc 50-gamma ozone was mixed with PRP/PPP solution for 30 seconds; the knee was prepared with a betadine swab and left for 5 minutes before being swabbed with chlorhexadine. 1% lidocaine was placed subcutaneously at the injection site to access the subpatellar space; a 21 gauge 1.5 inch needle with 10 cc syringe using 5 cc saline was used to locate the joint space; the patella was pushed laterally and needle was placed under the rim and advanced 2 cm. Once good flow was established using 2 cc saline, the syringe was replaced with the PRP/PPP/Ozone mixture and was placed using the same needle in location. The patella was gently moved from side to side mixing the PRP mixture into the subpatellar space. The patient was then asked to lay supine for one hour with her right leg raised 30 degrees. The patient was asked to follow the same instructions for the next 24 hours. There were no complications with the procedure.

Discussion

In this case, the first three weeks of treatment consisted of instrument-assisted soft tissue mobilization (IASTM) of the peripatellar capsule on the right. Over this period of time, the patient's VPRS score decreased from seven to four out of 10 and reported a 16.3% improvement in func-

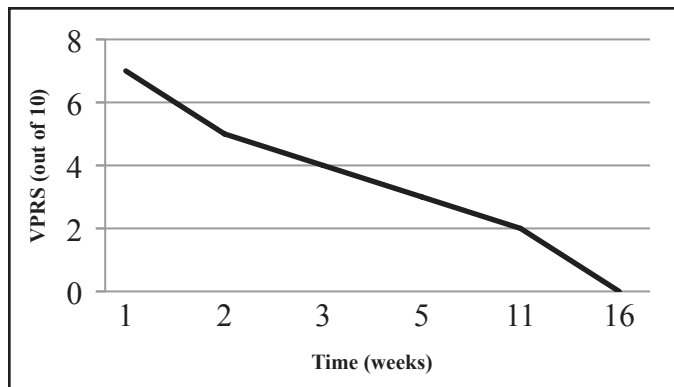


Figure 1.

Patient-reported Verbal Pain Rating Scale (VPRS) scores out of 10 over the course of sixteen weeks, including the initial three-week trial of care and subsequent PRP injections.

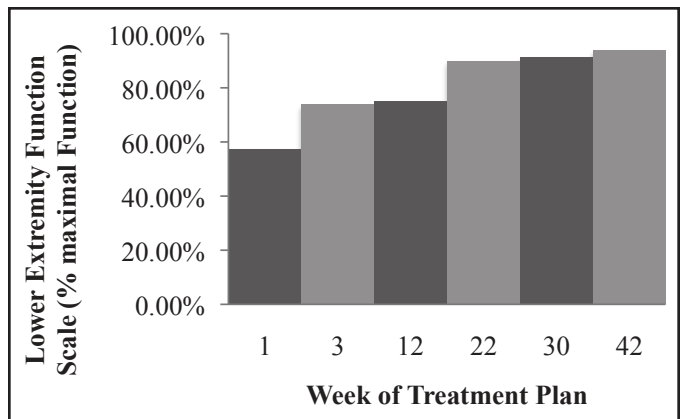


Figure 2.

Patient-reported Lower Extremity Functional Scale (LEFS) scores portrayed as percent of maximal function over the course of thirty weeks, including the initial three-week trial of care, pre-PRP1, 1-month post-PRP2, and 3 months post-PRP2.

