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Sleeve Gastrectomy and Liver Omentoplasty Effects on Pro-Inflammatory Markers in Rats with Obesity and Liver Fibrosis

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ABSTRACT

In 2018, more than 600 million people are clinically obese. Obesity cause liver fibrosis. The increase in glucose levels triggers the formation of pro-inflammatory factors. TNF-α and PDGF play role as inflammatory factors in liver fibrosis process. This study aimed to examine the effect of sleeve gastrectomy and liver omentoplasty on TNF-α and PDGF level in obese rats model. This study is a laboratory experimental study on 20 male wistar rats. The samples then divided into 2 control groups and 2 treatment groups. Rats were treated with a high-fat diet to become obese based on Lee's criteria >300 and liver fibrosis induction with CCl4, as well as liver sleeve gastrectomy and omentoplasty procedures. PDGF and TNF- α levels were measured by PCR method. TNF-α and PDGF level were found to be the lowest in the treatment 1 group. There was a significant difference on TNF-α and PDGF level across all the study groups. Mann Whitney analysis shows a significant decrease on PDGF level between the control group and the treatment 1 group (p=0,004). It was found that there is a difference on TNF-α level between control with treatment 1 and 2 groups. There is also a significant decrease between positive control and treatment 1 group, and between treatment 1 and 2 groups. Liver omentoplasty and sleeve gastrectomy could reduce the level of pro-inflammatory markers (PDGF and TNF-α) on rats with obese and liver fibrosis model.

1. INTRODUCTION

The prevalence of obesity worldwide is increasing from year to year with data from the World Health

Organization (WHO) in 2013 noting that around one billion people in the world are overweight and at least 300 million are clinically obese, while in 2018 this number has doubled. i.e. 1.9 billion people worldwide are overweight and of that number more than 600 million are clinically obese. Meanwhile, 39% of adults aged 18 years and over are overweight and obese [1]. Based on Riset Kesehatan Dasar (Riskesdas) 2020, the prevalence of obesity has increased from year to year where in 2016 it was 14.8 percent then increased in 2017 to 19.6 percent, in 2018 to 20.8 percent and to 21.8 percent in 2019 [2]. The impact arising from obesity where the excess energy is stored in the tissues in the form of fat causing liver fibrosis. Liver fibrosis is a common pathological consequence of chronic liver disease which is characterized by the progressive formation of scar tissue in the liver parenchyma as a response to wound healing due to chronic injury. Liver fibrosis is a result of all chronic liver injury, with manifestations of extracellular matrix protein deposition resulting in scar tissue at the site of injury, loss of tissue architecture and liver failure. Chronic injury can be caused by infection with Hepatitis B or C virus, parasitic infection, alcohol, drugs and toxins, venous obstruction, cholestasis, autoimmune or metabolic diseases such as obesity. Fibrosis that keeps ongoing can end up as cirrhosis [3].

Obesity is associated with high levels of extracellular glucose, which will trigger an increase in the production of ROS (Reactive Oxygen Species) that in turn will trigger cytokines secretion and induction of inflammation. Cytokines secreted by immune cells and adipose tissue adipokines increase tissue inflammation [4]. However, studies have shown the role of white adipose tissue is to produce certain bioactive substances called adipokines. Apart from adipokines, several mediators of inflammation and cell growth were also found, such as Tumor Necrosis Factor alpha (TNF-α) and PDGF [5].

TNF-α has been associated with acute phase response, in which it is secreted mainly by macrophages and lymphocytes in response to oxidative stress and inflammation in obesity, which could exacerbate oxidative stress [6]. TNF-α can cause decrease insulin sensitivity by decreasing autophosphorylation of insulin receptors to insulin receptor tyrosine kinase activity inhibitors, decreasing insulin sensitive glucose transporter (GLUT), increasing circulating fatty acids, changing B-cell function, increasing glyceride levels and reducing HDL levels. Proinflammatory mediators from adipose tissue, such as TNF- α contribute directly to vascular damage, insulin resistance, and atherogenesis [4]. TNF- α could causes hepatic inflammation and fibrosis through ROS-JNK pathway activation which induces hepatocyte apoptosis, necrosis and progress to hepatic fibrosis [7]. TNF- α has been reported to interfere with lipid metabolism and become an insulin antagonist, while PDGF is one of the cell growth factors. Obesity is known to induces PDGF expression in the adipocytes, which is the most potent factor involved in stimulating hepatic stellate cells (HSC) proliferation, differentiation, and migration. PDGF would additionally promotes collagen production and deposition, and also transforms HSC into myofibroblasts that induces liver fibrgenesis [8].

