Platelet Count and Mean Platelet Value of Rabbit Implanted Bali Cattle Bone Graft

(*JUMLAH PLATELET DAN MEAN PLATELET VALUE KELINCI PASCA IMPLANTASI BAHAN CANGKOK TULANG ASAL SAPI BALI*)

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Abstract

Platelets have an important role in fracture healing. However, its role is still under appreciated. Therefore, this study investigated the values of mean platelet volume and platelet count as the parameters in fracture healing. This study used 10 male local rabbits which were divided into 2 groups randomly, KI (control group) and KII (treatment group). Each femur of both groups was drilled a hole with a diameter of 5 mm, where KI without any substitutes and KII was implanted by demineralized bali cattle bone graft. Each animal was evaluated by collecting its blood from the central auricular vein into an EDTA tube at 24 hours, week 2, 4, and 6 postoperative determining the values of MPV and PC. Results show that treatment shows no differences to MPV and PC, yet time observation does. Overall, MPV and PC were increased in number during observation and are correlated positively.

Keywords: bone graft; fracture healing; mean platelet value; platelets

Abstrak

Platelet mempunyai peran penting dalam kesembuhan fraktur. Namun, perannya masih kurang dihargai. Oleh karena itu, penelitian ini akan memeriksa nilai dari *mean platelet volume* dan *platelet count* sebagai parameter dalam kesembuhan fraktur. Penelitian ini menggunakan 10 ekor kelinci lokal jantan yang dibagi atas 2 kelompok secara acak, KI (kelompok kontrol) dan KII (kelompok perlakuan). Setiap femur dari kedua kelompok dibor sebuah lubang dengan diameter 5 mm, dimana KI tanpa ada substitusi dan KII diimplantasi dengan bahan cangkok sapi bali demineralisasi. Setiap kelinci dievaluasi dengan cara mengambil darah dari vena aurikular sentral ke dalam tabung EDTA pada waktu pascaoperasi 24 jam, minggu ke-2, 4, dan 6 dengan menentukan nilai MPV dan PC. Hasil menunjukkan bahwa perlakuan tidak menunjukkan adanya pengaruh terhadap MPV dan PC, tetapi waktu pengamatan menunjukkan adanya pengaruh. Secara keseluruhan, MPV dan PC meningkat selama pengamatan dan berkorelasi positif.

Kata kunci: bahan cangkok; kesembuhan fraktur; *mean platelet value*; platelet

INTRODUCTION

Fracture is a bone condition when it loses its continuity. The condition could be fixed by bone graft application. Many kinds of bone graft have been studied clinically and experimentally, especially xenograft. A bovine xenograft is one of many kinds of xenografts which has been widely used for orthopedic purposes. An experimental study of Wirata *et al.* (2018) reported that animal studies were experiencing fracture healing by the usage of bovine xenograft. Fracture healing should be monitored routinely and could be assessed by many diagnostic instruments, yet a hematological parameter is the one to be used. To have a specific measurement of fracture healing,

platelets could provide clinicians helpful information to assess fracture healing on a patient.

Platelets are one of blood elements with a variety of densities and sizes (Ocak *et al.*, 2013). Platelets have an important role in wound healing, hemostasis, inflammation, and repair of mineralized tissue, as well as in fracture healing (Barnes *et al.*, 1999; Sharif and Abdollahi, 2010). The study of Gruber *et al.* (2002) reports that platelets contribute to the healing of skeletal defects and the regeneration of the grafted bone matrix. An experimental study demonstrated that the migration and proliferation of bone marrow cells (BMCs) cultured in osteogenic condition were increased by platelet releasate (PR) (Kark *et al.*, 2006). Platelets degranulation, anuclear cell fragments, affects bone metabolism by releasing different growth factors and chemoattractants (Sharif and Abdollahi, 2010).

Activated platelets will release growth factors such as platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), insulin growth factor-1 (IGF-1), insulin growth factor-2 (IGF-2), epidermal growth factor (EGF), and transforming growth factor-β (TGF-β) and mediators like thromboxane A_2 (TXA$_2$) and prostaglandins (PGs) are released due to platelets degranulation (Sharif and Abdollahi, 2010). These are known as PR, which can stimulate and enhance osteogenic cells recruitment and migration in bone regeneration (Kark *et al.*, 2006).