tion (LEFS) as depicted in Figure 1. IASTM is a common treatment method for myofascial restriction and is applied using various instruments designed to provide a mobilizing effect to improve range of motion, function, and to decrease pain.^{14,15} While the body of knowledge with respect to IASTM continues to grow, it is currently thought to stimulate connective tissue remodeling via resorption of excessive fibrosis, in addition to inducing repair and regeneration of collagen secondary to fibroblast recruitment.^{16,17} This in turn, will result in the release and breakdown of adhesions, fascial restrictions, and scar tissue.^{17,18} There are various IASTM tools and companies that bring their own approach to instrument design and treatment protocol, however, despite the variation between approaches or school's of thought, the overarching notion behind IASTM is to enhance myofascial mobility with limited adverse effects to the patient.¹⁹⁻²³ With respect to efficacy, a recent systematic review by Cheatham²⁴ whose intent was to appraise the current evidence examining the effects of IASTM as an intervention to treat a musculoskeletal pathology or to enhance joint ROM found that overall, IASTM groups displayed equal improvement when compared with control or comparison groups. Unfortunately, as Cheatham²⁴ notes, the "heterogeneity and paucity" among current IASTM studies makes it difficult

to form meaningful clinical implications. The variability in study methods including the population, type of IASTM intervention, dosage time, and outcome measures make it challenging to determine the optimal treatment protocol.

Prior to receiving her first PRP injection (PRP1), the patient reported 75% functionality. Six months after PRP2, she reported 93.75% functionality, as shown in Figure 2. PRP injections are an expanding therapeutic approach that have been used in an ever-expanding variety of clinical contexts, including orthopaedic surgery, sport medicine, dentistry, dermatology, and plastic surgery.²⁵⁻²⁷ Platelets are cells that contain over 300 bioactive proteins and growth factors that control revascularization, cell growth and differentiation, in addition to synthesis of connective tissue.²⁸ The mechanism of PRP action is elusive, however, Sanchez²⁹ maintains that several biological pathways may lead to clinical efficacy. PRP targets multiple regenerative processes by reaching different cell phenotypes and diluting and replacing pro-inflammatory cytokines with anabolic growth factors, therefore being able to modulate angiogenesis and inflammation.³⁰ Among the growth factors and cytokines that are relevant to osteoarthritis, PRP delivers platelet-derived growth factor-AB, transforming growth factor- β 1, vascular endo-

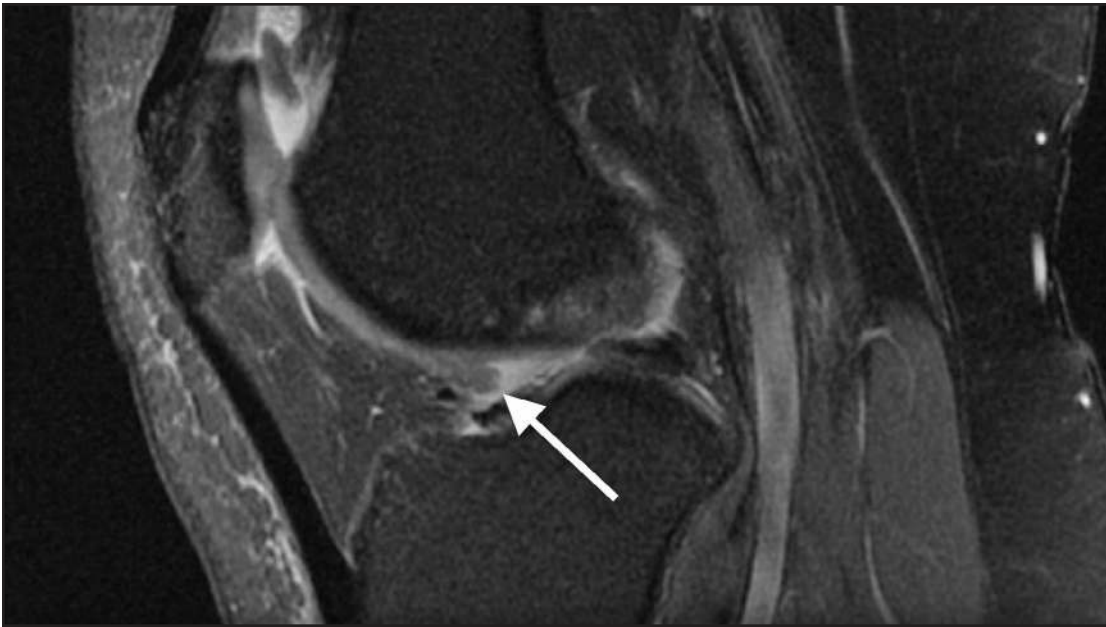


Figure 3.
MRI image (sagittal plane) depicting chondral fissuring and cartilaginous discontinuity in the patient presented in this case report.

