Clinical Evaluation of Platelet Rich Plasma Application on Autogenous Bone Graft in a Sheep Model

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Abstract

Autogenous Bone Graft is considered the safest material due to the compatibility of the material with the patient's body. A non-vascularized bone graft can apply growth factors such as Platelet-rich-plasma (PRP) to catalyze bone healing. In Indonesia, no research currently analyzes PRP's application to autogenous bone grafts in jaw bone in vivo.

To analyze the clinical effect of Platelet Rich Plasma (PRP) application in non-vascularized autogenous bone graft in the mandible of Ovis aries sheep as a human model.

A total of 24 Ovis aries sheep were classified as PRP and non-PRP groups. A defect on the sheep's mandible measuring 2 cm x 1.5 cm x 2cm was made, and the cut segment was then used as a scaffold. The cut segment was then refined into a mill using pestle and mortar and mixed with Platelet Rich Plasma (PRP). The analyzed variables were signs of infection before and after surgery and signs of inflammation such as wound temperature, hyperemia, and edema.

Based on the results of statistical tests, there was no statistically significant difference in temperature, edema, and hyperemia between the PRP and non-PRP groups. Conclusion: PRP applied to mandibular defects does not affect the bone healing mechanism clinically.

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Introduction

Unreconstructed defects in the mandible can cause severe morbidities such as impaired mastication, speech, and aesthetics[1]. The mandibular defect can be caused by trauma, infection, or pathological and congenital conditions. Reconstruction is required to correct the defect to improve the quality of life[2,3]. Many patients have a severe mandibular bone defects that are difficult to reconstruct and often require bone tissues and for functional and structural restoration.[4] The proposed method for mandibular reconstruction uses reconstruction plates, non-vascularized and vascularized bone grafts[5]. The reconstruction of the mandibular defect can also be performed by adding graft material. Grafting material can be classified as autogenous, allograft, and xenograft. An autogenous bone graft is a material that can be derived from the same individual body. Meanwhile, xenograft is a bone substitute material derived from inorganic parts of bone harvested from species that are genetically different from the graft recipient.[5]

Autogenous Bone Graft is considered the safest material due to the compatibility of the material with the patient body. An autogenous bone graft can be obtained from several anatomical areas such as symphysis of the mandible, ramus, or cancellous areas of the maxillary tuberosity and bones in the ramus-posterior mandibular body. In addition, the donor site for autogenous bone graft collection can also be obtained from the anterior iliac crest, tibia, fibula, and calvarium.[6]

Non-vascularized bone graft has several advantages compared to vascularized bone graft in mandibular reconstruction due to lower donor site morbidity, more straightforward

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instrumentation, and not requiring complex operator skills.[7] The use of non-vascularized bone graft can be done by application of growth factors to catalyze bone healing.[7,8]

Platelet-rich plasma (PRP) is a major source of growth factors that can be used to improve bone healing. It can release autologous growth factors in platelets without the risk of disease transmission or immunogenic reactions.[9] In Indonesia, no research currently analyzes PRP's application to autologous bone grafts in vivo. Based on the above background, this study aims to analyze the clinical effect of Platelet Rich Plasma (PRP) addition in non-vascularized autogenous bone graft in *Ovis aries* sheep as a human model.

Materials and methods

This research was conducted using a quasi-experimental design with a post-test control group design. The number of the subject was 24 *Ovis aries* sheep. A total of 12 sheep were classified as part of the experimental group (PRP), and the rest was part of the control group (non-PRP). We use a mixture of platelet-rich plasma (PRP) taken from sheep blood and the mandibular bones from the sheep. We made a defect on the sheep's mandible measuring 2 cm x 1.5 cm x 2cm; the cut segment was then used as a scaffold. The cut segment was then refined into a mill using pastel and mortar, mixed with Platelet Rich Plasma (PRP). The grafting materials are then covered with a membrane (Batan®). The analyzed variables were signs of infection before and after surgery and signs of inflammation (wound temperature and edema) one day, three days, and seven days after the surgery. Temperature measurements were taken on the postoperative wound at one day, three days, and seven days postoperatively. The observation was also made on whether there were signs of inflammation, such as hyperemia and edema, on the postoperative wound. Then a comparison was made between subjects with PRP and non-PRP. All data were then tested by normality test using Kolmogorov Smirnov and then homogeneity test with Levene's Test. Finally, data analysis was then performed using the Mann-Whitney test.

