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BONE METABOLISM MARKERS IN RESPONSE TO THREE AND SIX SESSIONS OF LOW ENERGY EXTRACORPOREAL SHOCKWAVE THERAPY IN INDIVIDUALS WITH POST ANTERIOR CRUCIATE LIGAMENT RECONSTRUCTION

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ABSTRACT

Precise information on the effects of extracorporeal shockwave therapy (ESWT) on biochemical status in humans is still lacking. Hence, this study investigated the effectiveness of three or six sessions of low energy ESWT on serum bone metabolism markers in individuals who have undergone post anterior cruciate ligament (ACL) reconstruction. Participants with ACL injuries were assigned into three groups (n=10 per group), i.e. physiotherapy alone without ESWT (control), three sessions of ESWT combined with physiotherapy (3ESWT), and six sessions of ESWT combined with physiotherapy (6ESWT) groups. Serum bone metabolism markers, i.e. osteocalcin (OCN), human Cross Linked C-telopeptide of Type 1 Collagen (CTX1), calcium, and phosphorus were measured at weeks 0, 2, 9, 12, and 6 months post ACL reconstruction. Serum OCN and CTX1 concentrations within the 3ESWT and 6ESWT groups across the five measurements were not significantly different. However, serum calcium concentrations at weeks 2, 9, and 12 were significantly higher compared to their respective baseline values for all three groups. Shockwave therapy had no significant effect on bone resorption and formation markers postoperatively. Six sessions of shockwave therapy (6ESWT) elicited the highest serum calcium level at week 12 post-operatively among all the groups. More studies are warranted to substantiate these findings.

Key words: Anterior cruciate ligament, bone metabolism, shockwave therapy

INTRODUCTION

Bone metabolism is a constant remodeling process that sustains the balance between the formation of new bones and the resorption of old or injured ones. This process occurs on the surface of the bone at specified sites, also known as bone metabolism units (BMUs). Biological bone metabolism markers can provide real-time assessment and be used as indicators of bone resorption, formation, and turnover (Christenson, 1997). Markers of bone formation are produced by active osteoblasts during different phases of their development and could represent different aspects of osteoblast function and bone formation (Shetty *et al*., 2016).

Bone resorption markers are produced during the bone resorption phase of bone remodeling, which includes by-products of osteoclast activity that are released during bone resorption. Bone resorption markers indicate osteoclast activity and/or collagen degradation (Christenson, 1997). Markers of bone resorption can be detected in the serum or urine (Banfi *et al*., 2010). Theoretically, bone turnover can be determined by comparing the number of substances that are released during resorption with the amount of formation-associated substances (Christenson, 1997). Additionally, bone is a major source of inorganic ions which include calcium and

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phosphate, and is actively involved in calcium homeostasis in the body. Serum osteocalcin (OCN) in the present study was measured as the biomarker of the bone formation while human Cross Linked C-telopeptide of Type 1 Collagen (CTX1) was measured as a biomarker of resorption. In addition, other bone metabolism markers such as phosphorus and serum total calcium were also determined.

It has been previously reported that extracorporeal shockwave therapy (ESWT) has the potential to be applied on the bone (Gollwitzer *et al*., 2013; Wang *et al*., 2014a; Yilmaz *et al*., 2017), tendon (Vetrano *et al*., 2011), and enthesis (Chow *et al*., 2014; Wang *et al*., 2014b) to accelerate the healing process. The mechanical stimuli produced by the shockwaves have been shown to induce physiological responses at the cellular level of bone in animal studies (Wang *et al*., 2011; Wang *et al*., 2013a; Wang *et al*., 2013b; Hsu *et al*., 2017; Lama *et al*., 2017; Wang *et al*., 2017). Nevertheless, to date studies investigating bone metabolism markers following ESWT intervention in humans are limited. Therefore, this current study was carried out to investigate the effects of three or six sessions of low energy ESWT on bone metabolism markers in individuals who had undergone post anterior cruciate ligament reconstruction. The measurement of bone metabolism markers may be helpful to identify the early changes related to bone tissues (Christenson, 1997; Cacchio *et al*., 2009) such as subchondral bone remodeling including osteogenesis (Wang *et al*., 2013b), regression of osteoarthritic knees (Wang *et al*., 2011), anti-osteoporotic effects (Lama *et al*., 2017) and healing of long bone non-union (Cacchio *et al*., 2009). Thus, it was hypothesized that ESWT could accelerate the healing of ACL injuries at post anterior cruciate ligament reconstruction (ACLR) with the observation of bone formation and resorption markers within six months post-operatively.

