INCREASING INTERLEUKIN-6 (IL-6) LEVEL AFTER PLATELET RICH PLASMA (PRP) ADMINISTRATION ON WISTAR RATS WITH PARTIAL HEPATECTOMY: AN EXPERIMENTAL STUDY

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Abstract

Purpose: To determine IL-6 levels after PRP administration in Wistar rats with partial hepatectomy (PH).
Methods: An experimental study with post-test only control group design that conducted at INA Lab Padang Laboratory and Biomedical Laboratory, Faculty of Medicine, Andalas University from March to May 2023. Eighteen Wistar rats were divided into three groups with 6 rats each group: 1) sham procedure, 2) PH without PRP administration, and 3) PH with PRP administration. IL-6 levels were examined from blood samples taken from the right atrium on the second day after PH.
Results: IL-6 levels in the sham procedure group were relatively equal to the IL-6 levels in the PH group (4.55 ± 0.84 ng/L; 3.80 ± 0.81 ng/L) meanwhile the PH + PRP group was 5.15 ± 0.79 ng/L. However, IL-6 levels in the PH and PH + PRP groups showed significant differences.
Conclusion: Administration of PRP can increase IL-6 levels on Wistar rats with partial hepatectomy.

Keywords: Interleukin-6, Platelet Rich Plasma, Partial Hepatectomy.

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1. INTRODUCTION

The liver is one of the human body's organs that has its own uniqueness.Liver receives dual blood supplies, Seventy five percent comes from the portal vein and the second comes from the hepatic artery (25%). The liver has ability to regenerate it self in case of damage or loss of cells.[1,2]

In a study, the liver can reach its original size within 3-6 months in adults and less than 3 months in children after liver resection. At the cellular level, liver regeneration after resection consists of compensatory hypertrophy followed by hyperplasia of the remaining hepatocytes. This phenomenon is described by three different phases: initiation (0-5 hours after resection), proliferation (5-144 hours after resection), and termination.[3]

Liver resection-induced injury triggers a signaling cascade that mobilizes immune cells to eliminate necrotic tissue, alters metabolic processes, and induces regeneration. This process is mediated by cytokines and growth factors within the first five hours after hepatectomy.[4] The proliferative phase begins 5 hours after resection and can be divided into a period in which hepatocyte and cholangiocyte proliferation is induced for 72 hours, and an angiogenic phase of 2-3 days, during which hepatic stellate cells (HSC), endothelial cells (EC), and Kupffer cells (KCs) proliferate in response to cytokines and growth factors produced by hepatocytes. In the termination phase, autonomous hepatocyte proliferation is controlled by anti-proliferative factors such as transforming growth factor-beta (TGF-β) released by HSC and KC, and activin, to ensure normal liver mass and function.[4,5]

In mice, it has been shown that IL-6 levels increase during the first hour of DNA synthesis in hepatocytes, starting 24 hours after hepatectomy. This is followed by strong activation of the transcription factors STAT3 and C/EBPβ/nuclear factor-interleukin 6 (NF IL-6), resulting in increased transcription of target genes. These results are involved in the G0/G1 phase transition of hepatocytes after hepatectomy.[3]

Platelet-rich plasma (PRP) is an autologous product rich in growth factors, obtained from a blood sample through centrifugation to separate the platelet-rich supernatant. PRP has thrombotic effects necessary for homeostasis and growth factors. The selection of PRP is based on its lower cost and easy availability, as well as the absence of rejection or immune response to autologous PRP. A recent study by Salem et al. (2018) examined mice with dimethylnitrosamine-induced liver fibrosis and found that PRP significantly improved liver enzyme changes, accompanied by a significant decrease in hepatic hydroxyproline content and IL-8 levels, and an increase in the anti-apoptosis marker Bcl-2. PRP also showed a significant decrease in fibrosis-related genes α-SMA and TGF-β, as well as a significant decrease in the inflammatory marker NF-κB1.[4] Hesami et al. (2014) also confirmed that PRP has no toxic effects on the liver and even has a protective effect against histological damage and oxidative stress by increasing glutathione and reducing lipid peroxidation in liver tissue.[5]

2. MATERIALS AND METHODS

This research is an experimental study with a post-test only control group design approach using white rats (Wistar strain) as the research object. This research was conducted at the Padang and INA Lab Laboratory Biomedical Laboratory, Faculty of Medicine, Andalas University. This research was conducted from March 2023 to May 2023.

The population of this study were white male Wistar rats (Rattus norvegicus) aged 8 weeks with a body weight of 200-

300 grams. Based on criteria of the World Health Organization (WHO), the minimum samples in the animal trial is five rats for each group.[1,3] We used six rats for each group. Inclusion criteria for rats were Rattus Norvegicus Wistar strain, male, in health condition, aged 8 weeks, body weight 200-300 grams and no anatomical disorders. The research materials were platelet rich plasma (PRP).