Up to now, no drug has been widely accepted as an anti-fibrotic agent in humans. Combination therapy can be synergistic, not additive. Antifibrotic therapy also raises the theoretical concern that inhibition of the lung tissue formation response will not prevent the encapsulation of the damaged region which will lead to the expansion of tissue damage. This makes the need for procedures to be carried out to be able to control or provide solutions, one of which is to carry out a sleeve gastrectomy procedure which is a bariatric procedure and also perform hepatic omentoplasty in the hope of repairing liver tissue that has fibrosis.

This study will examine the liver by performing Omentoplasty on the liver of obese rats to see the effect of hepatic Omentoplasty on TNF- α and PDGF in obese rats undergoing sleeve gastrectomy.

2. Methods

2.1 Study design

This research is an in vivo laboratory experimental study on experimental animals, male wistar (spargue) rats. This study aims to investigate the effect of TNF-α and PDGF on the occurrence of liver fibrosis in obese rats undergoing sleeve gastrectomy and hepatic omentoplasty. The sample in the study will be grouped into 2 control groups and 2 treatment groups. The control group consisted of a negative control group (normal) and a positive control group (high-fat feed and CCl4 induction). The treatment group consisted of treatment group 1 (feeding high-fat and induced CCl4 with sleeve gastrectomy) and treatment group 2 (feeding high-fat and induced CCl4 with sleeve gastrectomy and liver omentoplasty).

2.2 Research Sample Selection

20 male wistar rats (Sprague Dawley) were included as samples in this study. Wistar rats aged 6-8 weeks, weighing 200-300 grams, and male were selected with inclusion criteria, i.e rats with Lee index > 300 and rats with liver fibrosis after CCl¬4 induction. Mice were not included in the study sample if they were disabled, the liver was already cirrhotic, and the mice died during the study. All mice used in the study were obtained from the iRATco Animal Rat Provider.

2.3 Data Collection

Research and data collection was carried out for 1 month. Treatment and observations on wistar rats as well as tissue collection and masson trichrome staining were carried out at the Laboratory of the Faculty of Medicine, Gadjah Mada University. The data processing was carried out at SCCR, Faculty of Medicine, Sultan Agung Islamic University. TNF- levels were taken in liver tissue as measured by the PCR method (pg/ml) and PDGF levels were measured by the PCR method (pg/ml).

2.4 Statistical Analysis

Data analysis includes descriptive analysis and hypothesis testing. In the descriptive analysis, TNF- expression is presented in the form of a table of mean and standard deviation. Data normality test was carried out in each group with the Saphiro Wilk test and obtained normal / abnormal results. If the results are normal, then the analysis of hypothesis testing in this study uses the paired sample t test. Then the repeated measure or Friedman GLM test was carried out in paired group comparative analysis. Meanwhile, if the results are not normal, a non-parametric hypothesis test such as the Kruskal-Wallis in comparative analysis will be carried out and followed by the Mann-Whitney test. In addition, the correlation test between the expression levels of TNF-α and PDGF with the proportion of fibrosis was also carried out using the Pearson or Spearman statistical test.

3. Results

All rats sampled in the study met the Lee index to be considered obese. The levels of TNF- and PDGF of each group are presented in Tables 1 and 2. Based on the hypothesis test, there were significant differences in the levels of TNF-α and PDGF in all study groups.

The Mann Whitney test found that there was a difference in PDGF levels between the control group and treatment group 1 ($p = 0.004$), in the comparison of control group 1 - positive control, control 1 - treatment 2 did not find a significant difference, the same thing as the comparison in the control group. treatments 1 and 2 (Figure 1). Meanwhile, in TNF- levels, there were differences between TNF- levels between the normal control group and treatments 1 and 2. There were also differences between the positive control group and treatment 1, and between treatment groups 1 and 2 (Figure 2).

4. Discussion

20 male Sprague dawley rats aged 6-8 weeks were randomized into 4 groups. 5 male Sprague Dawley rats were given standard diet and 15 rats were fed a high-calorie and high-fat diet for 8 weeks in the form of pellets to achieve obesity and the liver fibrosis process which was then induced by intraperitoneal CCL4. 15 rats that had been declared obese and liver fibrosis were randomized into 3 treatment groups, namely K_2 was the positive control group, P_1 was the sleeve gastrectomy treatment group and P_2 was the sleeve gastrectomy and liver omentoplasty treatment group, which were after 10 days will be terminated and performed PCR examination to see the expression of TNF- and PDGF genes.

