COMPARISON OF LASER CLASS 1M AND CLASS 4 APPLICATIONS IN CANINE CRANIAL CRUCIATE LIGAMENT RUPTURE EVALUATED BY CLINICAL OUTCOMES AND ULTRASONOGRAPHY.

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A Thesis Submitted in Partial Fulfillment of the Requirements
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การเปรียบเทียบการใช้เลเซอร์ คลาส 1 เอ็มและคลาส 4 ในสุนัขที่มีภาวะเอ็นไขว้หน้าข้อเข่าขาด โดยประเมินทางคลินิกและภาพวินิจฉัยโดยคลื่นเสียงความถี่สูง

นายนพกฤษฎ์ เอกอัครวรพงศ์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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By	Mr. Noppakrit Ekakaraworapong
Field of Study	Veterinary Surgery
Thesis Advisor	Assistant Professor KUMPANART SOONTORNVIPART, Ph.D.

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นพกฤษฎ์ เอกอัครวรพงศ์ : การเปรียบเทียบการใช้เลเซอร์ คลาส 1 เอ็มและคลาส 4 ในสุนัขที่มีภาวะเอ็นไขว้หน้าข้อเข่าขาด โดยประเมินทางคลินิกและภาพวินิจฉัยโดยคลื่นเสียงความถี่สูง. (COMPARISON OF LASER CLASS 1M AND CLASS 4 APPLICATIONS IN CANINE CRANIAL CRUCIATE LIGAMENT RUPTURE EVALUATED BY CLINICAL OUTCOMES AND ULTRASONOGRAPHY.) อ.ที่ปรึกษาหลัก : ผศ. ดร.กัมปนาท สุนทรวิภาต

การศึกษานี้มีจุดประสงค์ในการทดสอบประสิทธิภาพของเลเซอร์ 2 ชนิด ในการรักษาภาวะข้อเสื่อมที่เกิดจากเอ็นไขว้หน้าข้อเข่าขาดก่อนการทำศัลยกรรม TPLO โดยทำการศึกษาในสุนัขที่มีภาวะเอ็นไขว้หน้าข้อเข่าขาดจำนวน 19 ตัว โดยการทดลองจะแบ่งออกเป็นระยะเวลา 4 สัปดาห์ต่อเนื่อง และแบ่งสุนัขออกเป็น 3 กลุ่ม คือ กลุ่มควบคุม จำนวน 4 ตัว กลุ่มที่ใช้เลเซอร์ คลาส 1 เอ็ม จำนวน 8 ตัวและกลุ่มที่ใช้เลเซอร์ คลาส 4 จำนวน 7 ตัวสุนัขทุกตัวจะได้รับการประเมินทางคลินิกซึ่งประกอบด้วย การประเมินระดับการเดินกะเผลก การวิเคราะห์น้ำไขข้อ การประเมินภาวะความเจ็บปวดโดยใช้แบบสอบถาม และประเมินด้วยภาพวินิจฉัยโดยคลื่นเสียงความถี่สูง โดยการประเมินภาพวินิจฉัยโดยคลื่นเสียงความถี่สูงจะทำการ ประเมินลักษณะต่างๆ โดยการให้คะแนน ซึ่งจะให้คะแนนในหัวข้อ คือ คะแนนน้ำในข้อเข่า คะแนนกระดูกอ่อน และคะแนนผิวกระดูก และประเมินด้วยภาพวินิจฉัยโดยคลื่นเสียงความถี่สูงในวันแรกร่วมกับการเจาะน้ำไขข้อมาตรวจวิเคราะห์และทำแบบประเมินภาวะความเจ็บปวดโดยใช้แบบสอบถาม หลังจากนั้นจะถูกแบ่งออกไปในแต่ละกลุ่มเพื่อทำการทำหัตถการตามแต่ละกลุ่มทดลองนั้นๆ ในส่วนของกลุ่มควบคุมจะได้รับยาแก้ปวดและในส่วนของกลุ่มเลเซอร์ทั้ง 2 คลาส จะทำตามแบบแผนการรักษาโดยจะทำเลเซอร์รักษาทั้งหมด 6 ครั้ง ประกอบด้วยสัปดาห์แรก 3 ครั้ง สัปดาห์ที่สอง 2 ครั้งและสัปดาห์ที่สาม 1 ครั้งและสัปดาห์สุดท้ายเป็นการประเมินด้วยคลื่นเสียงความถี่สูง เจาะน้ำไขข้อ การประเมินระดับการกะเผลก ทำแบบประเมินความเจ็บปวดในสุนัข ผลการศึกษาพบว่าการใช้เลเซอร์ทั้ง 2 คลาสมีความสามารถในการจัดการความเจ็บปวดและลักษณะการแสดงทางคลินิกได้เทียบเท่าการให้ยาแก้ปวด ซึ่งถือเป็นข้อดีในการลดการใช้ยาในสุนัขที่มีภาวะความผิดปกติของระบบอื่นๆ ร่วมด้วย

สาขาวิชา ศัลยศาสตร์ทางสัตวแพทย์ ลายมือชื่อนิสิต .....

ปีการศึกษา 2563 ลายมือชื่อ อ.ที่ปรึกษาหลัก .....

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Nineteen dogs diagnosed of cranial cruciate ligament rupture were included in this study before TPLO was performed. They were classified into 3 groups which consisted of control, low-level laser therapy class 1M and low-level laser therapy class 4 groups. The number of each group were 4, 8 and 7 dogs, respectively, and the duration of treatment protocol was 4 weeks consecutively. On the first day of the study, dogs were examined and assessed of the clinical signs such as lameness score, synovial fluid analysis, canine brief pain inventory questionnaire (CBPI) and ultrasonographic score. In ultrasonographic monitoring, ultrasonographic scores on synovial effusion, articular cartilage and bone surface were given. The control group received only NSAIDs while low-level laser therapy group received 3 weeks-protocol; the first week consisted of 3 visits alternatively, the second week was 2 visits alternately and the last week was 1 visit. On the last week of visit, clinical outcomes and ultrasonographic monitoring were performed. The results revealed low-level laser therapies either class 1M or class 4 had a potential of pain-relief and reducing inflammation in the same level of using NSAIDs. Additionally, dogs with unhealthy condition were taking advantage of lessening the adverse effect of drugs. The results of this study shown that two classes of low-level laser therapy had an efficacy in pain control and relieve clinical sign as the level of giving NSAIDs which benefit in unhealthy dogs' conidition.

Field of Study: Veterinary Surgery Student's Signature .....

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CHAPTER I

INTRODUCTION

Important and rationale

Low-level laser therapy (LLLT) has recently gained more popularity recently in veterinary rehabilitation. It has been used in many diseases for various purposes such as wound management, inflammation alleviation or pain relief. Controversial issues among LLLT doses are described for instance. Although there are many low-level laser therapy classes available in the small animal practice such as class I, III and IV and the capabilities of those low-level laser therapy devices are similar, however there are some differences among low-level laser therapy classes thus our study aims to evaluate those effect. The class I low-level laser therapy does no harm to the human or animal retina while low-level laser class IV does. In the application of using laser therapy devices in the hospital, the low-level laser class IV needs to have the individual room to operate, and the operator should always wear protecting glasses to prevent laser light get directly to the retina which causes permanent damage while class I is much more convenient. Nevertheless, both of class I and IV low-level laser therapy are non-invasive technique which can be done in any unhealthy condition patients. In orthopedics fields, there are many patients who cannot have surgery due to their health concerned problems.

One of the most common diseases that can frequently occurred in old age dogs is cranial cruciate ligament diseases. The incidence of canine cranial cruciate ligament disease was 56% of all lameness cases (Taylor-Brown et al., 2015). Canine cranial cruciate ligament rupture has the potency to induce the inflammation in the stifle joint and subsequently the osteoarthritis (OA). Synovial membrane plays an important role in the pathophysiology of osteoarthritis (Alves et al., 2013). Inflammation induces synovial cells to produce cytokines such as interleukin-1β (IL-

1β) and tumor necrosis factor alpha (TNF-α) and chemokines as well as matrix metalloproteinases. These cytokines and chemokines can be found in synovial fluid. The Low-level laser therapy has great potential utility in regulating interleukin and inflammatory mediator expression (Yamaura et al., 2009). Furthermore, it can also reduce inflammatory signs and symptoms in osteoarthritis (Alves et al., 2013). This study aims to evaluate laser therapy class 1M and 4 efficacies in pain relief and reduction of inflammation on cranial cruciate ligament rupture dogs having unhealthy condition before undergoing surgery.

CHAPTER II

LITERATURE REVIEW

2.1 Low-Level laser therapy

Low-level laser therapy (LLLT) has been used in veterinary fields for more than many decades. The laser is being used in rehabilitation to modulate the cellular function of the cells. The mechanism process is known as the photobiostimulation. The laser therapy can modulate a photon acceptor or chromophore resulted in biological processes, for example, mitochondrial respiration and adenosine triphosphate synthesis, wound and joint healing acceleration and muscle regeneration. A chromophore is a molecule that absorbs light such as chlorophyll, hemoglobin, myoglobin, cytochrome c oxidase, other cytochromes, flavin, and flavoproteins or porphyrins. Acute and chronic pain have also been treated by laser therapy. Properties of the laser and light are similar, but the origin of the wave is different. Laser is originated from an artificial light source which might be the elements that emit the photon by excited state of the electrons, such as helium-neon, gallium-arsenide, or gallium-aluminum-arsenide. Laser light is monochromatic, coherent, and collimated. With these properties LLLT can be penetrated through the skin surface without heating effects, no skin damage with few or no side effects.

Figure 1 A. Laser light is collimated. B. Natural light is divergent.

