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The Effect of Isometric Exercise Plantar Flexor on Osteoblast Activity Measured by Bone Specific Alkaline Phosphatase and Callus Formation in a Patient Post Open Reduction Internal Fixation with Non-articular Tibia Fracture

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Abstract

BACKGROUND: Post-operative non-articular tibia fracture several problems that can occur include; pain, muscles atrophy, muscles weakness, joint stiffness, delayed union, and non-union that makes patients limited in their daily activities. Several factors that play a role in the process of fracture healing include osteoblast activity and exercise. Isometric exercises plantar flexor might effectively prevent the problem. Markers of osteoblast activity are bone-specific alkaline phosphatase (BSAP) levels and Hummer scale callus formation. Not yet known the effect isometric exercise of plantar flexor on osteoblast activity in the post open reduction internal fixation of non-articular tibia fractures, due to the lack of studies in this field.

AIM: This research was conducted to investigate the effect of isometric plantar flexor on osteoblast activity and callus formation in patients post open reduction internal fixation nonarticular tibial fractures.

HYPOTHESIS: There are differences in osteoblast activity and callus formation between groups that do isometric exercises of plantar flexor muscles with those that without isometric exercises plantar flexor.

ANALYSIS: Hypothesis test used a paired t-test with a value of α 0.05 and a confidence level of 95%.

METHODS: This clinical trial was true experimental with pre-post test control group design divided into two groups, group I obtained treatment of isometric exercises of the plantar flexor muscle, range of motion knee joint, and ankle while group II obtained the range of motion knee joints and ankle. Osteoblast activity measured with bone-specific alkaline phosphatase level and callus formation.

RESULTS: The result of the study found to increase mean bone-specific alkaline phosphatase group I; 15.6 and group II; 5.2. A paired t-test of independent samples with α of 0.05 and confidence interval 95% was obtained p-value = 0.000, there is a significant difference in increased levels of bone-specific alkaline phosphatase group I obtained isometric exercises plantar flexor with group II without isometric exercises plantar flexor in patients post open reduction internal fixation of a non-articular tibia fracture. Radiographic examination of Hummer scale callus in group I who carried out isometric exercises plantar flexor had an average value of 2.63, whereas group II without isometric exercises plantar flexor average of 3.06. Wilcoxon test with α 0.05 and 95% confidence interval obtained p-value = 0.000, there is a significant difference in callus image in both groups of patients post open reduction internal fixation non-articular tibia fracture. The linear regression calculated of callus assessment with bone-specific alkaline phosphatase obtained the value of R quadrat = 0.793, which showed that the formation of callus Hummer classification could predict the change in bone-specific alkaline phosphatase value by 79%.

CONCLUSION: The research found isometric exercise plantar flexor in patients post open reduction internal fixation non-articular tibia fracture enhances osteoblast activity and callus formation that will likely short the healing process time and prevent delayed union or non-union.

Introduction

The incidence of non-articular tibia fractures increases from year to year. Tibia is the bone most often broken because of the superficial structure and position. The increased number of tibial fractures over time results in increased morbidity and disability rates. Fracture is one of the problems that humans are faced with in a risky life because of the development of the industrialisation of society, the increasing number of vehicles, and increased activity [1]. The incidence of tibial fracture is recorded at 16.9/100,000 per year [2].

Treatment days averaged 7.2 days [2]. The most common causes of cases of tibia fractures are due to traffic accidents, falls from stairs, skateboarding, and sports. Tibial bone fractures often occur in men aged 15 to 29 years [3]. Fractures in the tibia are often accompanied by skin, muscles, fat and fascia [4].

The problem that usually occurs in patients who experience non-articular tibia fractures is the length of the healing process, muscle atrophy, reduced bone density, delayed union, and non-union. The process of fracture healing is influenced by several factors such as; osteoblast activity, fracture type, nutritional status, care, psychological conditions, time of medical intervention and rehabilitation as early as possible [5], [6]. Patients after open reduction internal fixation of the tibia fracture are long enough for several weeks without weight bearing which can cause complications in the form of calf muscle atrophy, calf muscle weakness, osteopenia, limited motion of the knee joint, and ankle [7].