The results showed that there was a decrease in the mean level of PDGF in obese rat models treated with sleeve gastrectomy (1.08 ± 0.04) and sleeve gastrectomy + omentoplasty (1.30 ± 0.26) without finding a significant association. These results are in line with [9] who showed that there was no inflammatory reaction or hepatic fibrosis process after bariatric surgical intervention. Surgical intervention not only provides histologic changes but also cures disease globally by providing substantial changes in body weight, repairing hepatic endothelial damage, reducing insulin resistance, and reducing several associated cardiovascular risk factors. The PDGF signaling pathway exerts persistent activation in response to a variety of stimuli and facilitates the progression of liver fibrosis. Since this pathway modulates a broad spectrum of cellular processes, including cell growth, differentiation, inflammation and carcinogenesis, it has emerged as a therapeutic target for hepatic fibrosis and liver-associated disorders [8]. In this study, decreased PDGF levels after sleeve gastrectomy and omentoplasty treatment could indicate a reduction in the systemic inflammatory process.

The results of the analysis of TNF-α parameters showed that the sleeve gastrectomy treatment group had lower TNF-α levels (0.01 ± 0.01) than the control group (1.03 ± 1.26), the same thing as the sleeve gastrectomy group and liver omentoplasty which had lower TNF- α levels as well (0.05 ± 0.02). These results indicate that both sleeve gastrectomy and sleeve gastrectomy and hepatic omentoplasty treatments can reduce hepatic TNF-α levels. This study is in line with that reported by [10], where the study found that the sleeve gastrectomy procedure effectively reduced TNF-α levels at 1 year postoperatively in obese female patients, which is associated with loss of body fat. Fat mass can cause a decrease in serum TNF-α levels, with leaner study subjects having lower levels than obese subjects, indicating that adipose tissue has a major role in the regulation of serum cytokine concentrations. [11] showed that there is an improvement in TNF-α levels after undergoing laparoscopic sleeve gastrectomy in obese patients, which is in accordance with the hypothesis that sleeve gastrectomy is not only effective in reducing body weight, but most importantly improves low-grade inflammation in obese patients to prevent the occurrence of metabolic induced diseases. obesity bag, with one of them being liver fibrosis.

In the group of rats that underwent sleeve gastrectomy and hepatic omentoplasty, TNF-α levels were also found to be decreased compared to the control group, indicating the effect of this intervention on healing from liver fibrosis. Studies related to the use of hepatic omentoplasty in the treatment of liver fibrosis are still minimal. Some studies explains that this procedure is thought to aid the healing process of the liver surface and help transport macrophages to the site of damage. According to its function, the omentum can passively move to the site of intra-abdominal inflammation when attached. Omentum has been conventionally used in hepatic injuries and can be used by general surgeons. There was an effect of hepatic omentoplasty on the healing of liver fibrosis conditions [11].

5. Conclusion

Based on the results of this study it can be concluded that; Hepatic omentoplasty and sleeve gastrectomy on obese rats with liver fibrosis could reduce the level of platelet derived growth factor (PDGF) and tumor

necrosis factor alpha (TNF-α) in the liver.

Disclosure

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Conflict of Interest
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper or others.

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Table 1. Mean levels of TNF-α

Group	Mean ± SD	Median (min – max)	p£	Transf.
K_1	1,00	1,00	–	–
K_2	1,03 ± 1,26	0,28 (0,05 – 2,73)	0,086*	0,281*
P_1	0,01 ± 0,01	0,01 (0,001 – 0,01)	0,044	0,021
P_2	0,05 ± 0,02	0,05 (0,03 – 0,08)	0,625*	0,758*

*p > 0,05; significant

Then on the results of this descriptive analysis, the normality test of the data was also carried out in each group in the study. Based on the results of data analysis, it was found that the K2 group had a p value of 0.086, the P1 group had a p value of 0.044, and the P2 group had a p value of 0.625. The p value is considered significant if p > 0.05. Based on these results, it was found that only groups K2 and P2 had normal data distribution. Therefore, hypothesis testing will be continued with non-parametric analysis.

Table 2. Uji Mann-Whitney TNF-α

Kelompok		p
I	II	
K1	K2	0,577
	P1	0,005*
	P2	0,005*
K2	P1	0,008*
	P2	0,076
P1	P2	0,008*

*p < 0,05; signifikan

It was concluded that there were differences in TNF- levels between groups K1 and groups P1 and P2 with a significance value of p 0.005 and 0.005 respectively. However, in terms of the analysis of the relationship between groups K1 and K2, no significant differences were found with p-values of 0.577 each.