MATERIALS AND METHODS

In the present study, thirty male participants, age between 20 to 40 years old were recruited from the Orthopaedic Department in Hospital Universiti Sains Malaysia and Hospital Raja Perempuan Zainab II. These participants were patients with an ACL tear and underwent primary single autograft hamstring ACLR. Participants with primary single autograft patella tendon ACLR, revision ACL surgery, previous knee surgery which interferes with the knee functions, having multiple knee ligament injuries, medical problems, and participants on NSAIDs medication and/or consuming calcium supplementation were excluded from this study.

This is a quasi-experimental study and uses an opportunistic sampling method. A day before the operation, all potential participants were fully informed by the researcher about the nature of the experiments, purposes of the study, procedures, termination or withdrawal options, potential risks, and benefits as participants of this study. They were also informed that their participation in this study was voluntary, and they were allowed to withdraw from this study at any time during this study. If they agreed to participate, they were requested to complete the participant information and consent form. After that, they were screened to ensure that the inclusion and exclusion criteria were met. The qualified participants were randomly assigned into three groups with 10 participants per group. The groups were physiotherapy without ESWT (control), three sessions of ESWT combined with physiotherapy (3ESWT), and six sessions of ESWT combined with physiotherapy (6ESWT) groups. The estimation of sample size was calculated based on Wang *et al*. (2014b) by using GPower software (Erdfelder *et al*., 1996). The calculated sample size was 7 per group. The estimated dropout rate during the experimental period is 30%, i.e. three participants per group. Therefore, 10 participants were required to be recruited for one group. There were three study groups in the present study, thus the total number of participants recruited in this study was 30 participants. The Human Research Ethics Committee of Universiti Sains Malaysia and the Medical Research and Ethics Committee granted the ethical approval to conduct this study (USM/JEPeM/16090303 and NMRR-18-953-41870 (IIR) respectively). In addition, this study was also registered with the National Medical Research Register (NMRR).

ACL reconstruction (ACLR) procedures

All ACLR procedures were carried out by an orthopedic surgeon in HUSM and HRPZ II respectively based on a standard procedure. All the participants were examined to confirm the diagnosis of ACL tears. The reconstruction surgeries were conducted arthroscopically. Gracilis and semitendinosus tendon grafts were harvested and whip stitches were sutured at both ends. The grafts were folded in half and tensioned for 20 min. The femoral and tibial tunnels were drilled using the standard technique. The grafts were then secured to the femoral end with an endobutton while the tibial end was secured with a bioabsorbable screw (Suppiah *et al*., 2013). Concomitant meniscal injuries were addressed during reconstruction.

Extracorporeal shockwave therapy (ESWT)

The application of ESWT was administered to the participants once a week for three weeks (started at week 7) in the 3ESWT group. Meanwhile, for the 6ESWT group, the application of ESWT was administered to the participants once a week for six weeks (started at week 7). The ESWT was generated by a portable shockwave therapy unit (ShockMaster 300, GymnaUniphy, Germany). The applicator of the ESWT was put directly on the skin region, perpendicular to the femoral tunnel at the lateral side of the knee joint, directed latero-medially, after applying the ultrasound gel. Then, shockwaves were released as the applicator button was pressed. The therapy was performed without anesthesia. Total energy used is adapted from a study done by Wang *et al*. (2002) i.e. 0.18 mJ/mm^2 = 14 kV (equivalent to 4 bar as stated by Furia *et al*. (2009)) for the 6ESWT group meanwhile for the 3ESWT group, half of the dosage i.e. 0.09 mJ/mm^2 was used. ESWT was administered at 500 shocks, 1.5 bar, once per week for either 3 or 6 weeks. The therapy session took between 3 to 5 min. After the intervention, the treatment area was inspected again to observe if there was any redness/hematoma if occur. Patients in the control group did not receive ESWT after ACLR.