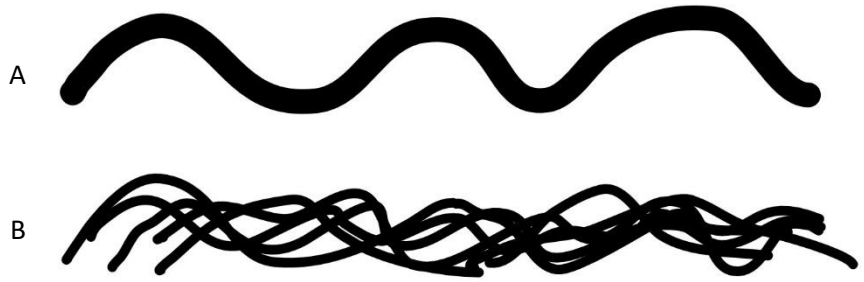


Figure 2 A. Laser light is coherent. B. Normal light is non-coherent.

Wavelength of the photons determines the effect of laser therapy. The target wavelength is approximately 600-1,200 nanometers which called the optical window that photons mainly absorbed by protein, nucleic acid, melanin, hemoglobin, and myoglobin. The optimum wavelength has been estimated at 810 nm (Cotler et al., 2015). Mitochondrial duty is to convert food molecules and oxygen into adenosine triphosphate (ATP) by phosphorylation. Cytochrome c oxidase is the primary photo-acceptor for the red-NIR wavelength (Wong-Riley et al., 2005). Nitric oxide (NO) produced in mitochondria can inhibit respiration by binding to COX and displace oxygen especially in injured or hypoxic cells. LLLT is proposed to have the potency of reversing the binding of NO and COX (Lane, 2006). The most important signaling in LLLT is ATP, cyclic AMP, NO and reactive oxygen species (ROS).

The biological effects of the laser therapy are also included the reduction of cyclooxygenase and prostaglandin E_2 production. Low-level laser therapy is highly effective of treating inflammatory arthritis with the correlation of inflammatory marker serum Prostaglandin E_2 reduction (Castano et al., 2007). LLLT has also shown the potency of enhancing cell proliferation of fibroblasts, keratinocytes, endothelial cells and lymphocytes by stimulation via mitochondrial respiration then leading to increasing growth factors (Wong-Riley et al., 2005). LLLT can stimulate neovascularization, and increase collagen synthesis in healing of acute and chronic wounds (Saygun et al., 2012).

To minimize all side effects of low-level laser therapy, the patients will not get any pain from the procedure comparing to acupuncture or force-feeding drugs. Low-level laser therapy shown the effect of reducing the inflammatory cytokine such as IL-1, IL-6, TNF-α, Metalloproteinases (MMP) and cox-2 enzyme (Laraia et al., 2012). The peripheral nerve endings may play a role in pain control as well. Nociceptors of myelinated A∂ and unmyelinated, slow-conducting C fibers lie within the epidermis. The direct contact of LLLT stimulated on the epidermal neural network. LLLT had the ability of inhibition of action potentials which had the 30% neural blockade within 10 to 20 minutes of application, and was reversed within about 24 hours (Bashiri, 2013). LLLT can have short-, medium- and long-term effects. Short term effects were neural blockade of the peripheral and sympathetic nerves and the release of neuromuscular contractions leading to a reduction of muscle spasms (Carrasco et al., 2009). The medium-term effects were to decrease local edema and a reduction of inflammation within hours to days (Carati et al., 2003). The long-term effect is to improve in tissue healing. While the disadvantages of using low-level laser therapy were never use on thyroid gland, cancer or tumor and pregnancy.

The power of laser is in a unit of time and expressed in watts. When applying to the surface area, it will be also called the power density or intensity. When combining the power and the surface area, it will be clinically called dose of laser energy. The classification is based on wavelength and power in the unit of watt. Dosage of anti-inflammatory effect is applied 1 to 6 J/cm^2 for acute and subacute. The dosage of analgesic effects for joint pain in acute pain was 4 to 6 J/cm^2 (Millis and Levine, 2013). The recommendation of treatment was 1-3 J/cm^2 daily for the first 7-10 days with a 1- to 2-day break (Millis and Levine, 2013). Recommendations for arthritic joints had ranged from 4 J/cm^2 to 30 J/cm^2 for the total of dosage (Millis and Levine, 2013).

Low level laser therapy is mainly divided into 4 classes, class 1, 2, 3 and 4 by power and wavelength. Class 1 is safe under all conditions of normal use such as supermarket scanners. Class 1M is also safe for all uses except when pass through a lens. The power of Class 1M is less than that of a Class 3B but cannot damage the retina unless focused. Class 2 laser is in the visible-light spectrum (400-700 nm) such

as a laser pointer. As for a visible light, laser Class 2 can generate blink reflex when expose the light less than 0.25 seconds. Class 2 lasers are limited to 1 mW continuous wave or emission time less than 0.25 seconds. Class 3B is either continuous light in the 315 nm to far infrared ranges but limits to 500 mW or pulsed laser 400-700 nm wavelength and limits to 30 mW. Protective eyewear is required when using Class 3B or above. Class 4 has the greatest potential to cause tissue damage due to the highest power. The power is typically around 1 to 15 W. Class 4 laser may cause permanent eye damage or skin burnt.

Class 1M, which M stands for multiradiant, diode is made of Gallium-Aluminum-Arsenide. It has multiple wavelengths of 905, 875, 640, 470 nm and the maximal power is 50 watts. It emits the laser as super pulse mode. Super pulse mode emits a series of radiation impulses with high amplitude in an extremely short duration typically 100 to 200 nanoseconds. It can provide the treatment with high concentration of photons, driven deeper into the target tissue without any risk of overheating. It stimulates blood microcirculation and reduces pain. In clinical studies, Super pulsed lasers beat Class 4 lasers in safety, controlling inflammation mitigating pain, infection control and wound care, setting a new standard for veterinary laser therapy. It can penetrate through various depth of tissue because of its multiple wavelengths.

Class 4 has the wavelength of 650, 808 and 905 nm and the maximal power of 1.2 watts. It emits the laser as continuous super-pulse, combined and synchronized MLS impulse. The wavelength of 808 nm has an anti-edemic and anti-inflammatory effect. The wavelength of 905 nm has an analgesic effect. There are synergistic energy combining the effects between 808 nm and 905 nm while minimizing the risk of thermal damage by synchronizing the continuous and pulsed emission. It can penetrate through the depth of 2-4 cm (Hudson et al., 2013).

In canine cranial cruciate ligament patient receiving low-level laser therapy also get better result in returning to function of the leg than the control group (Rogatko et al., 2017). Some of the study cannot conclude whether low-level laser therapy after tibial plateau leveling osteotomy in dogs improve the clinical outcome or not (Renwick et al., 2018). In this study, author is interested in using class 1M and class 4.

2.2 Osteoarthritis

Osteoarthritis (OA) is a chronic progressive disease that worsen the quality of life (Vijarnsorn et al., 2019). With the progression of disease, joint's structure and function are altered which then lead to abnormality in gait and posture. The alterations of affected joint structure are cartilage deterioration, synovitis, osteophyte formation and inflammation and thickening of joint capsule. As a result of alterations of affected joint structure changes, pain comes in as a major role that leads to decrease musculoskeletal abilities such that decrease in range of motion and mobility. OA is a chronic disease which progress worsen, on the other hand, nociceptor was stimulated for a period of time which progress to central nervous sensitization that leads to overall pain (Knazovicky et al., 2016).

Congenital or acquired musculoskeletal disorders are the origin of OA. Arthropathies of young dogs which lead to secondary OA, such as joint dysplasia, osteochondrosis dissecans, ununited anconeal process and patellar luxation (Martinez, 1997). For acquired musculoskeletal disorders as developmental abnormalities, progressive cartilage deterioration have played the major role (Martinez and Coronado, 1997). Direct trauma to the joint may also induce intra-articular lesion. Injuries of ligaments or intra-articular fracture have formed a consequence of joint instability. Later, the onset of OA has begun.

There are many factors that accelerate OA progression. The mechanical factors were thought to predominate in canine OA aetiopathogenesis (Henrotin et al., 2005). The abnormal mechanical strains induced osteochondral microfractures, abnormal bone and cartilage remodelling and ultimately cartilage loss and bone sclerosis (Henrotin et al., 2005). Excessive released joint matrix or damaged cartilage will trigger synovial macrophages and fibroblasts activation resulting in catabolic factors.

Pathophysiology of OA starts with the chondrocytes change their phenotypic modifications because of hypertrophic chondrocytes, a macrophage-like cells or apoptotic cell. As this happened, it leads to cartilage hypertrophy due to chondrocytes repair ability, degradation, and mineralisation (Braunstein et al., 1990). Hypertrophic cartilage is characterised by cell cluster formation, increased matrix hydration and an

accelerated matrix turnover (Henrotin et al., 2005). In normal chondrocytes, they will produce aggrecan and type II collagen but as in the repair phase, they also produce other molecules such as type I, IIA, III or X collagens and tenascin which usually absent in normal cartilage (Yasuda and Poole, 2002). As the production of those molecules excess, the cartilage will become more hypertrophy. As the repair process undergone excessively, the cartilage loss suggested that the cartilage repair reaction is transient and inefficeint (Kirsch et al., 2000; Aigner et al., 2001). Chondrocytes were produce more metalloproteinases which break the balance between catabolic and anabolic processes in the result of matrix degradation (Bluteau et al., 2001). OA chondrocytes are also over produce in interleukin-1 (IL-1) and tumor necrosis factor (TNF) receptors while decreasing in transforming growth factor (TGF) β -RII receptors (Wang et al., 2003a). IL-1 played major principal of activation of metalloproteinase synthesis as well as TNF-α, fibronectin fragments and microcrystals (Yasuda and Poole, 2002). IL-1 also needs other factors to fulfill its task as catabolic activity. Then IL-6 came in to complete the catabolic activity (Milner et al., 2001). Cartilage matrix can also undergo oxidative damage by reactive oxygen species (ROS) which is generated by chondrocytes. Subchondral bone has also play role in the OA characterized by subchondral bone sclerosis but there is not clear that bone changes are the cause or the consequence of cartilage lesions. Subchondral bone thickening was the result from increasing in osteoid volume and a low mineralization (Henrotin et al., 2005). The osteoblasts in subchondral bone were also changed in their phenotype such that they produce more insulin-like growth factor (IGF)-1 and urokinase (uPA) while IGF binding protein (IGFBP) and plasminogen activator inhibitor (PAI)-1 remains unchanged (Hilal et al., 1998). The imbalance between PAI-1 and uPA advocated the hydrolysis of IGFBP which lead to increase in free IGF-1 locally. IGF-1 acts as autocrine/paracrine pathway to enhance bone matrix formation (Hilal et al., 1998). In addition, OA osteoblasts were also resistant to parathyroid hormone stimulation in the end result of abnormal bone remodeling and bone sclerosis (Hilal et al., 2001).