thelial growth factor-A, hepatocyte growth factor, and insulin-like growth factor.²⁹ PRP also contains tissue inhibitors of metalloproteases (TIMP-1, TIMP-2, TIMP-3, and TIMP-4) and β -thromboglobulin, which neutralizes the action of destructive metalloproteases.³¹

Studies examining the effect of PRP *in vitro* demonstrated a strong positive effect on chondrocyte proliferation rate and an increase in chondrocyte marker expression; however, the maintenance of this expression has not been unanimously confirmed^{32,33}. Encouraged by results found *in vitro*, numerous case series and comparative trials utilizing different protocols have shown a positive effect of PRP, leading to an overall improvement of the symptoms.³⁴ When compared to hyaluronic acid, Liu³⁵ found that better histological outcomes and restoration of subchondral bone were achieved with pure PRP. Further to this, pure PRP was found to reduce inflammation in the joint cavity more than hyaluronic acid. As a result, Liu³⁵ concluded that pure PRP may be used as a potential substitute to hyaluronic acid in treating degeneration or damage of articular cartilage. A randomized controlled trial conducted by Patel³⁶ investigated the effect of PRP versus a saline placebo in early knee osteoarthritis and found that PRP was effective in the short term with respect to relieving pain, stiffness, and knee function, however, noted

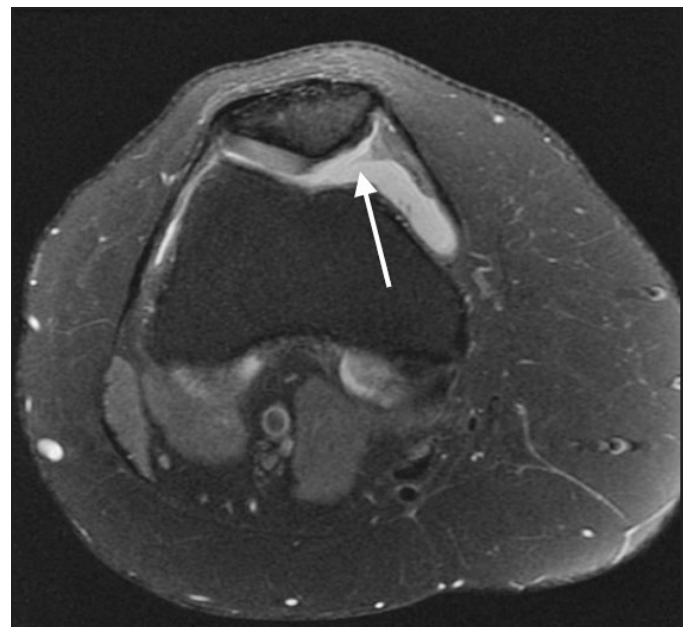


Figure 4.
MRI image (axial plane) showing direct visualization of subpatellar chondral thinning in the patient presented in this case report.

that the effect tends to taper off over time, leaving the option of future staged injections as a potential therapeutic regimen. The literature is limited in studies examining the efficacy of PRP on advanced OA, however, a recent study conducted by Jubert² found that in patients with late-stage knee OA, a single PRP intra-articular injection is effective for relieving pain and improving activity of daily living and quality of life.