The research procedure began with the acclimatization of the Wistar rats. Intravenous Ketamine was used to anesthesize the rats. Septic and antiseptic procedures was done using povidone iodine 10 %. Then, performed laparotomy by upper and lower midline incision. The technique used was the 70% hepatectomy model described by Higgins and Anderson which included resection of the left lateral and median hepatic lobes. The left lateral and middle lobes were tied with 4/0 silk thread a then 70% hepatectomy technique was performed so that only the right lobe and caudate lobe left. After checking for bleeding, the abdominal wall was closed layer by layer.

These were 3 groups of Wistar rat. Group 1 was the rats underwent sham surgery. Wistar rats in group 2 was only performed partial hepatectomy while group 3 was done partial hepatectomy with PRP. After surgery, the rats got fed orally for 24 hours. All rats were allowed to freely consume standard food and water. Platelet Rich Plasma was given for 3 days. After 72 hours, the rats were sacrificed and blood samples collected from right atrium for checking IL-6 level. A relaparotomy was performed through the same incision line under anesthesia and sterile conditions.

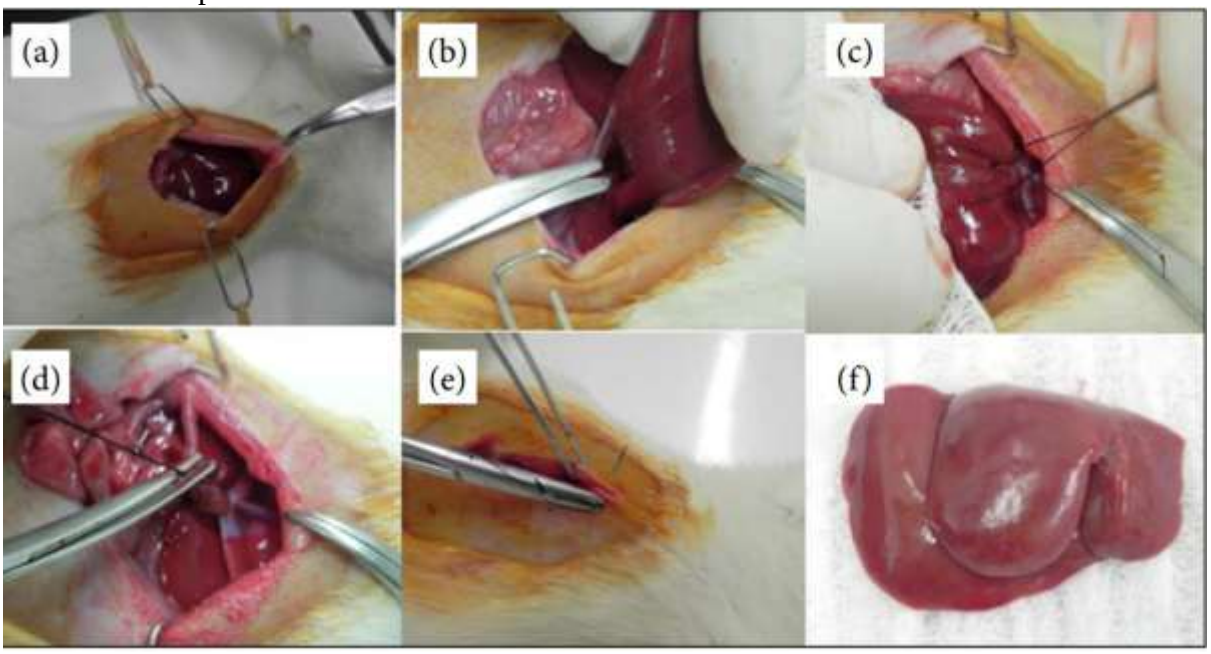


Figure 1. 70% Hepatectomy technique by Higgins and Anderson. (a) Median laparotomy and metal retractor insertion; (b) Dissection of liver and ligament sections; (c) and (d) Wire placement, ligation, parenchyma cutting, and specimen collection; (e) Abdominal wall closure; (f) Liver lobe resected 70% of liver parenchyma.[5]

Univariate analysis was a description of the description of each research variable. Bivariate analysis was used to determine the relationship between independent variable and dependent variable. Before entering into bivariate analysis, the assumption of normality of data distribution was tested with the Shapiro Wilk test and the homogeneity of variance with the 57 Levene test. The distribution of

the data and the homogeneity of the variants were normal and homogeneous so that they fulfilled the requirements of the paremetric test, namely one way ANOVA followed by Tukey's post hoc test. The analysis is said to be meaningful if the p value ≤ 0.05.