Clinical signs and symptoms of OA were seen as joint effusion, pain, and joint stiffness (Nakamura et al., 1999). The synovial fluid of most OA patients has increased in the number of mononuclear cells which are mainly macrophages, T lymphocytes

and inflammatory cytokines (Nakamura et al., 1999). The structural changes of synovial membrane are hyperplasia of synovial lining as an effect of inflammatory cells infiltration (Goldenberg et al., 1982).

2.3 Cranial cruciate ligament rupture patients

Cranial cruciate ligament rupture is the most common pathologic disease of the stifle joint. Cranial cruciate ligament disease is known for degenerative of cranial cruciate ligament extracellular matrix then leading to ligament rupture (Comerford et al., 2011). There was also an evidence of trauma case that make ligament rupture. Breed, body weight and neutered status are the risk factors of cranial cruciate ligament disease (Whitehair et al., 1993). Most commonly found breeds are Newfoundland, Rottweiler, Labrador Retriever, Bulldog and boxer but West Highland White Terrier is also over-represented (Witsberger et al., 2008). The mean age of large breed dogs (body weight >15 kg) was 5.5 years while in small breed (body weight <15 kg) was 7.4 years (Harasen, 2008). Neutered dogs especially female had higher prevalence in developing cranial cruciate ligament disease (Slauterbeck et al., 2004).

Cruciate ligaments are made of cells and extracellular matrix which are 60-80% water. In dry weight, 90% of the component is collagen which are mostly type 1 with smaller amounts of elastin, proteoglycans, glycoproteins and lipoproteins (Frank et al., 1985). Fibroblasts in cranial cruciate ligament are also plays an important role as they respond in mechanical environment and mechanical force for cellular arrangement. When there is an overstimulation of tendons, repetitive loads occur which may lead to degenerative cascade to tendinopathy (Wang et al., 2003b). Stimulation of tendon may produce catabolic gene expression which make a loss of tendon material properties (Egerbacher et al., 2008). Under-stimulation or disused may also cause an alteration between cell matrix then fibril will become damage in physiologic loading (Arnoczky et al., 2007). The most common site of rupture is in the middle of ligament. Decrease in cellularity in the form of apoptosis is also the cause of rupture. Alterations in cruciate ligament cell morphology as chondrocytic change may block the communication between extracellular matrix metabolism (Henrotin et al., 2005).

Cranial cruciate ligament has an ability to passage of macromolecule markers from synovial fluid to the substance between cranial cruciate ligament and blood. Thus, in osteoarthritis condition intra-articular osmotic pressure reduces, blood flow to cranial cruciate ligament is affected (Kobayashi et al., 2006). In extracellular matrix perspective, cranial cruciate ligament rupture has significantly higher amounts of immature collagen cross-links, total and sulphated glycosaminoglycans, water content and concentration of matrix metalloproteinase-2 when compare to normal ligament (Comerford et al., 2004). In healing, cranial cruciate ligament fails to heal because of the lack in fibrin-platelet plug formations in the joint (Murray et al., 2007).

Diagnosis of cranial cruciate ligament is classically done by palpation of the affected limb. In standing position, palpation of the affected limb should find muscle atrophy of gluteal and quadriceps muscle in chronic lame. Joint effusion might be seen in acute and chronic lameness in various degree. Then in lateral recumbency position, crepitation of stifle joint can be found. Pain and range of motion can also be found in various degree depend on chronicity (Vasseur et al., 1996). Cranial drawer sign should be done via using the fingers of the upper hand to firmly grasp the bony landmarks of lateral fabella, patella and distal femur and lower hand is grasped on the fibular head, tibial tuberosity, and tibia. Upper hand is stabilized, if lower hand can move cranially then it is a positive in cranial drawer sign. Cranial drawer sign should be done in extension and flexion position. If the flexion is positive, only the craniomedial band is rupture (Strom, 1990). Tibial compression test is also done by upper hand grasp on the tibial tuberosity and distal femur and lower press hock joint in 90-degree position which mimic walking pattern.

Radiographic changes of cranial cruciate ligament rupture patients include thigh muscle atrophy, joint effusion, periarticular swelling, loss of intra-patellar fat pad shadow, and periarticular osteophyte formation (Vasseur, 2003). Osteophytes are shown in distal patella, femoral trochlear ridges, fabellae, intercondylar notch, fibular head and tibial and femoral condyles. To clarify joints' structure, other methods have been developed for diagnosing and monitoring canine cranial cruciate rupture.

Arthroscopy, magnetic resonance imaging (MRI), Computed tomography (CT-scan), serum OA biomarkers and ultrasonography.

Ultrasonography is a desirable addition method to orthopedic and radiological examination for diagnosis stifle diseases (Reed et al., 1995; Kramer et al., 1999). Benefit of using ultrasound for examining a joint disease are non-invasiveness and capability of soft tissue visualization (Reed et al., 1995; Ramírez-Flores et al., 2017). It has been proposed for detection this subtle change in soft tissue structure in early OA joint (Arnault et al., 2009; Nishitani et al., 2014). The ultrasonographic image can be done by dynamic position such as flexion, extension, and rotation. The ultrasonographic image is routinely performed in 4 regions: suprapatellar, infrapatellar, lateral, and medial.

There are 2 ways to treat cranial cruciate ligament rupture as conservative treatment and surgical treatment. For medical treatment, approximately 80% of dogs weigh <15 kg successfully resolved clinical signs. Medical treatments are usually symptomatic support. Restriction of activity, weight reduction, analgesic medication can be done to minimize clinical signs. To be clear, osteoarthritis will be progressed by time. The disadvantages of not doing a surgery are the instability of the stifle joint and various degree of meniscal injuries which might result in painful and disused limbs (Jerram and Walker, 2003).

The surgical treatments including the extracapsular stabilization, the fibular head transposition, the intra-articular reconstruction, and the tibial plateau leveling osteotomy. One of the most techniques that has been used nowadays is the tibial plateau leveling osteotomy (TPLO). In the patients with the condition of cranial cruciate ligament rupture, there is an inflammation all over the joint especially synovial membrane, synovial fluid, infrapatellar fat pad (Schmidli et al., 2018). With the inflammation of the joint, the synovial membrane become thicker with the infiltration of neutrophils and macrophages (Schmidli et al., 2018). Then the synoviocytes function more and release the cytokine IL-1, IL-6, TNF-α, Metalloproteinases (MMP) to induce more inflammation and pain to the joint (Muir et al., 2005; El-Hadi et al., 2012). For the pain pathway, cox-2 enzyme has been activated and triggered the pain. As mentioned

above, those pathways repeated as a loop because cranial cruciate ligament which is the cause has not been removed. As the disease has progressed, it will eventually end up as osteoarthritis (Rayward et al., 2004).

There are many ways to reduce the inflammatory process of the joint such as cold compression, drugs, nutraceuticals, acupuncture, low-level laser therapy. As for using drug in the treatment of reducing inflammation, there are two common groups of drugs which are steroids and non-steroidal anti-inflammatory drugs. Giving the steroids to the patients will lead to increase the liver enzyme such as alanine transaminase, alkaline phosphatase (Ginel et al., 2002). On the other hand, the non-steroidal anti-inflammatory drugs also have adverse effects when administrated (Monteiro-Steagall et al., 2013). NSAIDs are used in various situation of relieved pain in dogs such as osteoarthritis pain. Its pathway of reduction of pain is via inhibition of the cyclooxygenase (COX) enzymes, blocking the prostanoids synthesis such as prostagalndin E_2 (PGE_2) and other inflammatory mediator. By using NSAIDs can also cause undesirable side effects such as gastrointestinal mucosa and kidneys damage (Autefage et al., 2011). Firocoxib is a specific COX-2 inhibitor. The efficacy of firocoxib in the treatment of canine osteoarthritis has been studied in long-term administration as the result of effectively and safely in given as long-term treatment. Hence, there was some controversy that using firocoxib in the dose of 5 mg/kg is significantly increases BUN and creatinine levels after 2 weeks of treatment (Vijarnsorn et al., 2019). The effect of firocoxib was equaled or exceeded when compared to carprofen (Pollmeier et al., 2006).

Nutraceuticals such as marine based fatty acid compound also known as PSCO-524 has play an important role of managing osteoarthritis patients. It riches in long-chain polyunsaturated omega-3 fatty acids (omega-3) extracted from the New Zealand green-lipped mussel (*Perna canaliculus*) (Vijarnsorn et al., 2019). It extracts by using the super-critical carbon dioxide method. It composed of numerous sterol esters, sterols, polar lipids, triglycerides and at least 91 different fatty acids including eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and eicosatetraenoic acid

(ETA) (Shei et al., 2018). It has shown its potency of anti-inflammatory effects in the mechanism of leukotriene and prostaglandin production in lipoxygenase (LOX) and cyclooxygenase (COX) reduction (Treschow et al., 2007). It has shown its benefits toward the improvement of clinical signs while using in osteoarthritis patients (Vijarnsorn et al., 2019).