Blood sampling

The participants were seated when 6 mL of venous blood sample was withdrawn from the antecubital vein following a 12-hr overnight fast and immediately before ACLR (T_0), 2 weeks post-ACLR (T_1), 9 weeks post-ACLR (T_2), 12 weeks post ACLR (T_3) and 6 months post-ACLR (T_4). The blood was withdrawn by a qualified laboratory technologist/staff nurse / medical doctor/physician to determine the concentrations of bone metabolism markers. The blood was collected into a plain tube without EDTA. Serum from the clotted blood in the plain tube was used for determining the levels of serum bone metabolism markers (osteocalcin) as the bone formation marker and human Cross Linked C-telopeptide of Type 1 Collagen as the bone resorption marker, serum total calcium, and serum phosphorus). Serum was collected by centrifuging the blood sample with a centrifuge (Hettich-Rotina 46RS, Germany) at 4000 rpm for 10 min before separating it into equal portions and stored in a -80°C freezer (ThermoForma, Model 705, USA).

Blood biochemical analysis

Serum osteocalcin was analyzed using N-MID® Osteocalcin ELISA, (Elabscience Biotechnology Co., USA) and the concentration was determined using a photometric microplate reader (Molecular Devices; Thermo Scientific Varioskan Flash by Thermo Fisher Scientific, Finland). Serum CTX1 was analyzed with an enzyme-linked immunosorbent assay (ELISA) kit (Elabscience Biotechnology Co., USA) and the concentration was determined by a photometric microplate reader (Thermo Scientific Varioskan Flash by Thermo Fisher Scientific, Finland). Serum calcium and phosphorus were analyzed using an automatic analyzer (Ci8200 Architect (Abbott), Germany) with commercially available calcium and phosphorus reagent kits by applying Arsenazo III and Phosphomolybdate methods respectively.

Statistical analysis

Statistical Package for Social Sciences (SPSS) version 26.0 was used for the statistical analysis. All the data are presented as mean ± standard deviation (SD). Mixed ANOVA was performed to determine the significant differences within and between groups. Statistical significance was accepted with a '*p*' value of <0.05.

RESULTS

A total of 25 male participants with a mean age of 26.7±5.8 years completed the study. Three participants from the control group, one participant from 3ESWT and 6ESWT groups respectively discontinued the participation in this study due to personal reasons.

Bone formation marker: Serum osteocalcin (OCN)

There was no significant interaction between time and group in serum osteocalcin (OCN) concentrations, ($F(8, 88) = 0.613$, $p=0.765$). In addition, the main effect of group ($F = 0.101$, $p=0.905$) and main effect of time, ($F(4, 88) = 0.655$, $p=0.625$) on serum OCN concentrations were not significant. Furthermore, the mean serum OCN across the five-time points within each groups was not significantly different (Table 1).