Traumeel and ozone were added to the PRP mixture in an effort to mitigate pain and inflammation. Nonsteroidal anti-inflammatory drugs (NSAIDs) are commonly relied upon to limit inflammation and control pain. Traumeel is a fixed combination of biological and mineral extracts, which aims to apply stimuli to multiple targets to restore normal functioning of regulatory mechanisms and is also used to treat the symptoms associated with acute musculoskeletal injuries, including pain and swelling.³⁷⁻⁴⁰ Traumeel has shown comparable effectiveness to NSAIDs in terms of reducing symptoms of inflammation, accelerating recovery, and improving mobility, with a favourable safety profile.³⁷⁻⁴⁰ Oxygen-ozone (O₂O₃) is a mixture of Oxygen (O₂) and Ozone (O₃) that has been used in the treatment of several painful conditions, such as low back pain and lumbar disc herniation.^{41,42} This gas combination is produced from pure O₂ passing through a high-voltage gradient (5-13 mV) in a medical generator. The analgesic and anti-inflammatory effects of O₂O₃ seem to be due to ozone's intrinsic chemical properties.⁴³ Gracer and Bocci⁴⁴ noted that when ozonated blood is re-injected into an inflamed tissue, it leads to a normalization of metabolism, cell proliferation, and synthesis of extracellular matrix. This activation of either erythrocytes or platelets enhances both delivery of oxygen and release of growth factors.⁴⁵

Summary

Knee complaints can represent an important part of a chiropractic practice. Due to the incongruence of its articular surfaces combined with its weight-bearing function makes the knee a common site for degenerative joint changes. In this case, IASTM coupled with PRP injections may have improved the patient's pain and function to 0 out of 10 and 93.75%, respectively. The body of knowledge surrounding IASTM is still emerging, however, current evidence demonstrates equal improvements when compared to control or comparison groups. IASTM

is thought to stimulate connective tissue remodeling and the repair and regeneration of collagen resulting in the release and breakdown of adhesions, fascial restrictions, and scar tissue. Furthermore, in the presence of PRP, chondrocytes have recurrently demonstrated favourable growth and survivability parameters.⁴⁵ This may be due to PRP's three known biological properties succinctly summarized by Xie³²: (1) PRP has an anabolic effect on chondrocytes, mesenchymal stem cells, and synoviocytes; (2) PRP may act as bioactive cell scaffold; (3) PRP has the potential to inhibit inflammation and alleviate osteoarthritis symptoms. This case demonstrated the successful implementation and management of a conservative plan of management for chronic degenerative knee pain. The generalizability of this treatment plan may be limited as it represents only a single case and consideration should be made for other factors that may have resulted in the patient's positive outcome.

References

1. National Board of Chiropractic Examiners (Internet). Greeley, CO: National Board of Chiropractic Examiners; 2015 (accessed February 20 2017). Available from: <http://www.nbce.org/practiceanalysis/>
2. Jubert NJ, Rodriguez L, Reverte-Vinaixa MM, *et al.* Platelet-rich plasma injections for advanced knee osteoarthritis: a prospective, randomized, double-blinded clinical trial. *Orthop J Sports Med.* 2017; 5(2).
3. Allen K, Golightly YM. Epidemiology of osteoarthritis: state of the evidence. *Curr Opin Rheumatol.* 2015; 27: 276-283.
4. Neogi T, Zhang Y. Epidemiology of osteoarthritis. *Rheum Dis Clin North Am.* 2013; 39: 1-19.
5. Prieto-Alhambra D, Judge A, Javaid MK, Cooper C, Diez-Perez A, Arden NK. Incidence and risk factors for clinically diagnosed knee, hip and hand osteoarthritis: influences of age, gender and osteoarthritis affecting other joints. *Ann Rheum Dis.* 2014; 73: 1659-1664.
6. Yu D, Peat G, Bedson J, Jordan KP. Annual consultation incidence of osteoarthritis estimated from population-based health care data in England. *Rheumatol (Oxford).* 2015; 54: 2051-2060.
7. Moore KL, Dalley AF, Agur AMR. Clinically oriented anatomy. 6th ed. Philadelphia: Lippincott Williams & Wilkins; 2010.
8. Yochum TR, Rowe LJ. Essentials of skeletal radiology. 3rd ed. Philadelphia: Lippincott Williams & Wilkins; 2005.
9. Mont MA, Radjadyaksha AD, Low K, LaPorte DM, Hungerford DS. Anatomy of the knee extensor mechanism: correlation with patellofemoral arthrosis. *J South Orthop Assoc.* 2001; 10(1): 24.