On the other hand, mean platelet volume (MPV), one of the platelets parameters is sometimes forgotten by clinicians. Mean platelet value (MPV) can be used as an indicator of platelet function or platelet activity (Mayda-Domac *et al.*, 2010; Ocak *et al.*, 2013). Thus, MPV can be considered as one of the important parameters to use in monitoring bone regeneration. A high MPV can be used to predict poor patient prognosis (Noris *et al.*, 2016). In this study, we wanted to determine the values of MPV and platelets count (PC) and its correlation on bone regeneration of rabbit post-implantation bali cattle bone graft.

RESEARCH METHODS

Animal Model Preparation

Ten male local rabbits weighed 2.5 kg ± 0.5 kg were used in this study and divided into two groups randomly, KI as the control group and KII as the study group. Rabbits in this study had permission as fracture animal model by the Ethics Committee, Faculty of Veterinary Medicine Udayana University (No. 293/KE-PH-Lit-1/V.2017).

Bone Graft Processing

Demineralized bovine bone graft processing used the same method of the study of Wirata *et al.* (2018). In brief, cortical bali cattle femur bone was cleared from muscles and fats and smashed with a hammer into powder. The powder was then washed in aquadest and NaCl 0.9%. Next, the powder was soaked into chloroform-methanol (ratio 1:1) for 12 hours to defatten and deproteinize. Then, the powder was soaked into HCl 5% for 72 hours at room temperature and HCl was changed with an interval 12 hours. The ratio of powder weight and solution volume was 50 g : 500 mL. The powder was then washed with aquadest and 70% alcohol and preserved into 70% alcohol until it was getting used.

Surgical Procedure

After adapted for a week, the surgical procedure was done without fasting as the same method of the study of Wirata *et al.* (2018). Each rabbit was administered a combination of Xylazine-Ketamine (5 mg/kg BW and 35 mg/kg BW) intramuscularly. The anesthetized rabbit was positioned in left lateral recumbency. The surgical site was shaved craniolaterally along the femur bone. The femur was exposed and diaphysis was drilled with a diameter of 5 mm. The bone was washed with sterile saline to prevent heat damage while drilling the bone. In the control group (KI), diaphysis of the femur was drilled

without implanting bone graft. While in the study group (KII), diaphysis of the femur was drilled and the defect was filled with demineralized bali cattle bone graft. Muscles were sutured with 0000 catgut chromic and skin was sutured with 3-0 silk. Each rabbit was administered 10-30 mg/kg BW of enrofloxacin for 5-7 days and 1.5 mg/kg BW of carprofen for seven days, both orally.

Sampling

Platelet parameters were measured by collecting blood 3 mL from the central auricular vein into an EDTA tube at 24 hours, week 2, 4, and 6 postoperative. Blood was analyzed with an automatic blood analyzer (iCell-800 Vet, iCubio, China). Platelet parameters such as platelet count (PC) and mean platelet volume (MPV) were determined and their values were recorded.

Data Analysis

Data analysis was performed with the regression-correlation test for the determination of the correlation degree among the platelet parameters. The multivariate test was also used for the analysis of the effect of treatment and time in MPV and PC and Duncan's test was performed when there was a significant. Spearman's test was performed to determine the correlation between MPV and PC to bone graft application. Significant p-value was considered less than 0.05. Statistical analysis was performed with SPSS Program version 22.

RESULTS AND DISCUSSION

Results

The mean of MPV of group KI and KII is presented at Table 1 and PC at Table 2. Treatment shows no differences both to MPV and PC ($p>0.05$). However, time shows differences significantly to MPV and PC ($p<0.05$). Interaction between treatment and time also shows no significance ($p>0.05$).

Results of Duncan's test show that MPV on 24 hours has no differences to other weeks ($p>0.05$) and week 4 has differences to week 2 ($p<0.05$), yet there is no differences to week 6 ($p>0.05$). On the other hand, MPV on 24 hours and week 4 show no differences to other weeks ($p>0.05$) and week 2 has differences to week 6 ($p<0.05$).

Treatment was correlated positively to MPV and PC. In other hand, treatment shows no significance to both MPV ($p = 0.770$) and PC ($p = 0.614$) ($p>0.05$). Regression results show that time affects both MPV and PC ($p<0.05$) with R value each was 0.455 quadratically and 0.396 linearly. In this study, MPV can be counted with the regression formula of $y = 5.526 + 0.003x - 0.259z + 0.051z^2$, which y is MPV, x is PC, z is time, and z^2 is time square.