Bone resorption marker: human Cross Linked C-telopeptide of Type 1 Collagen (CTX1)

There was no significant interaction between time and group on serum CTX1 concentrations, $F(8, 88) = 0.884$, $p=0.533$. In addition, the main effect of group ($F(2, 22) = 0.572$, $p=0.573$), and main effect of time ($F(4, 88) = 0.462$, $p=0.764$) on serum CTX1 concentrations were not significant. However, mixed ANOVA with selected cases has indicated that the serum CTX1 within the control group across the five-time points was significantly different ($p<0.05$). Bonferroni Post hoc test revealed that there was a significant difference in serum CTX1 at week 2 compared with serum CTX1 at week 9 in the control group, $p=0.039$. Serum CTX1 concentrations were significantly higher at week 9 compared with serum CTX1 concentrations at week 2 within the control

Table 1. Mean and comparison of serum osteocalcin (OCN) and Cross Linked C-telopeptide of Type 1 Collagen (CTX1) concentrations

Time	Osteocalcin (OCN) concentrations			*p*-value*	Cross Linked C-telopeptide of Type I Collagen (CTX1) concentrations			*p*-value*
	Mean (SD)				Mean (SD)			
	Control	3ESWT	6ESWT		Control	3ESWT	6ESWT	
Baseline	44.85 (31.92)	50.61 (31.79)	48.07 (44.21)		0.86 (0.27)	0.60 (0.22)	0.77 (0.33)	
2 weeks	32.74 (18.01)	47.47 (21.76)	46.61 (50.47)		0.67 (0.22)	0.63 (0.27)	0.72 (0.37)	
9 weeks	48.95 (51.85)	50.32 (31.06)	49.05 (46.25)	0.625	0.72 (0.22)#	0.69 (0.19)	0.68 (0.20)	0.764
12 weeks	42.26 (43.47)	43.45 (31.68)	57.35 (67.08)		0.78 (0.27)	0.63 (0.23)	0.68 (0.27)	
6 months	41.87 (25.31)	46.60 (28.62)	52.02 (48.30)		0.67 (0.17)	0.69 (0.26)	0.75 (0.24)	
p-value#	0.225	0.747	0.642		0.016#	0.657	0.829	
p-value&		0.905				0.573		

#, different from week 2 significantly at $p<0.05$.
p-value*: compared between mean CTX1 across the five readings (main effect of time) by Mixed ANOVA.
p-value#: compared within each group based on time by Mixed ANOVA with selected cases.
p-value&: compared between groups (main effect of the group) by Mixed ANOVA.

group. There were also no significant differences in serum CTX1 within the 3ESWT and 6ESWT groups across the five measurements (Table 1).

Serum total calcium

Regarding serum calcium concentrations, there was no significant interaction between time and group, ($F(8, 88) = 0.467$) on serum total calcium. In addition, there was no significant main effect of group ($F(2, 22) = 0.272$) on serum total calcium. However, the main effect of time on serum total calcium concentrations was significant ($F(4, 88) = 7.875$, $p<0.001$). Bonferroni Post hoc test revealed that serum total calcium at weeks 2, 9, and 12 was significantly higher compared to serum total calcium at baseline, $p=0.023$, $p=0.004$, and $p=0.001$ respectively in a comparison of time for all three groups. 6ESWT elicited the highest serum calcium level at week 12 post-operatively among all the groups (Figure 1).

Serum phosphorus

There was no significant interaction between time and group in serum phosphorus concentrations, $F(5.43, 59.76) = 1.838$, $p=0.114$. In addition, there was no significant main effect of group observed, $F(2, 22) = 0.641$, $p=0.536$. However, there was a significant main effect of time in serum phosphorus concentrations, $F(2.72, 59.76) = 10.341$, $p<0.001$. Corrected Bonferroni Post hoc test showed that serum phosphorus concentrations at week 12 and after 6 months was significantly lower compared to serum phosphorus at baseline, $p=0.041$ and $p=0.002$ respectively, and serum phosphorus concentrations at 6 months were significantly lower compared to serum phosphorus concentrations at week 2, $p<0.001$, and at 9 weeks, $p=0.019$ in a comparison of time for all three groups (Figure 2).