Results

In observing the temperature of PRP subjects, there was an increase in temperature on the first day postoperatively, which slowly decreased on the third and seventh postoperative days. In the non-PRP group, the highest average temperature was obtained on the first and third postoperative days. However, based on statistical analysis, no significant difference in temperature was found at one day, three days, and seven days postoperatively as shown in Figure 1.

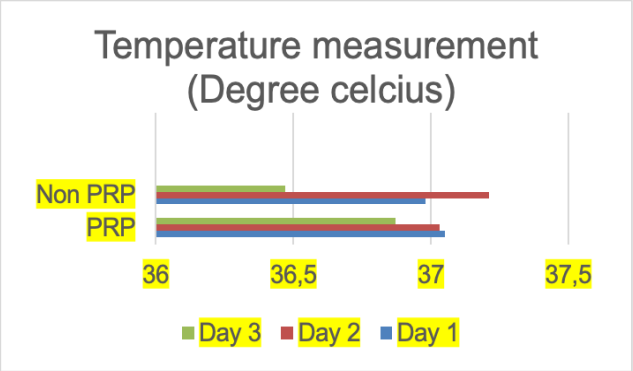

Figure 1. Temperature measurement of PRP and Non-PRP group (in degrees celsius).

In Figure 2, on observation of edema, the greatest edema occurred on the third postoperative day. This is consistent with the theory of the wound healing phase, where inflammation is usually resolved on the third day. Based on the results of statistical tests, it was concluded that there was no statistically significant difference in edema between the PRP and non-PRP group.

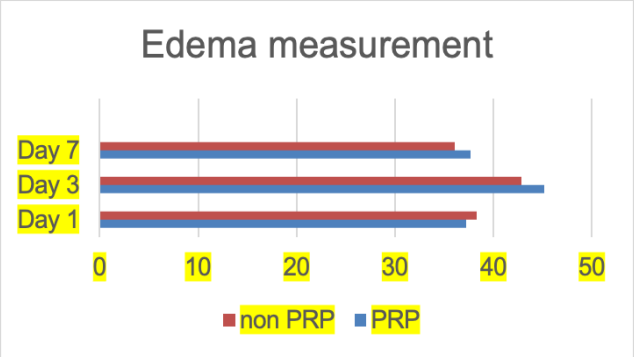

Figure 2. Edema measurement of PRP and Non-PRP group.

On observation of hyperemia, there was hyperemia in the wound area fading from the first day to the seventh postoperative day. Based on the results of statistical tests, it was concluded that there was no statistically significant difference in hyperemia between the PRP and non-PRP groups as shown in Figure 3.

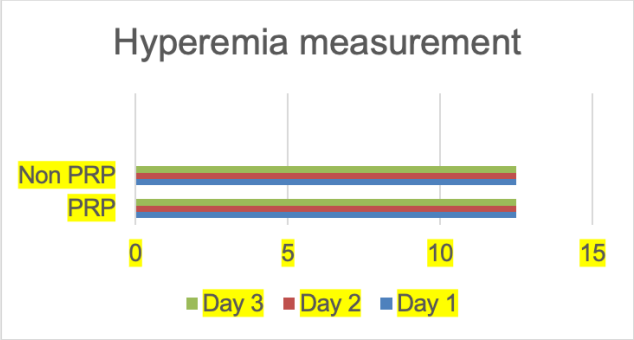

Figure 3. Hyperemia measurement of PRP and Non-PRP group.