Discussion

Bone is a highly vascularized tissue (Gruber *et al.*, 2002). Several types of fractures will cause blood loss as this study caused the fractured animal model bleeding. On 24 hours, a high number of MPV and PC on both groups could be happened because of immediate responses of platelets to fracture where the degranulation of platelets takes place during hematoma formation (Gruber *et al.*, 2002). In the case, activated platelets and their aggregates are surrounding the fracture site (Sharif and Abdollahi, 2010). The reaction of platelets orchestrated via complex signaling network including adhesion aggregation, granule release, and procoagulant expression (Weiss and Wardrop, 2010). Ocak *et al.* (2013) states that an increased MPV is indicating more reactive platelets (Ocak *et al.*, 2013).

On vascular injury, platelets will cover the exposed subendothelial matrix and mediate the recruitment of platelet and leukocyte. In result, a clot will be formed on the injury (Wagner and Burger, 2003). Ali *et al.* (2015) reports that inspite of platelets are commonly found intravascularly, they can greatly affect leukocyte recruitment to the inflammation are in many tissue. Platelets are activated at sites of the injury where they create a physical barrier to limit

blood loss and accelerate the generation of thrombin to intensify the coagulation process (Gruber *et al.*, 2002). Growth factors released from platelets degranulation are getting involved in clot formation and wound healing (Sharif and Abdollahi, 2010). To the fracture hematoma, growth factors that are abundantly storage in platelets can stimulate periosteal cells proliferation and differentiation and attract granulocytes and macrophages (Joyce *et al.*, 1990; Barnes *et al.*, 1999).

Evaluation results in the 2nd week show that both MPV and PC were getting decreased slightly in number from 24 hours, except in platelet evaluation on KII. However, MPV and PC were increased both groups on the following evaluation, except MPV of KII on week 6. This could be happened due to a wide variability of MPV and PC values because of multiple factors.

Thus, this could affect the result that it is shown that there is no differences between time to MPV and PC. A study of Arslan *et al.* (2017) reports that MPV and PC have a negative correlation in dogs with hemorrhagic enteritis. Contrary, this study shows that MPV is correlated positively to PC. The more PC is increased, the more MPV is increased. The size of platelet is determined by the level of the progenitor cells (Mayda-Domac *et al.*, 2010). MPV is also influenced by genetic polymorphisms and large platelets (Noris *et al.*, 2016).

In this study, thrombocytopenia was occurring on both groups, though MPV values were in the normal range. In the previous study of Wirata *et al.* (2018), exposure of radiation was performed due to fracture healing evaluation. We assumed that this condition can be caused by decreased platelet production with irradiation which could cause cell death and marrow suppression (Weiss and Wardrop, 2010).

CONCLUSION AND SUGGESTION

Conclusion

In summary, MPV and PC values of rabbits implanted bali cattle bone graft are correlated positively. Both values were increased during observation.

Suggestion

Further study needed about the correlation between red blood cells and their indexes to platelet parameters to elucidate the efficacy of bali cattle bone graft application and should be done in a longer observation. In order to limit blood loss and increase platelets activities, a vasoconstrictor drug should be considered to be administered postoperative and irradiation repetitively should be minimized.

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Tabel 1. Mean value of mean platelet value

Time (Week)	Mean Platelet Volume (Mean±SD) (fL)	
	KI	KII
0 (24 hours)	5.90±0.28	6.38±0.49
2	5.72±0.53	5.68±3.77
4	6.28±0.69	5.86±0.15
6	6.56±0.67	6.44±0.88
Mean±SD	6.11±0.62	6.09±0.60

Note: KI: control group without bone graft implantation; KII: study group with demineralized bone graft implantation on the defect. Reference range of MPV is 5.0-20.0 fL.

Tabel 2. Mean value of platelet count

Time (Week)	Platelet Count (Mean±SD) (x 10^9/L)	
	KI	KII
0 (24 hours)	234.20±44.14	218.60±50.25
2	196.80±64.55	224.40±68.46
4	262.80±44.28	273.80±56.46
6	299.80±19.00	248.20±60.24
Mean±SD	248.40±57.27	241.25±58.78

Note: KI: control group without bone graft implantation; KII: study group with demineralized bone graft implantation on the defect. Reference range of PC 270-656 x 10^9/L.