DISCUSSION

Initial alteration in bone metabolism markers is beneficial to detect the early changes in the bone following ESWT at a cellular level. Thus, it is believed that through bone metabolism markers, the potential positive outcome of ESWT can be predicted earlier than the radiology outcome after the treatment sessions. In this present study, we found that three and six sessions of low energy ESWT post-ACLR do not significantly affect the serum OCN and CTX1 concentrations within 6 months postoperatively. Nevertheless, we also observed that CTX1 level was significantly higher after 9 weeks than at 2 weeks in the control group with participants who did not receive ESWT throughout the study period. This observation seems to imply that ACLR alone may increase bone resorption.

Woelfl *et al*. (2017) reported that levels of bone turnover markers including bone alkaline phosphatase (bALP), c-telopeptide of type I collagen (β-CTX), band 5 tartrate-resistant acid phosphate (TRAP5b), as well as vitamin D3 and parathyroid hormone (iPTH) did not change after one year of the

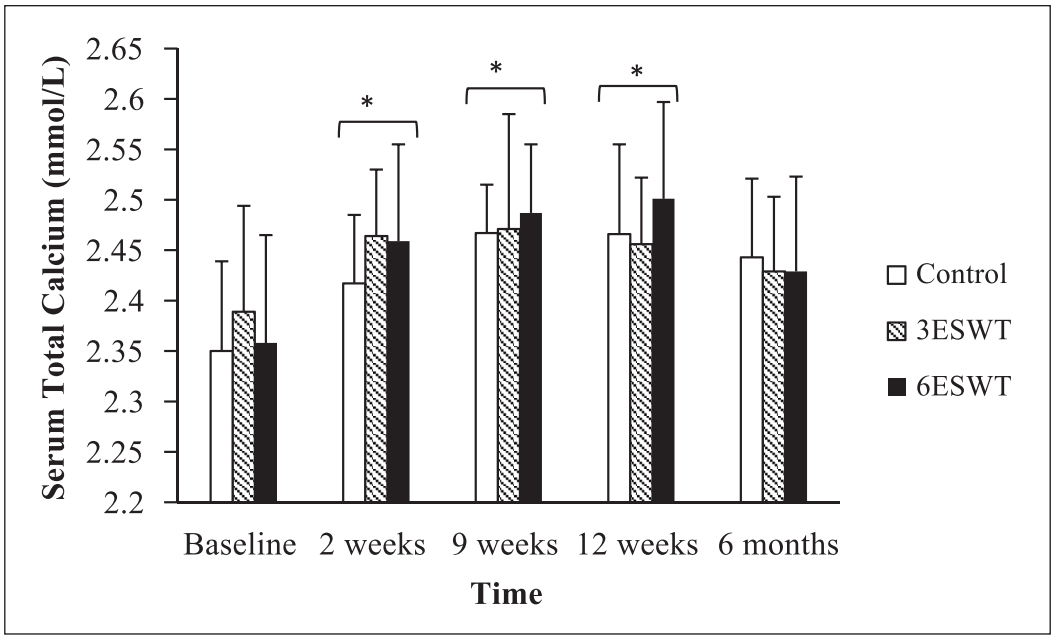

Fig. 1. Mean serum total calcium concentrations.
* significantly different compared to baseline value at $p<0.05$.