Programs and types of muscle training must be designed accurately and so that complications do not occur and the healing time can be achieved quickly. One type of muscle training given is the isometric exercise of the plantar flexor muscle. Research on factors that influence the processof osteoprogenitor differentiation and osteoblast activity is very important in the process of healing tibial fractures. It is not yet known the effect of plantar flexor isometric exercises on osteoblast activity and callus formation in patients with non-articular tibia fractures.

Hypothesis: 1. There is a difference in the increase in bone-specific alkaline phosphatase and callus formation grade in the group of patients who are doing isometric exercises plantar flexor with groups that do not do isometric exercises of the plantar flexor; 2. There is a relationship between callus formation and bone-specific alkaline phosphatase levels in a group of patients who are doing isometric exercises plantar flexor with a group of patients who do not do isometric exercise plantar flexor.

Material and Methods

This research was conducted in the Department of Physical Medicine and Rehabilitation at Zainoel Abidin General Hospital Banda Aceh Indonesian. The study included 34 patients over a period of 1 month. The subject was recruited from Orthopaedic inpatient room post open reduction internal fixation non-articular tibial fracture after obtaining written informed consent and approval of the institutional ethical committee. Patients included in the study were those of age group 20-45 years, normal body mass index with isolated post open reduction internal fixation non-articular tibial fracture. Excluded patient with; loss of consciousness, diabetic, anemia, and multiple fractures. Experimental research with pre-post test control group design with subject selection used systematically random. Group I with the treatment of isometric exercises of plantar flexor muscles and standard exercises range of motion knee and ankle joints while group II is only with standard exercises the range of motion knee and ankle joints.

Methods

Group I

1. Range of motion exercise.

The patient is lying supine with the lower limbs straight and the ankle joint in a neutral position or 0°. The therapist helps the dorsiflexion ankle movement to the pain limit or the patient's ability then return to the starting position, this movement is repeated up to 10 times. Furthermore, the therapist helps move plantar flexion ankle to the extent of the pain or the patient's ability then return to the starting position, this movement is repeated up to 10 times. Knee at position 0°. Furthermore, the therapist helps to flexion the knee joint to the limit of pain or patients ability then returns the extension to the original position, this movement is repeated up to 10 times. Every day a training session is conducted, where each exercise session moves the knee and ankle joints 10 times.

2. Isometric exercise plantar flexor

Patient is lying on his back with the position of the leg straight and ankle neutral position or 0°, used modified ankle-foot orthoses (Figure 1). Purpose use ankle-foot orthoses to able fixation of ankle joint so that no occur joint movement and easy to use. Modification by make a window on the back side to be able palpate muscle plantar flexor contraction. Patient performs an isometric contraction of the plantar flexor muscle. Assessment of plantar muscle contraction is done by palpating the calf muscle belly. The long contraction in a matter of 10 seconds use stopwatch casio HS3. Every day three sessions were carried out, one training session carried out 10 repetitions with a break every 10 seconds. Every session rests for two minutes. Educational exercises are carried out first on healthy feet.

Group II

The only range of motion exercise without isometric exercise.

The patient is lying supine with the lower limbs straight and the ankle joint in a neutral position or 0°.

Figure 1: Isometric Exercise Plantar Flexor

The therapist helps the dorsiflexion ankle movement to the pain limit or the patient's ability then return to starting position, this movement repeated up to 10 times. Furthermore, the therapist helps move ankle plantar flexion to the extent of the pain or the patient's ability then return to the starting position, this movement repeated up to 10 times. Knee at the straight position. Furthermore, the therapist helps to flex the knee joint to the limit of pain or patients ability then returns the extension to the original position, this movement is repeated up to 10 times. Every day a training session is conducted, where each exercise session moves the knee and ankle joints 10 times.