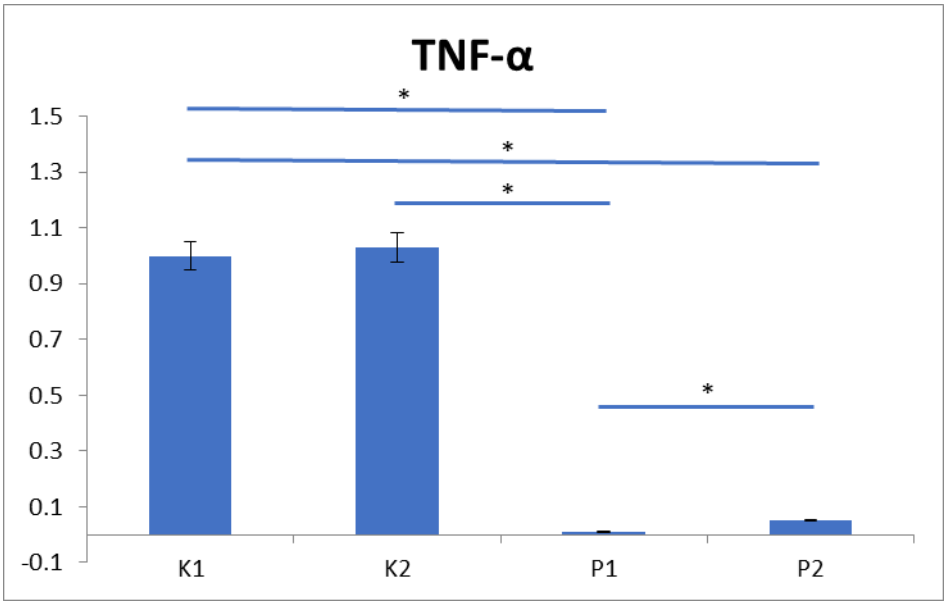

Figure 1. TNF-α levels

Comparison between the K2 group and the P1 and P2 groups. Where based on the results of statistical analysis found a significant difference between the comparison of the K2 group with the P1 group with a p value = 0.008. While the relationship between the K2 group and the P2 group was not found to be a significant relationship with a p value = 0.076. And in the analysis of the relationship between treatment group 1 and

treatment 2, it was found that there was a significant difference between the two groups with p value = 0.008.

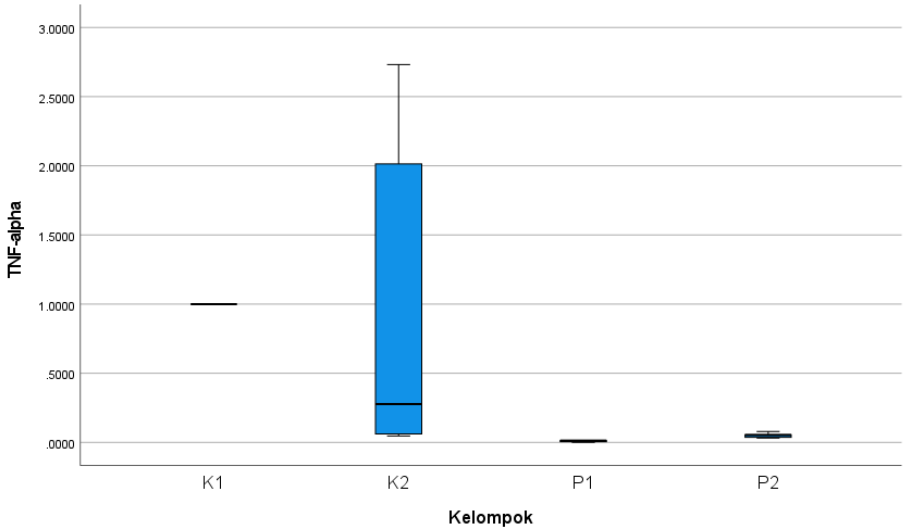

Figure 2. TNF-alfa

Description: K_1: Negative control group; K_2: Positive control group P_1: sleeve gastrectomy group; P_2: *sleeve gastrectomy* and *omentoplasty hepar* treatment group.

