ABSTRACT

Implants are widely used for orthopaedic applications such as fixing fractures, repairing non unions, obtaining a joint arthrodesis, total joint arthroplasty, spinal reconstruction, and soft tissue anchorage. Loosening cases in implant usage usually caused by bone resorption. Osteoclast have a pivot role in bone resorption. IL-6 is secreted by osteoblast as a response to metal ion. The purpose of this study is to know the differences between IL-6 express in tibial bone wistar rat applied titanium and stainless steel k-wire. This is an experimental study with Randomized post-test only control group design. Collected thirty wistar rat, divided into three groups, which are P0 treated with burr only in tibial bone, P1 treated with applied titanium k-wire Ø 1,25 in tibial bone, P2 treated with applied stainless steel k-wire Ø 1,25 in tibial bone. In the days 21 immunohistochemistry test was conducted and measured the expression of IL-6 on osteoblast. Statistic analysis with One Way Anova, The results shows p = 0,000. That means the differences of expression between three groups is significant (p < 0,05). This shows that this difference is statistically significant.This study conclude that there is a significance different expression of IL-6 between rat that applied titanium implant and stainless steel implant.

Keywords: Interleukin-6, Wistar rat, k–wire, Stainless steel, Titanium


INTRODUCTION

Implants are widely used for orthopaedic applications such as fixing fractures, repairing non unions, obtaining a joint arthrodesis, total joint arthroplasty, spinal reconstruction, and soft tissue anchorage. The primary aim of these devices is to provide mechanical stabilization so that optimal alignment and function of bone can be maintained during physiologic loading of bones and joints.

Biomaterials that widely used for orthopaedic surgery are stainless steel, pure titanium and titanium alloy. Implants used in medicine for bone osteosynthesis have to satisfy functional demands...
defined by the working environment of human body. Ideally, they should have biomechanical properties comparable to those of autogenous tissues without any adverse effects. The principal requirements are corrosion resistance, biocompatibility, biofunctionality, osseointegration. Implant implantation in bone can causes a local and systemic biological responses.

Inflammatory responses to implant debris over time have been attributed to macrophage reactivity and have been the primary focus of investigation over the past 40 years. Recent studies demonstrate a predominance of M1 macrophages in response to implant debris challenge (released metal ions and particles), which produce primarily pro-inflammatory mediators that affect other local cell around implants. Biomaterial surface properties play an important role in modulating the foreign body reaction in the first two to four weeks following implantation. An understanding of the foreign body reaction is important as the foreign body reaction may impact the biocompatibility (safety) of the medical device, prosthesis, or implanted biomaterial.

Loosening in implant implantation mostly caused by bone resorption. In bone resorption both active bone resorbing cells osteoclasts and osteoblastic cells play important roles. The osteoblastic cells regulate then osteoclast activity and formation by transmitting and amplifying osteolytic signals. Osteoclasts are activated by parathyroid hormone, 1,25(OH)2 vitaminD3, interleukins (IL-1, IL-6, IL-11), tumor necrosis factor-a (TNF-a) and prostaglandin E2 (PGE2). Interleukin Osteolytic reactions around implants are mediated by soluble factors such as interleukin-1. Metal ion concentration that related with toxic response of osteoblast can find in surrounding periprosthetic tissue.

**MATERIALS AND METHODS**

This study is an experimental study designed with Randomized post-test only control group design. Thirty wistar rat was collected, divided into three groups, which are P0 (group control) was treated with Burr only in tibial shaft, P1 was treated with applied titanium k-wire Ø 1,25 in tibial shaft, and P2 was treated with applied stainless steel k-wire Ø 1,25 in tibial shaft. In the end of the third week immunohistochemistry test was conducted and measured the expression of IL-6 on osteoblast.

This study was conducted in Farmacology laboratory of Medical Faculty Udayana University and Pathology laboratory of Veterinarian Faculty Udayana University. Starting from October 2015 – December 2015. The sample are wistar rat, male, age between 6 – 8 weeks, 250 gram weight. The results was analyzed for statistical significance using SPSS 22.0 for Windows. Descriptive analysis and inferential analysis one way Anova test was conducted.

**RESULTS**

Data of osteoblast that expression to IL-6 based on microscopic finding in each view. Results of of microscopic finding as shows in Figures 1,2,3. Figure 1; control grup, Figure 2; Grup 1 which apply titanium, Figure 3; Grup 2 which apply stainless steel.

Based on microscopic finding pointed that there are differences expression of IL-6 to osteoblast between group control and group one, group one also different with group two.

Numerik varibel test using One Way Anova; Tabel 1, explained that mean of IL-6 expression in control group is 10,10 ± 9,18, mean of IL-6 expression in group 1 is 13,10 ± 1,37, and in group 2 the expression is 25,30 ± 1,63. Results of Significance analysis with One Way Anova test shows that p value = 0,000. It means that the expression of IL-6 between those 3 groups are significans different (p < 0,05).

Post Hoc Test with Least Significant Difference – test (LSD) to identify the differences between three group, as shows in Tabel 2; Post-Hoc Test with Least Significant Difference – test (LSD) shows that there is significance different of IL-6 expression between control with group 1 and group 2. There is significance of IL-6 between grup titanium and grup stainless steel.