Bone Specific Alkaline Phosphataseanalysis

Bone-specific alkaline phosphatase (BSAP) is marker of activity osteoblast. This marker has value sensitivity 96% and specificity 80% and minimal diurnal variation. Measurement of isoenzymes BSAP by the calorimetry method using a spectrophotometer. The first blood collection from median cubital veins in both groups was examined for bone-specific alkaline phosphatase levels with the type of metra-sample stored that was performed on the second day after surgery. Second blood collection from median cubital veins in both groups for the examination of bone-specific alkaline phosphatase levels was carried out on thirty-first day after surgery.

Callus Formation Analysis

The first x-rays (AP/lateral position) of the tibia in both groups were examined on the first day after surgeryby the radiologist on the Cummer scale. The second x-rays (AP/lateral position) of the tibia in both groups were examined on the thirty-first day after surgery by a radiologist with Hummer scale. Reason chose Hummer because it has a valid level of measurement accuracy and Cohen's Kappa Hummer scale test results k = 0.65, CI 95% 0.59-0.75.

Statistical Analysis

Data analysis used: 1. Univariate which describing the distribution of the proportion of data; 2. Analysis of the homogeneity test against; bone-specific alkaline phosphatase, age, body mass index, and hemoglobin; 3. Analysis of hypothesis used a paired t-test with α of 0.05. and Confidence Interval 95%; 4. Analysis linear regression has used the relationship between the results of callus assessment with bone-specific alkaline phosphatase.

Results

The study population consisted group I of 13 (81%) males and 3 (19%) females with majority in the age group of 36-40 years 6 (37%) followed by 25-29 years 4 (25%), 41-45 years 3 (19%), 30-35 years 3 (19%) age groups. Group II (Control) of 12 (75%) males and 4 (25%) females with majority in the age group of 36-40 years 6 (38%) followed by 30-35 years 5 (25%), 25-29 years 4 (25%), 41-45 years 1 (6%) age groups (Table 1). Two samples drop out.

Table 1: Characteristics sample

Age (year)	GROUP I		GROUP II	
	Freq.	%	Freq.	%
25-29	4	25	4	25
30-35	3	19	5	31
36-40	6	37	6	38
41-45	3	19	1	6
Total	16	100 %	16	100 %
Gender				
Male	13	81	12	75
Female	3	19	4	25
Total	16	100 %	16	100 %

The level bone-specific alkaline phosphatase increased in both groups I and groups II (Table 2).

Table 2: Level Bone Specific Alkaline Phosphatase

GROUP I				GROUP II			
Sample number	BSAP 1	BSAP 2	Increase	Samplenum ber	BSAP 1	BSAP 2	Increase
1	14.9	28.8	13.9	2	27.3	31.1	3.8
3	15.7	27.3	11.6	4	31.6	33.8	2.2
5	26.2	29.5	3.3	6	16.1	21.9	5.8
7	20.1	44.9	24.8	8	28.3	38.4	10.1
9	23.4	45.4	22.0	10	23.1	26.6	3.5
11	16.4	34.0	17.6	12	30.0	35.5	5.5
13	20.1	26.4	6.3	14	27.4	28.8	1.3
15	28.3	36.6	8.3	16	22.3	26.2	3.9
17	30.7	52.9	23.2	18	31.7	36.7	5.0
19	29.4	37.4	8.0	20	27.4	30.7	3.3
21	28.4	41.5	13.1	22	19.6	22.2	2.6
23	44.0	98.3	54.3	24	21.7	29.0	7.3
25	16.9	29,5	12.6	26	19.6	30.3	10.7
27	30.8	33.0	2.2	28	31.1	34.4	3.3
29	19.9	48.1	28.2	30	20.5	28.2	8.2
31	26.5	27.5	1.0	32	out	out	Out
33	out	out	out	34	20.1	26.4	6.3

Homogeneity test results; bone-specific alkaline phosphatase, age, body mass index,and haemoglobin with alpha 0.05 and 95% confidence intervals. Homogeneous test conducted on both groups were obtained;

1. The level of bone-specific alkaline phosphatase obtained a value of p = 0.869 which showed the levels of bone-specific alkaline

phosphatase in both groups were homogeneous.