CHAPTER III

MATERIALS AND METHODS

3.1 Animals

This study included 19 dogs which did not undergo surgery due to having inappropriate condition or owner's financial problems. They were diagnosed of cranial cruciate ligament rupture and classified into 3 groups which are control, treatment with laser class 1M (TL1M) and treatment with laser class 4 (TL4). The number of dogs undergone by randomized sampling method in control group was 4, TL1M group was 8 and TL4 group was 7 dogs. Dogs included in this study were small breeds and any ages, body condition score and weight. The body condition score was determined based on modified nine-integer BSC scale system (Laflamme, 1997; Lund et al., 1999). The inclusion criteria for this study were the small breed dogs diagnosed as complete or partial cranial cruciate ligament rupture without any other orthopedics. The overall health condition was normal. The exclusion criteria were dogs given treatment in each group that has worsen the clinical signs which was evaluated either by researchers or Canine Brief Pain Inventory questionnaire. Dogs included in this study must not receive any anti-inflammatory drug or nutraceutical efficacy to control pain before study. If there were pain-relieved drugs or nutraceutical, 2 weeks of wash-out must be done to minimize interference. In control group, firocoxib of 5 mg/kg was given for 3 weeks consecutively. The cranial cruciate ligament rupture will be diagnosed by physical examinations diseases by cranial drawer test with or without radiographic examination.

Table 1 Classification of nine-integer body condition score scale system (Modification of Laflamme, 1997 and Lund et al., 1999)

Score	Description
1	Ribs, lumbar vertebrae, pelvic bones and all bony prominences evident from a distance. No observable body fat, dramatic waistline. Obvious loss of muscle mass. Excessively thin
2	Easily visible of ribs, lumbar vertebrae and pelvic bones. Some evidence of other bony prominences. Minor loss of muscle mass.
3	Easily palpation of ribs, pelvis and backbone. Evidence of top of lumbar vertebrae and prominence of pelvic bone. Obvious waist and abdominal tuck.
4	Easily palpation of ribs with minimal fat covering but not visible. Easily noted of waist from the top view. Abdominal tuck evident.
5	Ribs palpable without excess fat covering. Waist observed behind ribs from the top view. Abdominal tuck up when viewed from side.
6	Ribs palpable with slight excess fat covering. Waist discernible from the top view with no prominence. Appearance of abdominal tuck.
7	Difficult palpation of ribs and backbone under excess fat covering. Noticeable fat deposits over lumbar area and base of tail. Absent or barely visible of waist. Abdominal distention may be presented.
8	No palpation of ribs and backbone without pressure. Heavy fat deposits over lumbar area and base of tail. Absent of waist and abdominal tuck. Obvious abdominal distention may be presented.

9	Massive fat deposits over thorax, spine and base of tail. Absent of waist and abdominal tuck. Fat deposits on neck and limbs. Obvious abdominal distention. Severely obese

All patients were diagnosed and treated at the surgery unit, the Small Animal Hospital, Chulalongkorn University, Thailand. This study followed the guidelines for the care and use of laboratory animals and approved by the animal care and use committee of the Faculty of Veterinary science, Chulalongkorn University's, Bangkok, Thailand in No. 2031061. All patients included in this study must sign consent form to attend in this study.

3.2 Study designs

This study included 19 dogs for this study and classified into 3 groups. There was control (4 dogs), TL1M (8 dogs) and TL4 (7 dogs). The classification of each group was made by double blind random sampling method. All dogs included in each group started the treatment protocol after 1 week of cranial cruciate ligament rupture diagnosis. According to laser recommendation protocol, the duration of each treatment were 4 weeks consecutively. The first week consisted of 3 visits for low-level laser therapy alternatively. The second week, laser therapy performed twice a week and the third week performed once a week. In the last week clinical evaluation was performed. The programs of laser therapy in each group were shown in table 2. All dogs were recorded in video for evaluation of lameness score, gait and posture before and after the treatment of laser therapy in each experimental group. Video were evaluated by 3 veterinarians who had no conflict of interest to this study. The

ultrasound was done immediately before starting the laser therapy treatment and at the end of 4 weeks program. The clinical evaluations were ultrasonographic findings, synovial fluid analysis and lameness scores. The Canine Brief Pain Inventory questionnaire will be given before and after treatment. All treatment were terminated at the end of 4 weeks.

In control group, firocoxib was given in the dose of 5 mg/kg/day for 3 weeks. Ultrasonographic examination, synovial fluid analysis and lameness score were performed as same as the other treatment groups. The number of visits were the same as well.

Table 2 Laser therapy modes used in each visit for each experimental group. The laser dosage varied by product recommendation.

Treatment	Class 1M	Class 4
1^{st} - 2^{nd}	Swelling (1,000 Hz at 2 mins) + Inflammation (50 Hz at 2 mins)	Edema (18 Hz at 1 min) + Inflammation (18 Hz at 2 mins 30 sec)
3^{rd} – 6^{th}	Inflammation (50 Hz at 2 mins) + Severe pain (5,000 Hz at 2 mins)	Inflammation (18 Hz at 2 mins 30 sec) + Pain (acute) (18 Hz at 2 mins 30 sec)

3.3 Experimental designs

Clinical evaluation

a. Laser application

For applying laser probe, TL1M and TL4 were applied in the area around stifle joint as shown in the figure 3. Both TL1M and TL4 were applied by direct contact to the skin with the treatment protocol mentioned earlier. The laser probe was applied both medially and laterally of the patellar ligament through the stifle joint in the standing position.

All the treatment protocol was recommended by the laser company which has already tested for the results. In this study, the treatment protocol was used directly to treat the patients. The clinical evaluations were evaluated at first visit and last visit.

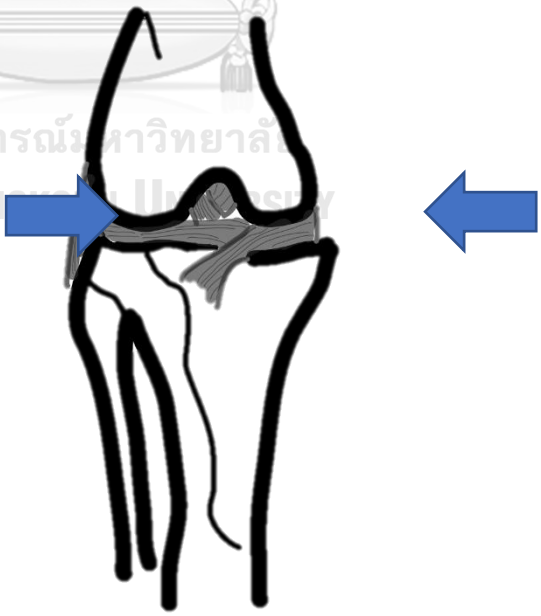


Figure 3 Laser probe application site as the arrows pointed.

b. Ultrasonographic examination

The ultrasound used in this study was Mindray Portable Ultrasound Z5. The ultrasound probe that used in this study was linear probe with the frequency of 10 MHz. The views that obtained in this study was suprapatellar view, infrapatellar view, and longitudinal view of both medial and lateral sides. After ultrasound was done, images were saved for further comparison of before and after treatments as the ultrasound score.

Table 3 Ultrasonographic standard views for evaluating the stifle joint (Modification of Reed et al., 1995; Kramer et al., 1999)

Standard regions / views	Implementation
Suprapatellar region	
(A) Parallel view on the femoral trochlea	Knee flexed at an angle of 45 degrees
Infrapatellar region	
(B) Infrapatellar view	Knee flexed at an angle of 90 degrees
(C) Maximal flexion view	Sagittal image Maximal flexion of knee Sagittal image

Table 4 Ultrasound scoring system of OA stifle joint (Modification of Gnudi et al., 2001 and Goranov et al., 2013)

Ultrasonographic findings	Score
Joint effusion	0 - absent 1 - mild 2 - moderate 3 - severe 4 - very severe
Articular cartilage of the femoral condyle (both medial and lateral sides) - at proximal femoral condyle - at distal femoral condyle	0 - anechoic 1 - hypoechoic 2 - hyperechoic 3 - heterogeneous
Bone surface of the femoral condyle (both medial and lateral sides) Irregular and/or rounded interruptions of the hyperechoic boundary of bones	0 - absent 1 - mild 2 - moderate 3 - severe 4 - very severe

Evaluation of a suprapatellar region with parallel view on the femoral trochlea, the stifle was flexed at the angle of 45 degrees. Ultrasound probe was placed on the femoral condyle in a sagittal view. With 45 degrees probing revealed joint effusion, proximal femoral condyles and articular cartilage (Figure 2). The images were obtained both in medial and lateral views.

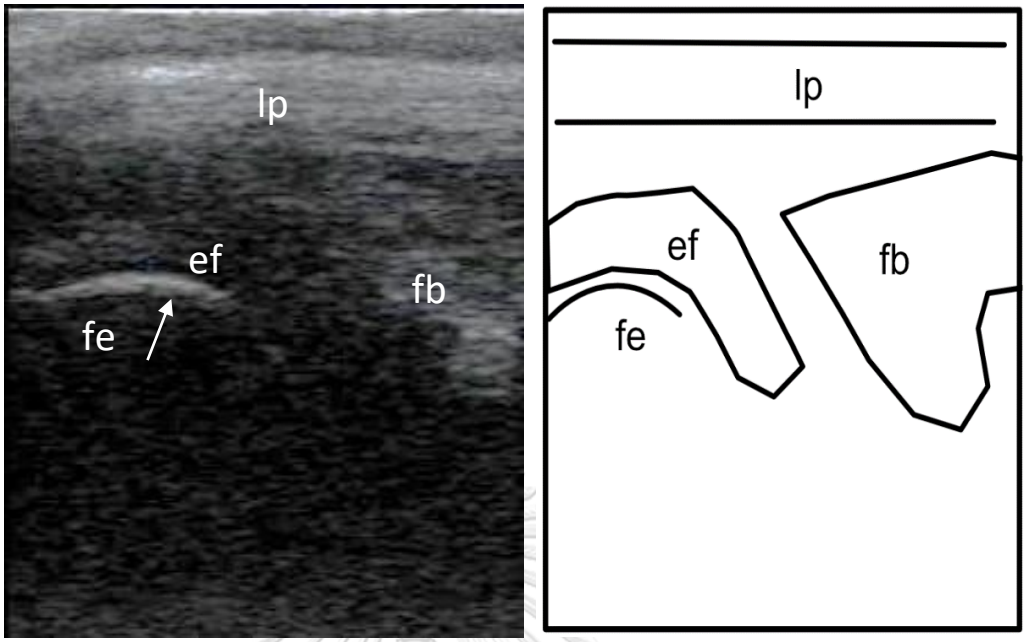


Figure 4 Suprapatellar view with sagittal image of stifle joint (A) shows joint effusion, femoral condyles and its cartilage. (ef = joint effusion; fe = surface of the femur; fb = fat body; lp = ligamentum patellae; white arrow = femoral cartilage)

Evaluation of infrapatellar region, the knee was flexed at the angle of 90 degree. Ultrasound probe was placed on the femoral condyle in a sagittal view. Ultrasonographic image showed the patella, the patellar ligament, the infrapatellar fat body, femoral cartilage surface and joint effusion (Figure 3). This view used for evaluation a score of articular cartilage and bone surface of both ridges of the distal femoral condyle. Joint effusion was also compared along with the suprapatellar view.