Table 3. Mean levels of PDGF

Group	Mean ± SD	Median (min – max)	p£	Transf.
K_1	1,00	1,00	–	–
K_2	1,77 ± 0,47	1,99 (0,93 – 1,99)	0,000	0,000
P_1	1,08 ± 0,04	1,06 (1,06 – 1,16)	0,000	0,000
P_2	1,30 ± 0,26	1,41 (0,92 – 1,55)	0,397*	0,309*

*p > 0,05; significant

Then on the results of this descriptive analysis, the normality test of the data was also carried out in each group in the study. Based on the results of data analysis, it was found that the K2 group had a p value of 0.000, the Pl group had a p value of 0.000, and the P2 group had a p value of 0.397. The p value is considered significant if p > 0.05. Based on these results, it was found that only the P2 group had normal data distribution. Therefore, hypothesis testing will be continued with non-parametric analysis.

Table 4. Uji Mann-Whiteny PDGF

Kelompok		p
I	II	
K1	K2	0,083
	P1	0,004*
	P2	0,095
K2	P1	0,095
	P2	0,067
P1	P2	0,161

*p < 0,05; signifikan

It was concluded that there was a difference in PGF levels between the K1 group and the P1 group with a significance value of p = 0.004. However, in terms of the analysis of the relationship between groups K1 and groups K2 and P2, no significant differences were found with p-values of 0.083 and 0.095 respectively.

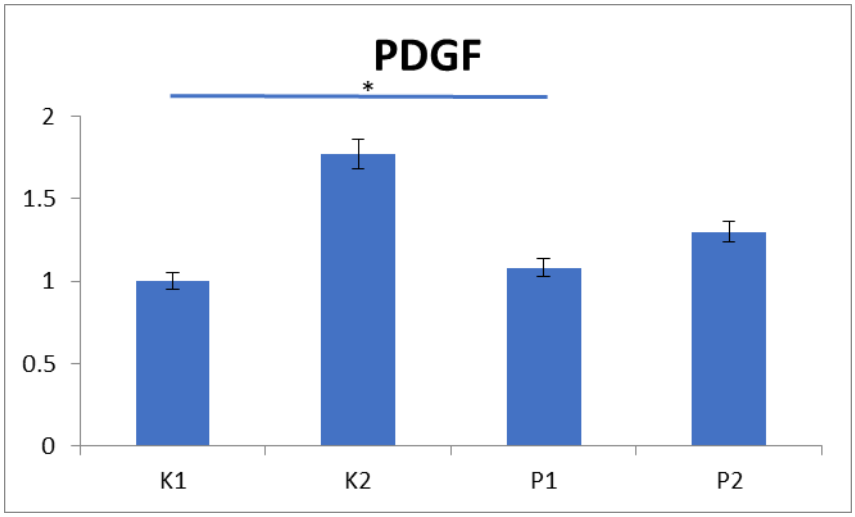

Figure 3. PDGF levels

A comparison was made between groups K2 with P1 and P2. Where based on the results of statistical analysis found no significant difference between the two comparisons. this is indicated by p-values of 0.095 and 0.067 respectively. And in the analysis of the relationship between the PI and P2 groups it was found that there was no significant difference between both groups with p value = 0.161.

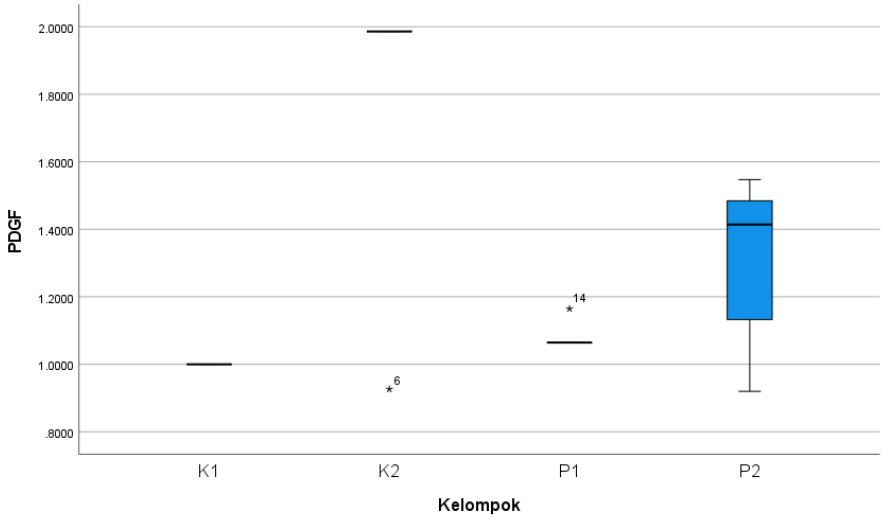

Figure 4. PDGF

Description: K_1: Negative control group; K_2: Positive control group P_1: *sleeve gastrectomy* group; P_2: *sleeve gastrectomy* and *omentoplasty hepar* treatment group.