2. Age in both groups obtained a value of p = 0.418 which showed the age in both groups is homogeneous.

3. Body mass index in both groups obtained p = 0.652 which showed that body mass index in both groups is homogeneous.

4. Hemoglobin levels in both groups obtained p = 0.158 also showed hemoglobin levels in both groups were homogeneous (Table 3).

Table 3: Homogeneity BSAP, Age, BMI, and Hb

No	Variable	Mean difference	Std.error	p
1.	Bone-specific alkaline phosphatase	0.869	2.29	0.869
2.	Age	0.418	2.06	0.418
3.	Bodymass index	0.652	0.38	0.652
4.	Hemoglobin	0.158	0.16	0.158

Because the distribution of bone-specific alkaline phosphatase levels in both groups are normal, then a paired t-test performed with α 0.05 and 95% confidence interval obtained the p-value = 0.000 (Table 4), which showed a significant difference in the increase in average bone specific levels alkaline phosphatase in the group that carried out isometric exercises plantar flexor with those who did not do isometric exercise plantar flexor in patients after open reduction internal fixation of non-articular tibia fractures.

The Paired t-test that was carried out on 2 groups was obtained bone-specific alkaline phosphatase levels in group I and group II with α 0.05 and CI 95% p-value = 0.000 which showed a significant difference in the increase in mean bone-specific alkaline phosphatase in the group that do isometric exercises plantar flexor with those who do not do isometric exercise plantar flexor in patients after internal open reduction fixation of non articular tibia fractures (Table 4).

Table 4: Paired t-test Bone Specific Alkaline Phosphatase Group I and group II

	Group I			Group II			p
	N	Mean	St.deviation	N	Mean	St.deviation	
Pct_BSAP	16	66.34	44.99	16	22.34	13.93	0.000

Analysis of callus assessment uses the Wilcoxon test on the group I who did the isometric exercise plantar flexor and group II did not do isometric exercises plantar flexor obtained the following results are obtained.

In the group, I the Hummer scale callus results obtained an average value of 2.63, the standard deviation of 0.72, and variance of 0.52, while in group II the results of the Hummer scale callus examination obtained an average value of 3.06, the standard deviation of 0.25, and a variant of 0.06. The Wilcoxon test obtained a p-value of 0.000, which showed a significant differences in callus formation between the two groups (Table 5).

Table 5: Wilcoxon test for Formation Callus of Group I and Group II

GROUP I			GROUP II			Wilcoxon
Mean	St.Deviasi	Variance	Mean	St.Deviasi	Variance	P value
2.63	0.72	0.52	3.06	0.25	0.06	0.000

Linear regression calculation results of callus examination with the results BSAP obtained by the value of R 2 quadrat = 0.793, which showed Hummer classification from callus formation able to predict a change BSAP in value 79%.

Discussion

The number of samples of patients with non-articular tibia fractures in this study we found 25 male (78%) and 7 female (22%). The highest age was 36-40 years old as many as 12 people (38%). Other researchers also reported more tibial fractures in male than female [8]. Chauhan et al., 2016 [9] reported that the results of epidemiological studies of tibia fracture were found in male as much as 78% (n = 156) and female 22% (n = 43) with the highest age in the range of 21-30 years. The study conducted by Amin MQ in Pakistan in 2015 out of 2120 cases of tibia fractures, 1980 male (93.4%) and 140 female (6.6%) with an average age of 33.28 ± 21.02 years. Clelland et al., 2016 [10] reported that the results of research on male incidents were almost four times higher than female. The incidence of the male is more dominant because of more work activities outside the home so that the risk of having an accident is greater. The incidence of non-articular tibia fractures is generally in the productive age or working age.