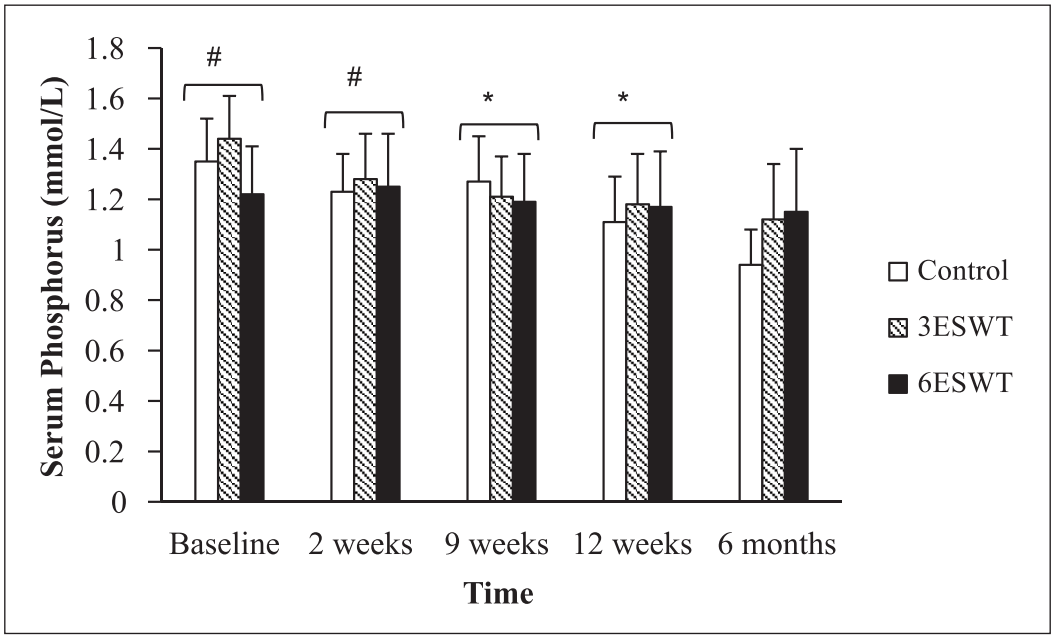

Fig. 2. Mean serum phosphorus concentrations.
* significantly different compared to baseline value at $p<0.05$.
\# significantly different compared to the value at 6 months at $p<0.05$.

study period. This might be attributed to the follow-up time being one year post-treatment, which was extended too long after the intervention period. However, they also reported that the application of a single, high-energy ESWT with 0.55mJ/mm^2 and 3000 shocks immediately after surgery could improve the serum levels of bone turnover markers in participants with low BMD compared to individuals with normal BMD. It was speculated that shockwave could stimulate changes in bone metabolism markers in serum level which contribute to normalizing bone mass in the low bone mineral density individual. Thus, based on these findings, they postulated that ESWT does not affect the serum bone metabolism markers in participants with normal BMD.

Parallel to the findings of Woelfl *et al*. (2017), and in vitro study by Hofmann *et al*. (2015) found that osteocalcin was expressed by human osteoblasts which were isolated from a hypertrophic type of non-union fracture in both treated and non-treated groups. The treated groups received 0.06 mJ/mm^2 and 0.5 mJ/mm^2 energy flux density (EFD) respectively with 500 impulses. The control group did not receive any ESWT exposure. Nevertheless, there was no significant difference between the groups. These results showed that at the cellular level, low energy of ESWT had the potential to alter the activity of the osteoblasts in vitro although it was not statistically proven. Thus, bone metabolism markers can be the indicators for healing in non-union fractures and bone mass in low BMD patients.

Contradictory to the abovementioned studies, OCN and bALP was used to predict the bone tissue response to ESWT at an early stage in the management of patients with long bone non-unions in the study carried out by Cacchio *et al*. (2009). After 4 sessions of ESWT, i.e. once a week for 4 weeks with 4000 impulses and 0.40 mJ/mm^2 EFD, it was found that 26 patients healed completely. However, no bone tissue response was found in the remaining 8 patients. They also found that the OCN and bALP concentrations were significantly higher in the healed group compared with the non-healed group during the first 2 months after ESWT treatment. Therefore, these researchers speculated that insufficient dosage used might be the underlying factor that leads to the poor bone tissue response in the non-healed group.

In a previous animal study, Wang *et al*. (2011) observed that serum OCN concentrations were significantly higher in the ACL transection (ACLT) plus ESWT group compared to the ACLT alone group. However, no significant changes were found in serum ALP concentrations. The ESWT was administered once a week for twelve weeks with the dosage of 800 impulses at 14kV. The regression of osteoarthritic knees after ESWT was marked by the changes in biomarkers of bone at 24 weeks. Their findings also demonstrated that serum OCN was more sensitive than serum ALP after receiving stimulation by the shockwave.