3. RESULT AND DISCUSSION

This research was about IL-6 levels after PRP administration on white Wistar rats with partial hepatectomy. The study was conducted on 18 white Wistar rats divided into 3 groups including sham procedure, hepatectomy, and hepatectomy plus PRP administration. IL-6 levels were examined from a blood sample taken from the right atrium. IL-6 levels were checked on the 3rd day after hepatectomy. The results of examining IL-6 levels between the following groups of rats are presented descriptively as follows:

Table 1. Description of IL-6 Levels (ng/L) between Groups

Rat Group	Minimum	Maxium	Mean± SD
Sham procedure	3,76	5,71	4,55 ± 0,84
Hepatectomy	2,48	4,64	3,80 ± 0,81
Hepatectomy + PRP	3,97	5,86	5,15 ± 0,79

SD = Standar Deviation

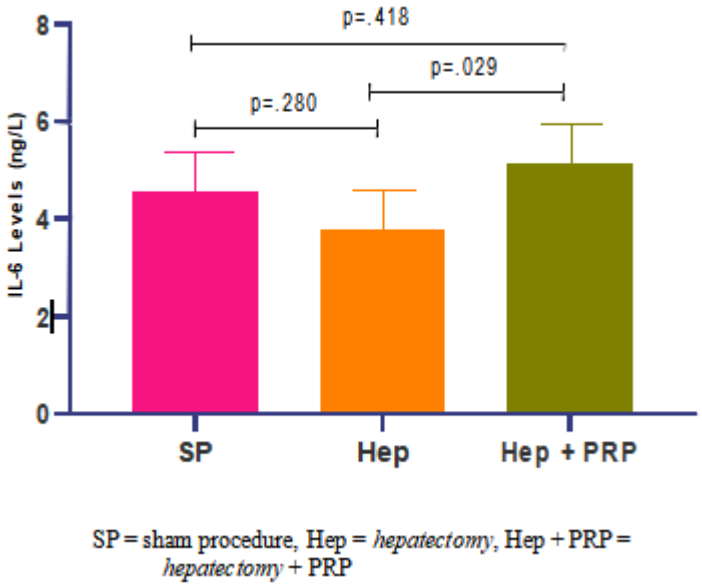
Based on Table 1, it is known that IL-6 levels in rats were treated with hepatectomy and given PRP had IL-6 levels between 3.97 to 5.86 ng/L with the highest average (5.15 ± 0.79) than the sham procedure rat group (4.55 ± 0.84 ng/L) and the hepatectomy rat group (3.80 ± 0.81 ng/L).

Next, an analysis of the normality of the distribution of the data was carried out, because the type of data used was ratio-scale data and an analysis of the homogeneity of variance because it was unpaired inter-group data. The results of the normality analysis of data distribution using the Shapiro Wilk test (n < 50) and the homogeneity of variance (Levene test) are shown as follows:

Table 2. Results of Analysis of Normality of Data Distribution and Homogeneity of Variants of IL-6 Levels p-value

Rat Group	Shapiro-Wilk	Levene test	One-way anova
Sham procedure	0,105		
Hepatectomy	0,578	0,939	0,036
Hepatectomy + PRP	0,217		

The results of Shapiro Wilk's analysis on data on IL-6 levels between groups of rats obtained a p>0.05 value indicating that IL-6 levels in each group had a normal distribution. The Levene test results also showed a p > 0.05 value, which means that the IL-6 level between the three groups of rats had a homogeneous variant. Furthermore, to see a comparison of IL-6 levels between the three groups, a one-way ANOVA test was carried out and a value of p = 0.036 (p <0.05) was obtained, meaning that the IL-6 levels between the three groups were significantly different, so a follow-up test was carried out to compare the average between the two groups. Group used Tukey's post hoc test with the results shown in Figure 2.



SP = sham procedure, Hep = *hepatectomy*, Hep + PRP = *hepatectomy* + PRP

Figure 2. Graph of comparison of the average IL-6 levels between groups

Further comparison of the mean between the two groups using the Tukey test showed that only the hepatectomy group and the hepatectomy group with PRP showed a significant difference in average IL6 levels (p = 0.029). IL-6 levels in the hepatectomy with PRP were significantly higher than IL-6 levels in the hepatectomy without PRP. Thus, the research hypothesis is accepted that there are differences in the healing and regeneration process of liver tissue in experimental animals (white rats) with PRP and rats without PRP.