Discussion

Large defects in the jaw bone can result in severe morbidity and functional impairment. Therefore, it is necessary to reconstruct the defect. Reconstruction of the defect can use bridging plates and free nonvascularized and vascularized bone grafts.[10]

Recent research has developed many bioengineering techniques in the development of bone grafts, including in mandibular reconstruction.[11] Bone graft material can reconstruct and trigger the osteogenesis process. Various bone graft materials exist, but autogenous bone graft is still considered the gold standard. The bone healing process in the graft area can occur through three mechanisms, namely osteoconduction, osteoinduction, and osteogenesis. Osteogenesis is a healing mechanism that occurs through the formation of bone tissue by new cells. Specific proteins are needed to start the osteogenesis process. Platelet Rich Plasma (PRP) is a protein known to accelerate the osteogenesis process. PRP is a protein that can be obtained from blood after a centrifugation process.[12] Local application of PRP in autogenous bone graft aims to release various growth factor such asbone morphogenetic proteins (BMP), platelet-derived growth factor, transforming growth factor-h, and insulin growth factor-I, which mediate osteogenesis processes. [13, 14] The biological process of bone healing is a complex mechanism that involves a specific regenerative pattern and expression of thousands of genes.[15]

Inflammatory process usually occurs immediately after trauma and peaks within 24 hours lasting until 7 days. During this process, Polymorphonuclear neutrophil (PMN) secrete inflammatory and chemotaxis mediators, such as IL-6 and CCL2, to recruit a second wave of inflammatory cells, including monocytes and macrophages, which infiltrate the wound healing area.[16] However, an excessive number of neutrophils can interfere with bone healing. The acute inflammatory phase and the formation of a hematoma are critical for cleaning the hematoma area from the fracture and eliminating the acute inflammatory phase.[17]

Several research studies have studied the administration of PRP in mandibular defects. The results of these studies have been inconsistent, with some indicating that PRP accelerates the healing process and others finding no significant effect. This variability in results can be attributed to several factors, such as differences in research methods, lack of standardization in PRP preparation, and variations in the number of platelets present in individual plasma samples.[18,19] However, an *in vivo* study showed that incorporating PRP to an autogenous bone graft can increase production of collagen in mandibular bone healing in sheep.[20]

Based on clinical examination, our study showed no significant difference in signs of inflammation, such as temperature increase and edema, between samples treated with PRP and those without PRP at 1-, 3-, and seven days post-surgery. While a slight increase in local temperature (about 1 °C) was observed in all samples, there was no difference in temperature on the forehead between the two groups. In addition, on statistical analysis, there was no overall difference in temperature between PRP and non-PRP samples. The greatest edema occurred three days post-surgery, consistent with the theory that the inflammatory phase lasts up to 3 days. However, statistically, there was no significant difference in edema between the two groups of samples.

Postoperative hyperemia was found in all sample sheep during observation for seven days postoperatively, starting from day +1 post-surgery to day +3 post-surgery and slowly fading. This is in accordance with the theory of the

wound healing process; namely, the inflammatory phase occurs on the first to the third day (Physiology of Wound Healing Christine).[21] In this phase, signs of inflammation, such as redness, increased temperature, and swelling, usually occur in the operating area. Adding PRP to the autogenous bone graft applied to the mandibular defect does not affect the clinical healing process. The addition of PRP to the autogenous bone graft can be accepted by the sheep's body and is not considered a foreign object because the bone graft comes from the mandibular bone of the *Ovis aries*.

Adding PRP to the autogenous bone graft applied to the defect is an easy and inexpensive reconstruction method for mandibular defects. PRP processing can be performed in the operating room, thus reducing the operation time. When inserted into the defect, the PRP and autogenous bone graft mixture will become denser but can still be shaped, making it easier to apply to the mandibular defect.

There was no significant difference in inflammation signs at pre- and post-surgery closure of the mandibular defect between PRP and non-PRP use. JW Stoelinga and JP Fennis conducted a similar study, using male goats and radiographic evaluation to assess bone healing in mandibular reconstruction with autogenous bone graft mixed with PRP or without PRP, and the results showed no difference.[22]

Conclusions

It can be concluded that PRP applied to mandibular defects does not affect the bone healing mechanism clinically.

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Declaration of Interest

The authors report no conflict of interest.

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