The similar dosages used by Wang *et al*. (2011) were applied to the intervention groups in few other animal studies such as Wang *et al*. (2013a), Wang *et al*. (2013b), Hsu *et al*. (2017), and Wang *et al*. (2017). The effects of ESWT with a different number of sessions were investigated by Wang *et al*. (2013a). It was found that OCN level significantly decreased in rats with ACLT and medial meniscectomy (MM) without ESWT, and in rats with ACLT and MM which received ESWT three times a week for 3 sessions of treatments. However, no significant change in OCN level was observed in control rats with sham arthrotomy without ACLT or MM and received no ESWT group. This study demonstrated that one session per week for one week and 2 sessions per week for two weeks of ESWT is the optimal number of ESWT sessions that caused a significant increase in OCN level in rats with ACLT and MM. The results obtained reflect that the OCN level was dose dependant and was affected by the number of ESWT sessions.

Several other animal studies explored the effects of ESWT on different sites of ESWT application i.e. at distal femur, proximal tibia, and both distal femur and proximal tibia in rats with an osteoarthritic knee. It was found that serum OCN level in all the treated groups was significantly higher compared with the non-treated group at 12 and 24 weeks (Wang *et al*., 2013b). Thus, it was speculated that the early detected changes in OCN level following ESWT could cause improvement in subchondral bone remodeling which includes osteogenesis in ACLT induced-OA changes of the knee.

A significant increase in bone formation markers such as osteopontin (OPG) and ALP was observed at 2 weeks post ESWT compared with the non-treated group in early osteoarthritis of the knee in rats (Hsu *et al*., 2017). It was speculated that ESWT has the potential to modulate the biological functions of osteoblasts in the treatment of the early OA of the knee. Thus, it is believed that ESWT could enhance osteogenic factors that lead to local stimulation of bone formation.

Additionally, Wang *et al*. (2017) found that the application of ESWT on subchondral bone significantly regulated bone remodeling through osteogenesis biomarkers, i.e. OCN at 12 weeks post shockwave treatment. OCN significantly influenced the level of molecular expression in different locations of ESWT application, i.e. medial tibia, lateral tibia, lateral femur, lateral tibia and femur, and medial and lateral femur. They observed that ESWT regulated the bone volume and porosity in subchondral bone, as well as correlated with the expression of osteocalcin from various locations. The EWST was applied one week after surgery with the dosage of 800 impulses at 0.22 mJ/mm^2 EFD.

Regarding effects of combined ESWT with supplementation, Lama *et al*. (2017) reported that the altered serum bone remodeling parameters were restored by ESWT alone or combined with raloxifene in the ovariectomized rats. Five sessions of ESWT with 0.33 mJ/mm^2, 3Hz, and 1000 shocks were applied at each treatment session. They found that ESWT alone or combined with supplementation promotes bone formation and suppresses resorption in ovariectomized rats. Thus, the authors concluded that ESWT with or without supplementation managed to produce anti-osteoporotic effects. The results of the aforementioned studies were obtained from small

animals. As the anatomy and physiology of the knee in animals may not necessarily resemble that of the human participants, the effects of ESWT in humans may be different. Collectively, the discrepancy between the current study and the aforementioned studies could be due to differences like the study design such as subjects, model and type of ESWT prescribed, etc.

The importance of supplementation or dietary intake that contained calcium and phosphorus to overcome orthopedic problems has been emphasized (Wang *et al*., 2015; Shi *et al*., 2017). However, to date, studies investigating the circulating serum calcium and phosphorus following ESWT in human or animal studies are lacking. In this present study, a significant increase in circulating serum total calcium was found after 2, 9 and 12 weeks post ACLR compared with baseline values. In addition, the highest value of serum total calcium was observed in six sessions of the ESWT group after 12 weeks post ACLR. It was also observed that serum phosphorus levels showed a decrement trend towards the 6 months post ACLR. However, the changes for these two bone metabolism markers were within the normal range. It was postulated that the natural healing of enthesis post ACLR was dominant than the effects of ESWT generally as no significant differences were found between groups. Nevertheless, the present observation of the highest serum total calcium level observed in six sessions of the ESWT group after 12 weeks post ACLR implied that a higher dosage and a longer period of ESWT may be needed for eliciting a greater effect on calcium level in the blood.