Stages of process osteoblast differentiation include; increase in cell number, matrix maturation, and matrix mineralization. The matrix maturation characterized by peak level of bone-specific alkaline phosphatase. Evaluation of bone healing processes is generally based on patient subjective statements in the form of complaints of pain and findings in the radiographic examination. Levels of bone-specific alkaline phosphatase as chemical marker in the early post-traumatic bone can predict fracture healing. Clinical measurement of isoenzymes bone-specific alkaline phosphatase is important to estimate the progress of bone healing after internal open reduction fixation of non-articular tibia fractures. Isoenzyme phosphatase is an isoenzymes group that plays a role in increasing the activity of serum or plasma bone-specific alkaline phosphatase. Clinical studies also show that serum levels of bone-specific alkaline phosphatase correlate with assessing bone formation processes [11].

Increase level bone-specific alkaline phosphatase in relation to trauma is a consequence of bone cell response to trauma, stress response, type of injury and surgery. Bone-specific alkaline phosphatase is very important for bone mineralization and is considered a biochemical marker useful for bone formation, thus examining this enzyme in serum used in evaluating bone healing process from fractures. The level of bone-specific alkaline phosphatase in fracture depends on the stability of bone fragments, bone type, and the number of bone fractures. Studied increase level in bone-specific alkaline phosphatase in relation to the type of trauma and concluded that the initial decrease in bone-specific alkaline phosphatase level was not only a consequence of bone response to trauma but also the total stress response associated with injury and surgery [12]. Another study noted significant bone-specific alkaline phosphatase levels decreased until several days after injury, on the eighth day the enzyme returned to the values of the first day and then continued to increase until the twelfth week in tibial fractures [13]. In a group, I who received isometric exercises plantar flexor for 4 weeks, knee and ankle joint motion exercises had an increase in the average value of bone-specific alkaline phosphatase levels that exceeded that of group II who only gained a range of motion in knee and ankle joints. These data illustrate that there was an increase in osteoblast activity that was higher in group I than in group II.

Exercise can increase cytokine reactions, it is based on research that after a while physical exercise increases IL-1 and IL-6. Other researchersfound that IL-1 also increased at the same time in all high-intensity training groups and IL-6 levels had a tendency to rise immediately after exercise. Osteoblast activity is also affected by bone morphogenetic protein (BMP), Platelet Derived Growth factor (PDGF), as well as by physical loading that work through mechanical sensors. These biological and mechanical signals have been shown to stimulate proliferation, migration, and differentiation of mesenchymal stem cell (MSC) against osteoblasts. The cell response to mechanical load of exercise plays a very important role in MSC development process. Plasticyti of bone, supression, fixation, shear, and velocity fluid flow are main factors that act as stimuli for cell growth during bone repair. Mechanical stimulation with low magnitude has been shown to be anabolic to bone [14]. Osteoblasts have a pressure receptor. The receptor receives a mechanical load. Mechanical load stimulates some physical signals that induce the activation of osteoblasts. Bone is a porous network consisting of a liquid period, a solid matrix and cells.

Bone is very dependent on mechanical information that guides the population of cells to adaptation, maintenance, and improvement [15]. Mechanical transductive signals prove important for affect certain cell responses and the underlying mechanism. The pathway of mechanotransduction is very interesting to explain the burden of muscles contraction has an effect such as changes in bone mass, increased bone formation, and osteoblast activity [16]. Physical exercise affects the effects on physiological, hormonal, and immunological processes. Exercises involving skeletal muscle contraction will occur in the process of synthesis and release of interleukin-6 and interleukin-15. After muscle damage was induced by exercising/exercising the IL-6 ratio in myofibers immediately increased, with a peak in 12 hours. Simultaneously in the blood, there is an increase in the number of neutrophils that stimulate and trigger the release of macrophages and lymphocytes. Macrophages moving to the injury site perform phagocytic functions and chemotactic attract satellite cells. The interaction between satellites and macrophages is mediated by IL-6. These cytokines are also involved in increasing protein degradation, multiplying satellite cells that can support muscle regeneration. Satellite cells follow a series of proliferation, migration, and incorporation into adult myofibers which leads to the growth of muscle fibers or hypertrophy.