Partial hepatectomy causes drastic changes in hepatic blood flow, causing friction between blood flow and the surface of liver sinusoid endothelial cells, known as shear stress, which leads to increased IL-6 release and induces an increase in portal venous pressure and intestinal permeability, there by increasing intestinal PAMPs including lipopolysaccharide (LPS). Lipopolysaccharide was recognized by Toll-like receptor (TLR) on Kupffer cells and triggers the release of IL-6 and TNF-α which are hepatotropic factors of liver regeneration after hepatectomy. However, in this study, IL-6 levels did not show a significant difference compared to IL-6 levels in non-hepatectomy control rats because IL-6 is a regulator of liver regeneration in the primary stage or within 4 hours after hepatectomy, while IL-6 levels in this study were measured 2 days later.[6] Without improvement in the patient's condition, IL-6 is reduced, which can interfere with the liver regeneration process. Interleukin-6 (IL-6) and TNF-α are two major proinflammatory cytokines that play a central role in the activation of STAT-3, NF-κB, phosphoinositide 3-kinase (PI3K) and Akt pathways during liver regeneration.

Deficiency of one of these two cytokines in bone marrow-derived blood cells can impair liver regeneration.[7] and IL-6 administration can reduce hepatocyte damage so that liver enzyme levels are in a lower range enzyme levels are in the lower range than in the group of rats not given IL-6 that were not given IL-6. Liver enzyme levels increased significantly on day 7 post hepatectomy compared to before hepatectomy and declined with improvement.[8] The study of Al-Ghamdi et al. showed that Sprague-Dawley rats that underwent a 70% resection resection without IL-6 induction had levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline

phosphatase (ALP) levels, which were significantly were significant compared to similar rats that were induced with IL-6 for 4 (four) days. Increased levels of ALT and bilirubin also occurred on the second day post hepatectomy. IL-6 administration to hepatectomized rats hepatectomy rats can improve liver enzymes, decrease apoptosis and increase the regeneration of liver cells.[9] IL-6 levels in hepatectomy rats treated with PRP increased significantly compared to hepatectomy rats treated with PRP.[10]

IL-6 levels in hepatectomy rats treated with PRP increased significantly compared to hepatectomy rats that were not treated with PRP. PRP works to enhance the recruitment, proliferation and differentiation of cells in tissue regeneration. Another process involved in wound healing is the role of PRP in suppressing the release of cytokines to prevent inflammation through macrophages to stimulate tissue healing, regeneration and epithelialization PRP also has anti-inflammatory properties through its effect on the NFkB signaling pathway. [11]PRP has the ability to act as an anti-inflammatory due to the presence of HGF in PRP that can suppress NFkB activity. HGF is able to inhibit inflammation in the kidney, preventing inflammatory cell infiltration through interference with NFkB activity. When HGF is present, there is a decrease in the production of the proinflammatory cytokine IL-6 followed by an increase in the anti-inflammatory cytokine IL-10, however IL-6 is normally involved in the production of HGF which acts to inhibit inflammation following stress. 11 HGF binding to its receptor activates the PI3K signaling pathway leading to phosphorylation of Akt and GSK3β which causes the activation of CREB. CREB will then interact with CBP and suppress NFkB, resulting in an increase in the anti-inflammatory cytokine IL-10 leading to the inhibition of inflammation.**[12]** This is because various types of platelet RNA that migrate to hepatocytes transformed into functional proteins (growth factors) that can promote liver regeneration through regulation of the inflammatory response by activating inflammatory cells. Growth factors contained in PRP increased significantly compared with hepatectomized rats that were not given PRP. because various types of platelet RNA that migrate to hepatocytes transformed into functional proteins (growth factors) that can promote liver regeneration by regulating the inflammatory response by activating inflammatory cells.activating inflammatory cells.[6] Growth factors contained in PRP stimulates IL-6 in liver sinusoid endothelial cells and Kupffer cells to activate STAT3 in hepatocyte proliferation. Endogenousplatelets accumulate in the liver immediately after hepatectomy. Which move fromsinusoid space to the Disse space and release various growth factors such as Insulin-like growth factor (IGF-1) and Hepatocyte growth factor (HGF) through direct contact with hepatocytes, thus inducing the initiation of hepatocyte mitosis.

Murata et al. study can also show the role of platelet administration (platelet not rich plasma) in patients with hepatocellular carcinoma and liver cirrhosis who performed partial hepatectomy. Platelet administration can restore liver function as indicated by liver enzyme levels such as AST, ALT, cholinesterase, albumin and prothrombin time which are relatively similar between 4 (four) weeks postoperatively and before surgery.[13] Post hepatectomy TNF-α activates IL-6 expression especially in Kupffer cells. TNF-α and IL-6 through the gp80/gp130 receptor complex then activate intracellular pathways important in hepatocyte proliferation post hepatectomy.[14] Another role of platelets is that when activated, they will secrete growth factors and other substances that function to accelerate the wound healing process by increasing cell proliferation, matrix formation, osteoid

production, connective tissue healing, angiogenesis, and collagen synthesis.[15]

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