In vitro studies involving human mesenchymal stem cells (MSCs), the ESWT treatment increased ALP activities and calcium deposits in the treated cells compared with the untreated cells (Hu *et al*., 2016; Catalano *et al*., 2017). The greater calcium depositions were observed in the treated cells compared with the untreated cells. It was postulated that the differentiation of human adipose-derived stem cells (hASCs) into osteoblasts which then caused calcium deposition was induced by ESWT and osteogenic medium (Catalano *et al*., 2017). The sensitivity of MSCs to ESWT is following the sequence: tendon-derived stem cells (TDSCs), followed by bone marrow mesenchymal stem cells (BMSCs), and then adipose-derived stem cells (ADSCs) (Hu *et al*., 2016). Besides, it was speculated that shockwaves increased osteoblast migration and penetration into artificial grafts (Muzio *et al*., 2010). Therefore, it was observed that shockwaves have the potential to boost bone formation through osteoblast stimulation in MSCs. Thus, these may be the factors that might contribute to bone tissue repair in humans. However, to date, the exact mechanisms of shockwave on bone biochemical markers are still debatable.

Besides ESWT, another source of physical/mechanical stimulations has been investigated to explore its benefits on the bone at cellular levels. It was found that short-term overloading of mechanical stimulation could open the cell membrane calcium channels and release calcium stores. It elevates intracellular calcium levels, and thus promoting the proliferation and differentiation of cells to a greater extent than the effect of apoptosis of the osteoblasts (Liu *et al*., 2017). Consistently, the present study also found that serum total calcium level was the highest with the higher dosage and longer period of ESWT among all the study groups.

Low-magnitude high-frequency vibration (LMHFV), a non-invasive biophysical intervention could alter the expression of osteocyte-specific markers in osteoporotic fracture healing in rats. Choy *et al*. (2020) reported that with the significant enhancements of the calcium/phosphorus ratio, LMHFV could promote mineralization in the osteoporotic group. These findings showed that mechanical stimulations through vibrations and short-term overloading are involved in the mobilization of these mineral components of bone.

Comparison between the findings of the present study and previous studies showed that varied outcomes were observed as there were differences in the number of treatment sessions, the dosage of shockwave prescribed, time of treatment given, sites treated, and time to follow up across the studies. It seems that individuals may respond differently to shockwave and the dosage used might be insufficient for certain participants and may need to be modified to obtain effectiveness. Furthermore, shockwave treatment showed dose-dependent and site-specific effects, as the positive changes could only be seen with optimal dosage at the particular areas treated. Nevertheless, it can be observed that the initial changes of bone metabolism markers at the cellular level can be detected as early as two weeks post-treatment in the animal studies and two months post-intervention in human studies.

There are a few limitations in this current study. For instance, the participants in the present study were not randomly selected and allocated into groups as limited numbers of patients met the inclusion criteria and not many of them agreed to participate in this study. Due to the aforementioned problems, the sample size for this study was relatively small. Nevertheless, sample size calculation has been performed based on a previous related study before the commencement of this study. In addition, as this is a novel study that applied low energy ESWT on a knee post-ACLR surgery, the dosage prescribed in this study might be inadequate to generate significant

positive changes on bone formation and resorption markers. Thus, future studies with higher ESWT dosage, randomization, and a large sample size are warranted to confirm and validate these findings.

CONCLUSION

Both three and six sessions of low energy ESWT did not elicit additional benefits on bone formation and resorption markers post ACLR. In addition, the highest serum calcium level at 12 weeks post-operatively was observed after six sessions of shockwave therapy.

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