Apart from playing a role in muscle growth, interleukin affects the activity and differentiation of osteoblasts [17]. Changes in increase in mechanical forces stimulation the development of bone marrow mesenchymal stem cell against osteoblastogenesis [18]. These local mechanical exercises or stimuli produce mechanotransduction, which is the conversion of physical signals into a typical signal of intracellular biochemistry that produces a biological response to the bone. The mechanotransduction process also involves fluid movement (lamellar fluid flow) which stimulates detector cells, but the transduction of mechanical factors into cellular stimulation is not yet fully understood (Isaksson, 2012) [19].

The burden of muscle contraction on the surface of the bone will cause fluid flow through changes in intramedullary pressure [20]. This mechanical force will increase the expression of nitric oxide synthase in osteocyte so that the production of nitric oxide increases. Nitric oxide suppresses osteoclast activity and increases osteoblast activity which is very important in the process of fracture healing. Plantar flexor isometric exercises can also induce prostaglandin synthesis by increasing cyclooxygenase2 (COX2) activity, the level of COX2 levels depends on extracellular regulated kinase (ERK) phosphorylation. Prostaglandin stimulates osteoblast activity through the Insulin Growth Factor. Insulin hormone growth factor increases during mechanical stimulation [20].

Sclerostin signals decrease in the stimulation of muscle contraction exercises. Sclerostin is an osteocytic protein that can inhibit Wnt signals. This signal plays an important role in the proliferation and differentiation of osteoblasts. The ratio of RANKL and Osteoprotegerin in osteocyte decreases in strains. If mechanically stimulated, osteocyte will produce TGF ß which plays a role in inhibiting osteoclastogenesis [21]. Transforming Growth Factor Beta (TFG-β) that regulate osteoblast activity including proliferation, differentiation, and tissue migration. After bone fracture will occur hematoma , expression of TFG-β increases and continues to be expressed throughout the remodeling phase. TFG-β plays a role in the process activation of mesenchymal stem cells (MSC).

Pressure from muscle contraction will be transferred through the muscle to the tendon that attaches the muscle to the bone, and therefore pressure is also applied to the bone specifically at the location of the adjacent bone surface [22]. The isometric contraction of the plantar flexor muscle will provide mechanical stimulation of pressure on the bone where mechanical stimulation is channeled through the extracellular matrix to osteoblasts, osteocytes, periosteal cells and osteoclasts. In the osteoblast cell wall where the pressure received at the receptor will trigger activity rather than osteoblasts. This type of mechanical stimulation can increase the proliferation and anabolism of osteoblasts to facilitate reconstruction of bone tissue, contributing to the process of homeostasis in bone tissue healing.

Effect isometric exercise plantar flexor and healing process of the tibia bone, activities osteoblast cells produce osteoid tissue and increase the amount of content alkaline phosphatase, which in the reaction of mineralization this enzyme prepares an alkaline atmosphere in the formed osteoid tissue. This enzyme in the bone increases phosphate concentration in the form of calcium-phosphate bonds in the form of hydroxyapatite which is based on mass law so that it plays an important role in depositing calcium and phosphate into the bone matrix.

The formation callus results were seen in group I which carried out plantar flexor isometric exercises had an average value of 2.63, whereas group II who did not do isometric plantar flexor exercises rated an average of 3.06. Bone-specific alkaline phosphatase levels in the blood of group I who obtained isometric plantar flexor exercise found a higher increase compared to group II who did not do isometric exercises of the plantar flexor muscle. This is because the activity of osteoblasts build bone by forming type I collagen and proteoglycans as a bone matrix or tissue osteoid through a process called ossification, and while actively producing osteoid tissue, osteoblasts increase the amounts of content alkaline phosphatase [23], [24].

In conclusion, isometric exercise plantar flexor 30 times a day for 4 weeks can increase osteoblast activity and callus formation in patients post open reduction internal fixation of non-articular tibia fractures. Thisexercise will prevent delayed union and non-union in non-articular tibia fractures.

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