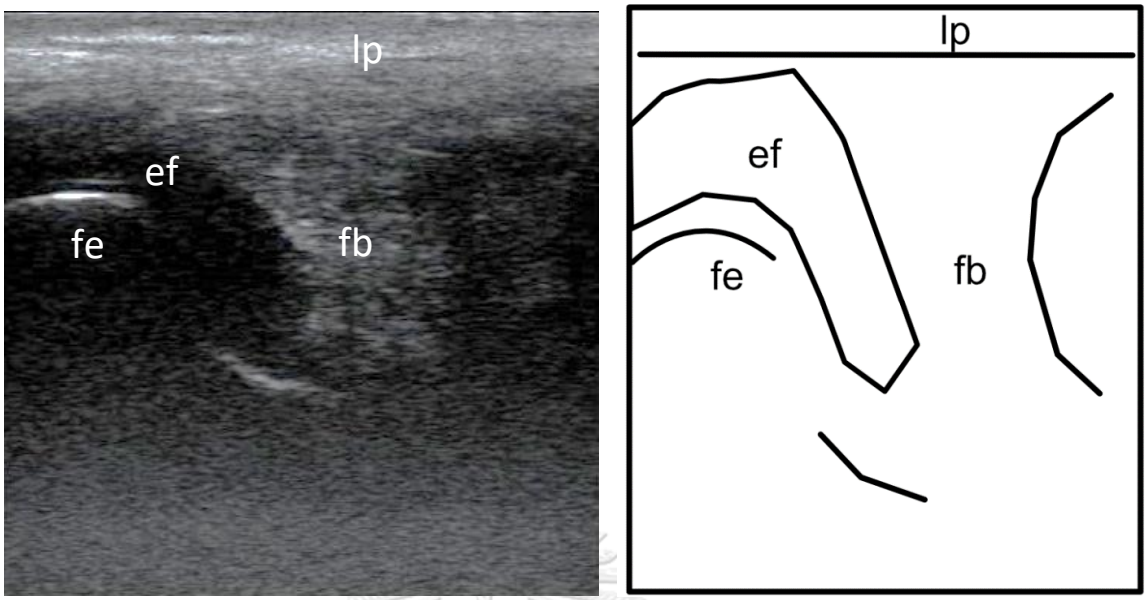


Figure 5 Infrapatellar view with sagittal image of stifle joint showed joint effusion, articular cartilage of the femoral condyle. (ca = cartilage of the femoral condyle; ef = joint effusion; fb = fat body; fe = surface of the femur; lp = ligamentum patellae)

Evaluation of the joint structure region, the knee was flexed in the maximal flexion position. Ultrasound probe was placed on the patellar, patellar ligament and tibial tuberosity in a sagittal view. Ultrasonographic image showed the patellar, the patellar ligament, the infrapatellar fat body, joint effusion and cranial cruciate ligaments (Figure 3). This view was used for evaluation cranial cruciate ligament, infrapatellar fat pad and joint effusion. Joint effusion was also compared along with the suprapatellar view and infrapatellar view.

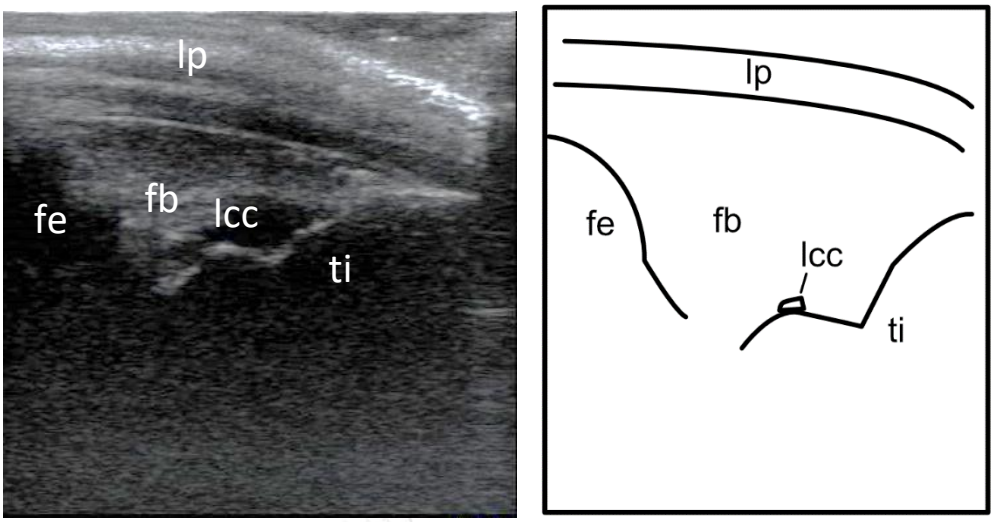


Figure 6 Infrapatellar view with sagittal image of stifle joint showed fat pad, ruptured cranial cruciate ligament. (fb = fat body; fe = surface of the femur; lcc = stump of the ligamentum cruciatum craniale; lp = ligamentum patellae; ti = surface of the tibia)

c. Synovial fluid analysis

Arthrocentesis was performed at the stifle joint. The synovial fluid was collected for appearance and cytologic evaluation during before and after the treatment. Volume, color, viscosity was noted. Spectrophotometry was used to measure the total protein concentration in the synovial fluid. For cytologic evaluation, total nucleated cell differential count was performed and classified (Cowell et al., 2007b).

d. Lameness scores

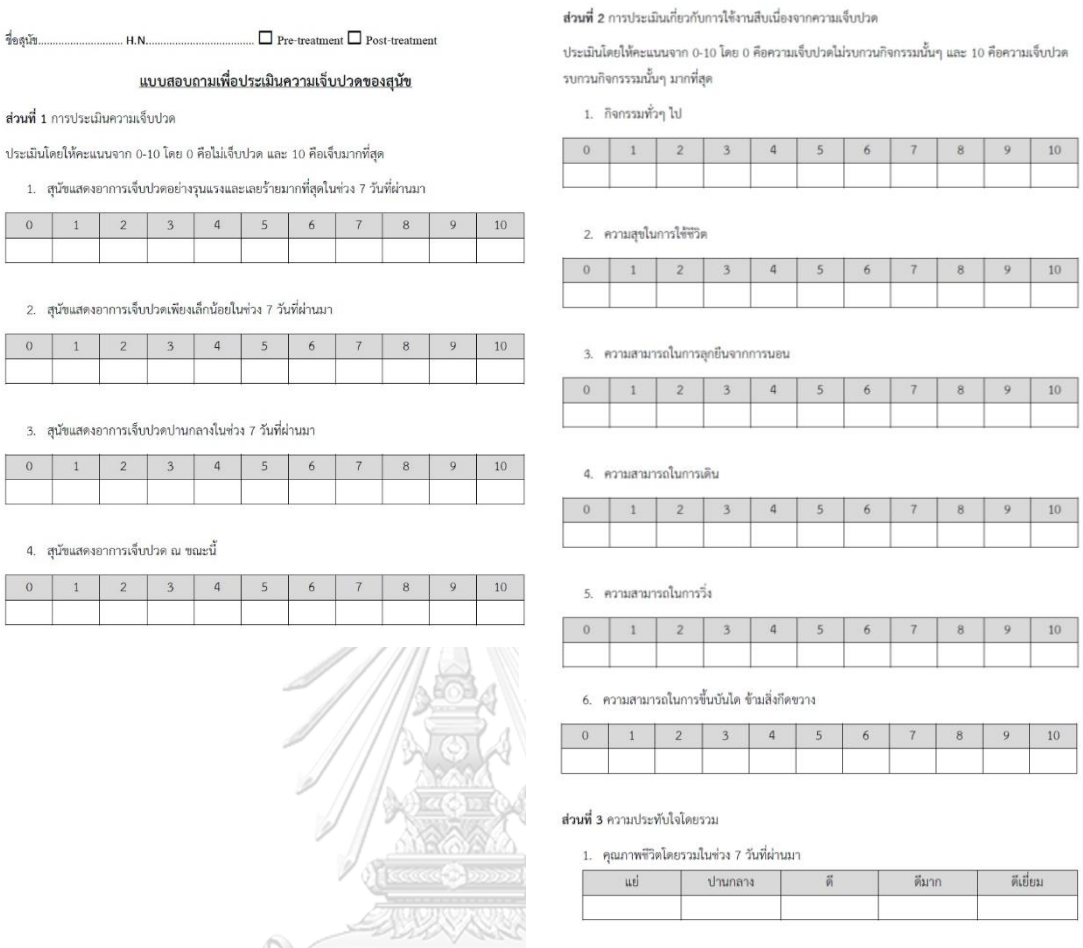
In each visit, lameness scores was evaluated and recorded by 3 veterinarians which was the same for the whole study with blinded tests based on Impellizeri et al., 2000 and Tinga et al.,2020 in Table 5.