### Tabel 1 One Way Anova Test

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean IL-6 Expression</th>
<th>95% CI</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>13.10 ± 1.37</td>
<td>12.12</td>
<td>14.08</td>
</tr>
<tr>
<td>Group 2</td>
<td>25.30 ± 1.63</td>
<td>24.13</td>
<td>26.47</td>
</tr>
<tr>
<td>Control</td>
<td>10.10 ± 1.28</td>
<td>9.18</td>
<td>11.02</td>
</tr>
</tbody>
</table>

### Tabel 2 Post Hoc Test with Least Significant Difference – test (LSD)

<table>
<thead>
<tr>
<th>Group</th>
<th>Group</th>
<th>Mean</th>
<th>95% CI</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>Group 2</td>
<td>-12.20</td>
<td>-13.52</td>
<td>-10.88</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>3.000</td>
<td>1.68</td>
<td>4.32</td>
</tr>
<tr>
<td>Group 2</td>
<td>Group 1</td>
<td>12.200</td>
<td>10.88</td>
<td>13.52</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>15.200</td>
<td>13.88</td>
<td>16.52</td>
</tr>
<tr>
<td>Control</td>
<td>Group 1</td>
<td>-3.000</td>
<td>-4.32</td>
<td>-1.68</td>
</tr>
<tr>
<td></td>
<td>Group 2</td>
<td>-15.200</td>
<td>-16.52</td>
<td>13.88</td>
</tr>
</tbody>
</table>
Identifying better materials for implants to reduce the risk of implant failure will aid in preventing mechanical failure or further complications.

Biomaterial that widely used in orthopaedic surgery are stainless steel (AISI 316L), pure titanium (CP-Ti) and titanium alloy (Ti6Al4V). In this study using krishner wire titanium and stainless steel apply to tibial bone wistar rat than measured the IL-6 expression. This study using IL-6 antibody, NB600-1131, Novus Biologicas. This antiserum detects recombinant and native IL-6 present in body fluids and cell supernatants in various assays and can apply in immunocytochemistry test.

Over the years, rats have been used in many experimental studies, which have added to our understanding of genetics, diseases, the effects of drugs, and other topics in health and medicine. Laboratory rats have also proved valuable in psychological studies of learning and other mental processes. Mice age between 6 - 8 weeks, Ages related to adrenocortikal response to stress is varies; glucocorticoid released by adrenal gland to inhibit IL-6 release.

One way anova test results explained that mean of IL-6 expression in control group is 10,10 ± 9,18, mean of IL-6 expression in group 1 is 13,10 ± 1,37, and in group 2 the expression is 25,30 ± 1,63. Results of Significance analysis with One Way Anova test shows that p value = 0,000. This study demonstrate the significance different between titanium and stainless steel (p value <0,05). Regarding orthopaedic implant loosening it has been hypothesized that particle-activated macrophages release interleukin-1 (IL-1) and tumor necrosis factor-a (TNF-a), This in turn stimulates osteoblasts to release interleukin-6 (IL-6) and prostaglandin E2 (PGE2). These mediators recruit and activate osteoclasts and may therefore lead to bone resorption and loss of implant fixation.

Schmidt et all in their study tested stainless steel, cobalt–chromium alloy (CoCrMo), commercially pure titanium (cpTi), Ti–6Al–7Nb and Ti–6Al–4V processed in the same manner as corresponding clinical implants. After 12 and 24 h the cells had actively secreted IL-6 and PGE2. There were no clear differences among the implant materials or with the plastic control.

From the experimental results it can be seen under the conditions specific both titanium and stainless steel stimulate IL-6 production. A relatively constant lower rate for titanium and a three fold increase for stainless steel by day six was observed. An increase in osteoblast activity may also infer a coupling mechanism thereby releasing...
growth factor for osteoblast precursor. Elevated levels of IL-6 are involved in bone destruction.\textsuperscript{11}

Orthopaedic biomaterials are associated with local and remote adverse tissue responses. Generally, these adverse effects are which are primarily generated by wear and corrosion. The debris can be present in different forms and wear particles.\textsuperscript{12}

The biocompatibility of titanium implants over stainless steel implants has been demonstrated in some studies. An in-vivo rabbit tibia study showed that titanium screws improved bone contact and had higher removal torques than stainless steel.\textsuperscript{13} Recently it has been shown that Ti has an anti-inflammatory interaction in a rat arthritis model. A histological study by Millar et al. compared the tissue response of titanium and stainless steel screws when inserted into the calvaria of dogs for different periods. They found no discernible difference in the tissue reaction between the two types of screw.\textsuperscript{13}

Study of Granchi et al. to determine level of cytokine bone resorption elevate in patien with aseptic loosening and the relation with type of implant. The results shows there is non significance different.\textsuperscript{14}

**CONCLUSION**

Based on the results of this experimental study conclude that there is significance different of IL-6 expression between rat that applied implant titanium and stainless steel and expression of IL-6 in mice that applied titanium implant lower than stainless steel.

As recommendation in this study, implant choices for orthopaedic surgery implant titanium recommended as first choice due to risk of inflammation less than stainless steel.

**REFERENCE**