10. Kerrigan DC, Lelas JL, Karvosky ME. Women's shoes and knee osteoarthritis. *Lancet*. 2001; 357(9262): 1097.
11. Bergman AG, Willen HK, Lindstrand AL, Pettersson HT. Osteoarthritis of the knee: correlation of subchondral MR signal abnormalities with histopathologic and radiographic features. *Skeletal Radiol*. 1994; 23: 445.
12. Yeung TSM, Wessel J, Stratford P, Macdermid J. Reliability, validity, and responsiveness of the lower extremity functional scale for inpatients of orthopaedic rehabilitation ward. *J Orthop Sports Phys Ther*. 2009; 39(6): 468-477.
13. Gleberzon B, Ross K. Manual of diversified diagnostic and therapeutic procedures. Toronto: Candian Memorial Chiropractic College; 2010.
14. Baker RT, Hansberger BL, Warren L, *et al*. A novel approach for the reversal of chronic apparent hamstring tightness: a case report. *Int J Sports Phys Ther*. 2015; 10(5): 723-733.
15. Baker RT, Nasypany A, Seegmiller JG, *et al*. Instrument-assisted soft tissue mobilization treatment for tissue extensibility dysfunction. *Int J Athl Ther Training*. 2013; 18(5): 16-21.
16. Howitt S, Jung S, Hammonds N. Conservative treatment of a tibialis posterior strain in a novice triathlete: a case report. *J Can Chiropr Assoc*. 2009; 53(1): 23-31.
17. Strunk RG, Pfefer MT, Dube D. Multimodal chiropractic care of pain and disability for a patient diagnosed with benign joint hypermobility syndrome: a case report. *J Chiropr Med*. 2014; 13(1): 35-42.
18. Papa JA. Conservative management of De Quervain's stenosing tenosynovitis: a case report. *J Can Chiropr Assoc*. 2012; 56(2): 112-120.
19. Stow R. Instrument-assisted soft tissue mobilization. *Int J Athl Ther Train*. 2011; 16(3): 5-8.
20. Markovic G. Acute effects of instrument assisted soft tissue mobilization vs. foam rolling on knee and hip range of motion in soccer players. *J Bodyw Mov Ther*. 2015; 19(4): 690-696.
21. Lee MS, Choi T-Y, Kim J-I, *et al*. Using Guasha to treat musculoskeletal pain: a systematic review of controlled clinical trials. *Chinese Med*. 2010; 5:5.
22. Amshel CE, Caruso DM. Vietnamese "coining": a burn case report and literature review. *J Burn Care Rehabil*. 2000; 21(2): 112-114.
23. Odhav A, Patel D, Stanford CW, *et al*. Report of a case of Gua Sha and an awareness of folk remedies. *Int J Dermatol*. 2013; 52: 892-893.
24. Cheatham SW, Lee M, Cain M, *et al*. The efficacy of instrument-assisted soft tissue mobilization: a systematic review. *J Can Chiropr Assoc*. 2016; 60(3): 200-210.
25. Anitua E, Sanchez M, Nurden AT, *et al*. New insights into and novel applications for platelet-rich fibrin therapies. *Trends Biotechnol*. 2006; 24(5): 227-234.
26. Cole BJ, Seroyer ST, Filardo G, *et al*. Platelet-rich plasma: where are we now and where are we going? *Sports Health*. 2010; 2(3): 203-210.
27. Mei-Dan O, Mann G, Maffulli N. Platelet-rich plasma: any substance into it? *Br J Sports Med*. 2010; 44(9): 618-619.
28. Wesner M, Defreitas T, Bredy H, *et al*. A pilot study evaluating the effectiveness of platelet-rich plasma therapy for treating degenerative tendinopathies: a randomized control trial with synchronous observational cohort. *PLoS ONE*. 2016; 11(2): e0147842.