Table 5 Modified Lameness score criteria (Impellizeri et al., 2000; Tinga et al., 2020)

Score	Signs
0	Normal gait when walking and trotting
1	Slightly lameness gait when walking and normal gait when trotting
2	Obvious lameness gait with partial weight bearing when walking and normal gait when trotting
3	Obvious lameness gait with non-weight bearing when walking and algetic gait when trotting
4	Non weight bearing when walking and trotting

e. Owner questionnaires

The owner will be assessed the questionnaire of Canine Brief Pain Inventory (Canine BPI) by University of Pennsylvania which created by Dr. Dorothy Cimino Brown aimed to detection of the severity of pain in day 0 and day after treatment. Canine BPI was translated to Thai for the owners to be more understandable and presented in Figure 4 (Cleeland, 1990; Cleeland, 2006).



ชื่อสุนัข..... H.N..... ☐ Pre-treatment ☐ Post-treatment

แบบสอบถามเพื่อประเมินความเจ็บปวดของสุนัข

ส่วนที่ 1 การประเมินความเจ็บปวด

ประเมินโดยให้คะแนนจาก 0-10 โดย 0 คือไม่เจ็บปวด และ 10 คือเจ็บมากที่สุด

1. สุนัขแสดงอาการเจ็บปวดอย่างรุนแรงและเลยร้ายมากที่สุดในช่วง 7 วันที่ผ่านมา

0	1	2	3	4	5	6	7	8	9	10

2. สุนัขแสดงอาการเจ็บปวดเพียงเล็กน้อยในช่วง 7 วันที่ผ่านมา

0	1	2	3	4	5	6	7	8	9	10

3. สุนัขแสดงอาการเจ็บปวดปานกลางในช่วง 7 วันที่ผ่านมา

0	1	2	3	4	5	6	7	8	9	10

4. สุนัขแสดงอาการเจ็บปวด ณ ขณะนี้

0	1	2	3	4	5	6	7	8	9	10

ส่วนที่ 2 การประเมินเกี่ยวกับการใช้งานสืบเนื่องจากความเจ็บปวด

ประเมินโดยให้คะแนนจาก 0-10 โดย 0 คือความเจ็บปวดไม่รบกวนกิจกรรมนั้นๆ และ 10 คือความเจ็บปวดรบกวนกิจกรรรมนั้นๆ มากที่สุด

1. กิจกรรมทั่วๆ ไป

0	1	2	3	4	5	6	7	8	9	10

2. ความสุขในการใช้ชีวิต

0	1	2	3	4	5	6	7	8	9	10

3. ความสามารถในการลุกยืนจากการนอน

0	1	2	3	4	5	6	7	8	9	10

4. ความสามารถในการเดิน

0	1	2	3	4	5	6	7	8	9	10

5. ความสามารถในการวิ่ง

0	1	2	3	4	5	6	7	8	9	10

6. ความสามารถในการขึ้นบันได ข้ามสิ่งกีดขวาง

0	1	2	3	4	5	6	7	8	9	10

ส่วนที่ 3 ความประทับใจโดยรวม

1. คุณภาพชีวิตโดยรวมในช่วง 7 วันที่ผ่านมา

แย่	ปานกลาง	ดี	ดีมาก	ดีเยี่ยม

Figure 7 Modified from Canine Brief Pain Inventory (Canine BPI) by Dr. Dorothy Cimino Brown (Cleeland, 1990; Cleeland, 2006). Canine BPI was translated to Thai for better understanding.

3.4 Statistical analysis

Demographic data were presented in descriptive statistic of mean and standard deviation in each group. Body condition score was presented in median. The parameters of lameness score and joint effusion score were analyzed by using Wilcoxon matched paired test. Synovial fluid analysis parameter was calculated by Paired-t test. Furthermore, the parameters of lameness score and joint effusion score were tested among pre-treatment and post-treatment group by Kruskal-wallis test. Synovial fluid analysis for pre-treatment and post-treatment were also tested within

group and among groups by one-way ANOVA. Canine Brief Pain Inventory score questionnaire was calculated using Wilcoxon signed rank test. All statistical analysis were performed using SPSS Statistic version 22. *P-value* < 0.05 is considered as statistically significant.

CHAPTER IV

RESULTS

19 dogs were enrolled in this study. They were classified into 3 groups which were control, TL1M and TL4 groups. The control group consisted of 4 dogs, whereas both TL1M and TL4 group had 8 and 7 dogs in each group, respectively. There were 2 Yorkshire terrier, 4 Chihuahua, 5 Pomeranian, 3 Shih Tzuh, 2 Poodle, 1 Jack Russell terrier, 1 West Highland terrier, 1 mixed breed. The control group consisted of 2 Chihuahua and 2 Pomeranian. There were 1 male and 3 females. The mean ± SD of age was 8.0 ± 2.4 years and body weight was 5.17 ± 2.2 kilograms. TL1M group consisted of 1 Pomeranian, 3 Shih Tzuh, 1 Poodle, 1 Jack Russell Terrier, 1 West Highland terrier, and 1 mixed. The mean ± SD of age was 10.1 ± 3.5 years and body weight was 6.9 ± 3.6 kilograms. TL4 group consisted of 2 Yorkshire terrier, 2 Chihuahua, 2 Pomeranian and 1 Poodle. The mean ± SD of age was 8.6 ± 9.2 years and body weight was 8.6 ± 9.2 kilograms. Mean ± SD of age and body weight of dogs and gender among all groups were not significantly different in the table 6 while there is a significant difference in BCS among groups (p = 0.07).

Table 6 Demographic data of patient in each group showing in Mean ± SD of age, body weight and gender of dogs in control, TL1M, TL4 groups

Demographic Data	Control group (N=4)	TL1M group (N = 8)	TL4 group (N = 7)	p-value
Age (year)	8.0 ± 2.4	10.1 ± 3.5	8.4 ± 1.9	0.38
Body weight (kg)	5.2 ± 2.2	6.9 ± 3.6	3.4 ± 1.3	0.07
Body condition score (median)	5.5	5	3	0.23

Gender (N)	Male(N)	1	3	3	0.85
	Female(N)	3	5	4	

4.1 Lameness score

Lameness score was evaluated at the first visit and last visit within 4 weeks. 3 Veterinarians were observed and evaluated lameness score for all cranial cruciate ligament rupture dogs. Most of lameness scores were improved when compared raw data. Wilcoxon Matched pairs test was calculated for pre-treatment and post-treatment. There were significant differences in TL1M and TL4 in pre-treatment and post-treatment ($p<0.05$). All P-value was shown in Table 7.

Table 7 Lameness score of all treatment group

TL1M		P-value	TL4		P-value	Control		P-value
Pre	Post		Pre	Post		Pre	Post	
2.5 ± 0.5	1.8 ± 0.7	0.01*	2.14 ± 0.7	1.50 ± 0.8	0.04*	2.25 ± 0.5	1.5 ± 0.6	0.08

4.2 Ultrasonographic examination

In ultrasonographic images, they were all done in 3 views such as suprapatellar region, infrapatellar region, and max flexion position. All views were done in the same ultrasound setting. Ultrasonographic findings were shown in the figure below of pre-treatment and post-treatment in each group.

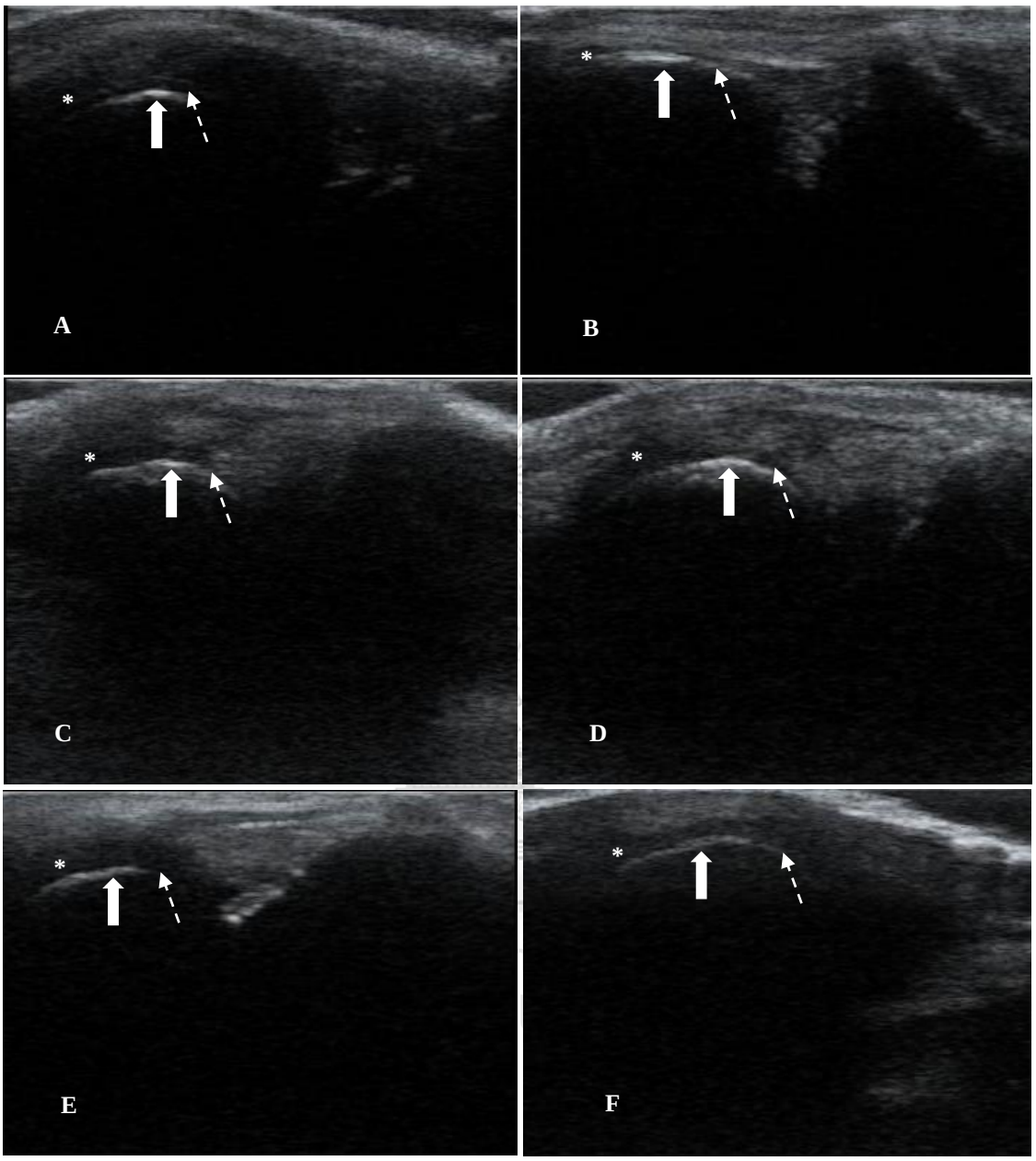


Figure 8 showed supracondylar region. A and B showed before and after treatment of TL1M respectively. C and D showed before and treatment of TL4. E and F showed before and after treatment of control group. Solid white arrow showed subchondrol bone. Dotted white arrow showed joint cartilage. Star showed supracondylar joint effusion.

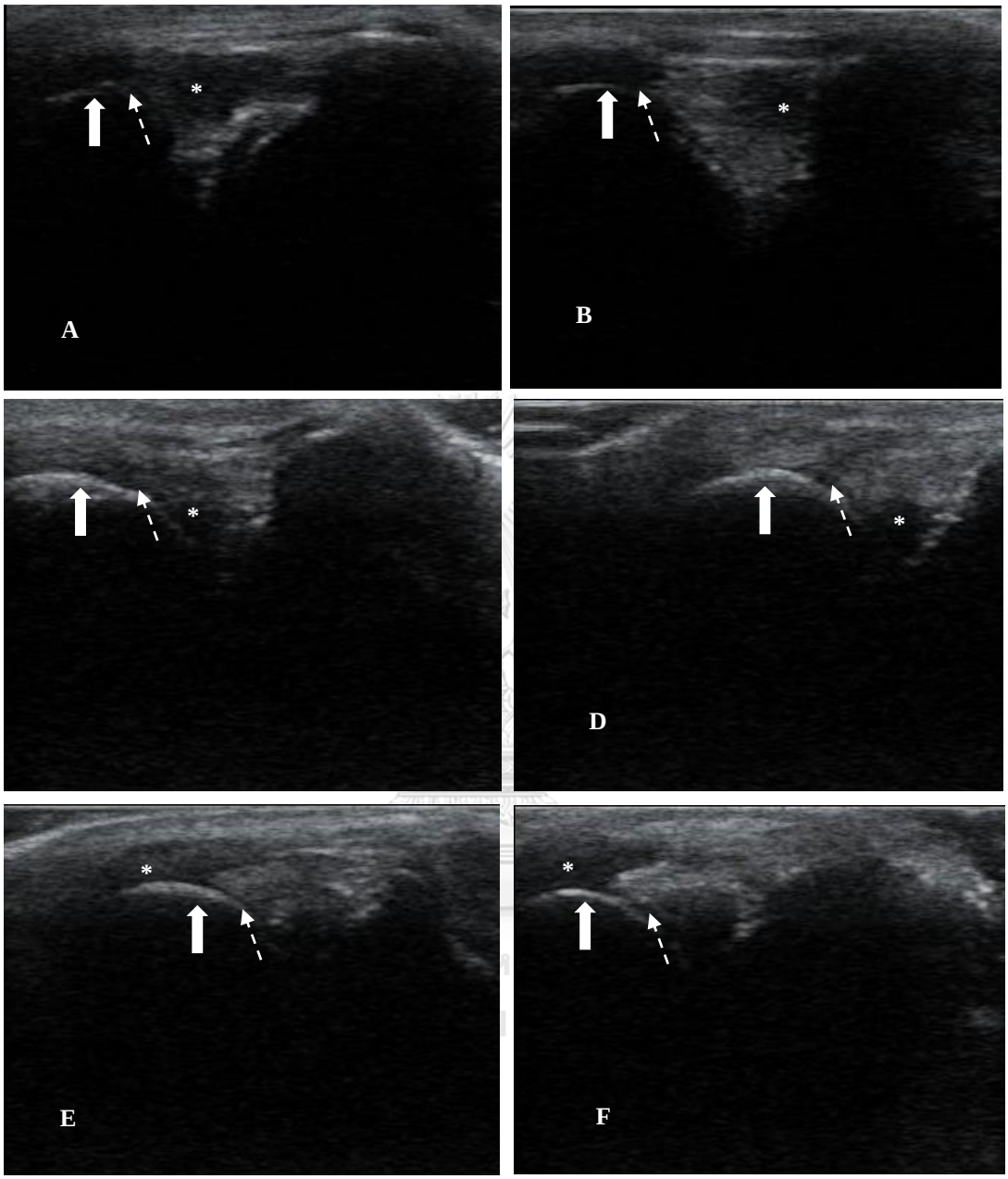


Figure 9 showed infracondylar region. A and B showed before and after treatment of TL1M respectively. C and D showed before and treatment of TL4. E and F showed before and after treatment of control group. Solid white arrow showed subchondrol bone. Dotted white arrow showed joint cartilage. Star showed supracondylar joint effusion.

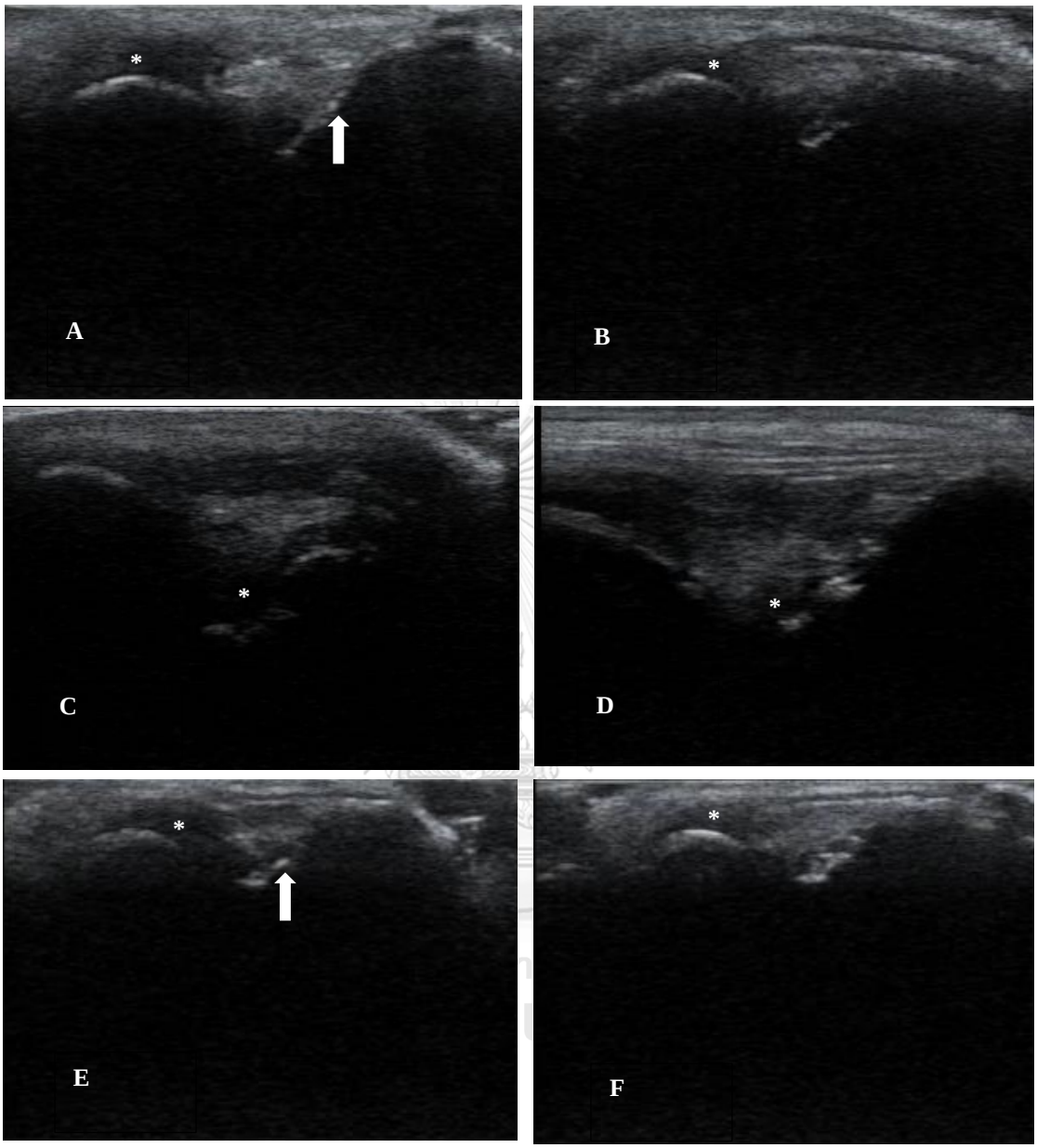


Figure 10 showed max flexion position. A and B showed before and after treatment of TL1M respectively. C and D showed before and treatment of TL4. E and F showed before and after treatment of control group. Solid white arrow showed remnant of cruciate ligament. Star showed supracondylar joint effusion.

Evaluation of joint effusion score, proximal and distal articular cartilage of femoral condyle and bone surface of femoral condyle were obtained in pre- and post-treatment in all group. Joint effusion score was the only parameters that had changed while the others remained the same. P-value of joint effusion score for pre- and post-treatment of TL1M group and TL4 group was statistically significant at P-value 0.03 and 0.04, respectively. While p-value of joint effusion score for control group was 0.1 which considered no statistically significant as shown in the table 8.