29. Sanchez M, Guadilla J, Fiz N, *et al*. Ultrasound-guided platelet-rich plasma injections for the treatment of osteoarthritis of the hip. *Rheumatology*. 2012; 51: 144-150.
30. Andia I, Sanchez M, Maffulli N. Tendon healing and PRP therapies. *Expert Opin Biol Ther*. 2010; 10: 1415-1426.
31. Richette P, Ravaud P, Conrozier T, *et al*. Effect of hyaluronic acid in symptomatic hip OA: a multicenter randomized, placebo controlled trial. *Arthritis Rheum*. 2009; 60: 824-830.
32. Xie X, Zhang C, Tuan R. Biology of platelet-rich plasma and its clinical application in cartilage repair. *Arthritis Res Ther*. 2014; 16:204.
33. Vannini F, Di Matteo B, Filardo G. Platelet-rich plasma to treat ankle cartilage pathology – from translational potential to clinical evidence: a systematic review. *J Exp Orthop*. 2015; 2:2.
34. Marmotti, A, Rossi R, Castoldi F, *et al*. PRP and articular cartilage. *BioMed Res Int*. 2014; 2015.
35. Liu J, Song W, Yuan T, *et al*. A comparison between platelet-rich plasma (PRP) and hyaluronate acid on the healing of cartilage defect. *PLoS ONE*. 2014; 9(5): e97293.
36. Patel S, Dhillon MS, Aggarwal S, *et al*. Treatment with platelet-rich plasma is more effective than placebo for knee osteoarthritis. *Am J Sports Med*. 2013; 41(2): 356-364.
37. Schnieder C. Traumeel – an emerging option to nonsteroidal anti-inflammatory drugs in the management of acute musculoskeletal injuries. *Int J Gen Med*. 2011; 4: 225-234.
38. Arora S, Harris T, Scherer C. Clinical safety of a homeopathic preparation. *Biol Ther*. 2000; 18: 222-225.
39. Lussignoli S, Bertani S, Metelmann H, *et al*. Effect of Traumeel S, a homeopathic formulation, on blood-induced inflammation in rats. *Complement Ther Med*. 1999; 7(4): 225-230.
40. Porozov S, Cahalon L, Weiser M, *et al*. Inhibition of IL-1beta and TNF-alpha secretion from resting and activated human immunocytes by the homeopathic medication Traumeel S. *Clin Dev Immunol*. 2004; 11(2): 143-149.
41. Paoloni M, Di Sante L, Cacchio A, *et al*. Intramuscular oxygen-ozone therapy in the treatment of acute back pain with lumbar disc herniation: a multicenter, randomized, double-blind, clinical trial of active and simulated lumbar paravertebral injection. *Spine*. 2009; 34: 1337-1344.

42. Murphy K, Elias G, Steppan J, *et al.* Percutaneous treatment of herniated lumbar discs with ozone: investigation of the mechanisms of action. *J Vasc Interv Radiol.* 2016; 27: 1242-1250.
43. Invernizzi M, Stagno D, Carda S, Grana E, Picelli A, *et al.* Safety of intra-articular oxygen-ozone therapy compared to intra-articular sodium hyaluronate in knee osteoarthritis: a randomized single blind pilot study. *Int J Phys Med Rehabil.* 2017; 5: 385
44. Gracer RI, Bocci V. Can the combination of localized “proliferative therapy” with “minor ozonated autohemotherapy” restore the natural healing process? *Med Hypotheses.* 2005; 65: 752-759.
45. Durant TJS, Dwyer CR, McCarthy MR, *et al.* Protective nature of platelet-rich plasma against chondrocyte death when combined with corticosteroids or local anesthetics. *Am J Sports Med.* 2016; 45(1): 218-224.