Table 8 Joint effusion score in all treatment group

TL1M		P-value	TL4		P-value	Control		P-value
Pre	Post		Pre	Post		Pre	Post	
3.3 ± 0.9	2.5 ± 0.9	0.03*	2.4 ± 1.1	1.4 ± 0.5	0.04*	2.5 ± 1	1.5 ± 0.5	0.1

As the results showed in table 9. The data remained at the same value as pre- and post- treatment in all group. There were no differences at all. The articular cartilage of femoral condyle that had been eroded remained eroded as well as bone surface of femoral condyle remained the same.

TL1M						TL4						Control					
Proximal Articular		Distal Articular		Bone Surface		Proximal Articular		Distal Articular		Bone Surface		Proximal Articular		Distal Articular		Bone surface	
Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
1.5 ± 1.6		1.5 ± 1.6		1.1 ± 0.6		2.6 ± 1.1		2.6 ± 1.1		1.3 ± 0.8		1.5 ± 1.7		1.5 ± 1.7		1.5 ± 0.6	

Table 9 Ultrasonographic scoring of all groups as mean ± SD

4.3 Synovial fluid analysis

Synovial fluid was obtained from all dogs at the beginning of the treatment and after treatment. All dogs' (100%) synovial fluid color was yellowish. Synovial fluid was analyzed in the criteria of total nucleated cell count, total protein via spectrophotometer, viscosity, and volume at pre- and post- treatment.

The before and after of total nucleated cell count for TL1M and TL4 was statistically significant with the P-value of 0.001 and 0.002 respectively. In control group, it was not statistically significant in the value of 0.069. While the other parameters such that total protein, viscosity and volume were no statistically difference in any stances. For repeated ANOVA of total nucleated cell count, total protein, viscosity and volume, there was no statistic difference among groups. For all cytology of each groups shown mainly neutrophils with some macrophages. (Table 10, 11, 12)

Table 10 Total nucleated cell count of all treatment

TL1M		P-value	TL4		P-value	Control		P-value
Pre	Post		Pre	Post		Pre	Post	
2,675.0 ± 755.46	1,287.5 ± 644.62	0.002*	2,271.4 ± 485.5	1,100.0 ± 355.9	0.001*	1,650.0 ± 238.0	1,475.0 ± 340.3	0.069

Table 11 Total protein of all treatment

TL1M		P-value	TL4		P-value	Control		P-value
Pre	Post		Pre	Post		Pre	Post	
4.4 ± 1.8	4.5 ± 1.3	0.82	3.8 ± 1.0	3.3 ± 0.6	0.11	3 ± 0.7	2.7 ± 0.2	0.38

Table 12 Viscosity of all treatment

TL1M		P-value	TL4		P-value	Control		P-value
Pre	Post		Pre	Post		Pre	Post	
2.4 ± 0.9	3.6 ± 2.8	0.17	2.7 ± 1.0	2.4 ± 1.0	0.58	6.9 ± 3.9	6.0 ± 2.9	0.26

Table 13 Volume of all treatment

TL1M		P-value	TL4		P-value	Control		P-value
Pre	Post		Pre	Post		Pre	Post	
0.2 ± 0.1	0.2 ± 0.1	0.10	0.24 ± 0.2	0.18±0.12	0.44	0.5 ± 0.8	0.4 ± 0.6	0.39

Kruskal-wallis test was analyzed for lameness score evaluation. Paired-ANOVA test was analyzed for the parameter of joint effusion, total protein and TNCC. P-value was listed in the table below. There was no statistically significant between Pre-treatment groups and Post-treatment among groups.

Table 14 Pre-treatment and Post-treatment were analyzed in all groups.

	Lameness		Joint effusion		Total protein		TNCC	
	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment
TL1M	0.51[a]	0.79 [a]	0.23[a]	0.06[a]	0.27[b]	0.06[b]	0.10[b]	0.49[b]
TL4								
Control								

[a] and [b] were calculated by Kruskal-wallis test and one-way ANOVA respectively.

4.4 Canine Brief Pain Inventory

Canine BPI was used for the comparison in before and after treatment in each group. Wilcoxon signed rank test was used to perform the statistic test. The statistic tests were tested by separation into two categories: pain and function. TL1M and TL4 had shown statistically significant in both pain and function categories before and after treatment while control group neither shown statistically significant.

Table 15 Canine BPI tested in criteria of pain and function in all groups

	Pain	Function
TL1M	0.01*	0.01*
TL4	0.03*	0.03*
Control	1.00	0.65

* shown statistically significant

CHAPTER V

DISCUSSION

Canine cranial cruciate ligament rupture is a common orthopedics disease of the stifle joint. It can occur in any breeds but mostly in large dog breeds. Once there is an abnormality to the joint, then osteoarthritis has begun which result in inflammation and pain. The recommendation for canine cranial cruciate ligament rupture is surgical treatment. All Dogs included in this study were in unhealthy or inappropriate conditions, thus they were delayed in surgical treatment. Theoretically, Low-level laser therapy has a potential in attenuating inflammation and pain, so it was used before all dogs have the surgery. Therefore, low-level laser therapy become a treatment of choice for reducing inflammation and pain which is the key to improve quality of life.

In this study, age, body weight and gender among groups were no significant different. Most breeds are Pomeranian ranks number one 26.3% of all dogs. There was 73.7% of cranial cruciate ligament rupture happened in the right. The age of study was age and ranges from 5-14 years (the mean age was 8.03 years). This result can be implied that dogs at the age of 8 years were likely to encounter with cranial cruciate ligament rupture. There was a study shown that the peak prevalence was in 7 to 10 years old (Whitehair et al., 1993). There was also study revealed that the mean age of small breed dog was 7.4 years (Comerford et al., 2011). In term of body condition score, there was also a difference in every score that effect the result of forces act on canine stifle joint.

The results of lameness score were statistically significant only in TL1M and TL4 groups. Most of the data shown improvement in lameness score. TL1M and TL4 has shown the ability in improvement of clinical sign. While given NSAIDs have the ability for reduction of inflammation and pain management in control group but no improvement of was found.

The ultrasonographic results in significantly reducing joint effusion in TL1M and TL4 while there was no significant result in control group. In control group, there was also a trend in reducing joint effusion while there was 1 dog with the same joint effusion score. For that dog with same joint effusion score might be the effect of highly potential in reducing inflammation and pain so that dogs were more comfortable to use limbs better. For more intense of using the limbs, the inflammation went on repeatedly in the vicious cycle. Other parameters such as proximal and distal cartilage score, bone surface score remains the same as before and after treatment. The pathological changes of the stifle joints are the results of osteoarthritis. As the lesion of osteoarthritis has started, there is no way to return to normal stifle joints.

As for Canine BPI, all TL1M and TL4 shown significant improvement by the owners. While in control group, there was no significant difference that might depend on number of dogs included. Every breed differs in behavior so hyperactive breed such as Jack russel terrier may imply not improving in the results. As in the conclusion of canine BPI, both TL1M and TL4 had potency in the owner aspect.

Synovial fluid analysis revealed the gross appearance of yellowish color for all dogs which various of volume that can be obtained from arthrocentesis. The volume of normal canine stifle joint effusion was less than 1 milliliter (Cowell et al., 2007a). In this study, the volume obtained was varied from 0.1 – 1.6 milliliters. 1.6 milliliters synovial fluid was obtained from chihuahua, that in small breed dogs usually obtained 0.1 – 0.5 milliliters. As that 1.6 milliliter synovial fluid was the results of severe inflammation while the others with less than 1 milliliters might also had severe

inflammation but infrapatellar fat pad conceal the synovial fluid. For further analysis of synovial fluid, total nucleated cell count was performed. There were statistically significant in decreasing TNCC of TL1M and TL4 while there was no statistically significant in control group. The normal joint TNCC was <3,000 cells/μl (Cowell et al., 2007a). TL1M and TL4 shown evidence of reducing inflammation in terms of reducing cells infiltration. As the study of Colter et al., stated that low level laser therapy has shown medium-term effect of reducing inflammation. In control group, there was also a trend in reducing inflammation but there was low in number of dogs. For total protein, there was not statistically significant. There was also a fluctuation of the data which did not match the improvement or worsen. The normal total protein is 1.8 – 4.8 g/dl measured by refractometer (Cowell et al., 2007a). For viscosity, there was no significant result in all group which might imply that viscosity cannot be the indicator of improvement in clinical signs because most dogs were not exceed the normal value. The normal value of viscosity was at least 2.5 cm (Cowell et al., 2007a).

As for low-level laser therapy, laser dosage has play an important role to obtain the optimal therapeutic level. The equation of dosage is $D(J/cm^2) = E_p(J) \times f\ (Hz) \times t\ (s) / A\ (cm^2)$ whereas E_p is the average pulse energy, t is time, A is the area of the irradiated surface on the tissue (Pascu, 2000). The average pulse energy can be computed by multiplying the peak power per pulse to the pulse time width and dividing the result with a factor m is frequency (f) x pulse duration (PD) so the equation is for TL1M is

$$D = (Peak\ power(W) \times time(s))/(Area(cm^2))$$

For laser class 1M, the recommendation with scanning technique, the area should not exceed 50 cm^2. The dosage of TL1M is inversely proportional to the irradiated surface area. As the surface area gone smaller the dosage of TL1M is higher. As in the raw data of dogs included in TL1M, large breed dogs' lameness score did not improve and also in ultrasonographic scoring; category of joint effusion. TL1M dogs which small breed may reach to 5 J/cm^2 while the surface area is 6 cm^2. Meanwhile

TL4, the dosage of each protocol is calculated to 4 J/cm^2 in each treatment. As in the data of TL4, in lameness score and joint effusion mostly shown the improvement.

In conclusion, both Laser class 1M and class 4 has an efficacy of reducing inflammation and pain relief as given NSAIDs. Furthermore, as the ethics issue of experimental disease, the study cannot let animals be in painful condition without any analgesic drugs. The outcome of the study did not show the significant between test group and control group. To clarify the hypothesis, further study should be performed.

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