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Lipid Profile and IL-1β Levels in Wistar Rats with Atherogenic Diet

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Abstract: ***Background:*** ***Atherosclerosis is a pathological condition characterized by active, chronic, progressive inflammation and plaque in the artery walls. High cholesterol diet is one of the factors that can initiate inflammation and the production of proinflammatory cytokines such as IL-1β to cause atherosclerosis. This study aims to determine the value of lipid profile and IL-1β as a marker of atherosclerosis in male wistar rats fed atherogenic diet. As a positive control, a statin group atorvastatin 7.2 mg/kg, was used.*** ***Methods:*** ***The design of this research is a true post test control study laboratory experimental. Wistar male rats aged 10 weeks with body weight 150-200 grams were divided into 3 groups, normal, atherogenic (high cholesterol) and atorvastatin groups. Induction was carried out for 60 days and the administration of atorvastatin for 30 days. After 90 days of treatment, serum total cholesterol and IL-1β levels were measured. Results: There were significant differences in total cholesterol levels in the atherogenic group, normal group, and atorvastatin group (331.793 ±19.118 vs 113.864±6.615 vs. 144.132±35.214 mg/dL; P<0.001). Triglyceride levels showed significant differences in the atherogenic group, normal group, and atorvastatin group (133.335±20.080 vs. 65.38475±4.098 vs. 73.077±3.854 mg/dL; P<0.001). LDL cholesterol levels in the atherogenic group, normal group, and atorvastatin group showed significant differences (263,887±19,032 vs 22,369±17,1901 vs 68,192±24.947 mg/dL; P<0.001). HDL cholesterol levels also showed significant differences in the atherogenic group, the normal group, and the atorvastatin group (41.239±1.894 vs. 78.419±10.683 vs. 61.324±10.241 mg/dL; P<0.001). IL-1β showed significant differences in levels in the atherogenic group, normal group, and atorvastatin group (11,733±2,217 vs 4,348±0,273 vs 4,860±1,766 pg/mL; P<0.001). Pearson correlation showed that IL-1β was positively correlated with increasing levels of total cholesterol (r = 0.940; P<0.001), triglycerides (r = 0.910; P<0.001), and LDL cholesterol (r = 0.936; P<0.001).*** ***Conclusion:*** ***There were significant differences in lipid profiles in the atherogenic group against the normal control group and the atorvastatin group against the atherogenic group. The levels of IL-1β as a proinflammatory cytokine in atherosclerosis showed high values and were significantly different in the atherogenic group. Atorvastatin showed significantly lower IL-1β levels (P<0.05) compared to the atherogenic group. Based on the correlation test, total cholesterol, LDL, and triglyceride levels were significantly positively correlated with IL-1β secretion (P<0.05). So that in the future lipid profile and IL-1β can be used as markers of the severity of atherosclerosis.***

Keywords: Lipid Profile, IL-1β, Atorvastatin

1. Introduction

Atherosclerosis is a pathological condition characterized by active, chronic, progressive inflammation and plaque formation in the artery walls. High cholesterol diet is one of the factors that can initiate inflammation in blood vessels to cause atherosclerosis [1]. Atherosclerosis is the leading cause of heart attack or stroke, with an annual total of 13 million deaths worldwide [2]. Atherosclerotic Cardiovascular disease (ACD) includes two main conditions: ischemic heart disease (IHD) and cerebrovascular disease (especially ischemic stroke). IHD and stroke were the world's first and third causes of death, respectively, 247.9 deaths / 100,000 people in 2013, representing 84.5% of cardiovascular deaths and 28.2% of all-cause mortality. Cardiovascular disease is projected in 2030 causing 23.6 million deaths [3] [4].

The levels of cholesterol in the blood is an indicator of the development of atherosclerotic vascular inflammation. The possibility of plaque development is high due to higher LDL levels and lower HDL concentrations [5]. Increased levels of total cholesterol until the formation of oxidized LDL (Low Density Lipoprotein) in plasma is one of the main risk factors for atherosclerosis caused by the accumulation of lipids in the arterial walls [6]. The inflammatory process in the early phase of atherosclerosis occurs due to exposure oxidized LDL in macrophages, and then in the later phase, inflammation increases with that exposure. Continuous exposure to oxidized LDL causes macrophages to become more active and produce proinflammatory cytokines such as IL-1β, IL-6, IL-17 and TNF-α [7]. There was a relationship between total cholesterol in the blood and the potential for atherosclerosis to increase the production of IL-1β cytokines. IL-1β plays a key role in the development of atherosclerosis through its action on endothelial cells, smooth muscle cells and macrophages to produce other proinflammatory cytokines such as TNF-α, IL-6, adhesion molecules such as VCAM-1, ICAM-1 and induce autoinflammation [8].

Jiang et al (2019) research using atheroma cell culture that IL-1β levels in cells with atherogenic inflammation showed a value of 5 pg/mL. Shu et al (2020) in their research showed the normal level of IL-1β in the mice used was 20 pg/mL. The mice were then induced to become CHD and IL-1β levels increased to 80 pg/mL. Al Batran et al (2014) showed a significant increase in total cholesterol levels in the atherogenic group 26.21 ± 0.29 mmol/L (P<0.05) compared to the normal group. Likewise, levels of IL-1β 84.33 ± 0.67 pg/mL were significant (P<0.05) than normal controls. This study aims to determine the value of lipid profile and IL-1β

as a marker of atherosclerosis in male wistar rats fed atherogenic diet.

2. Material and Methods

2.1 Material

The tools used were Genesis 40 Vis Thermo Scientific spectrophotometer, Thermo Scientific ELISA reader. The materials used were 96% alcohol, 70% alcohol, standard feed, atherogenic feed, CHOD-PAP cholesterol reagent, IL-1β ELISA kit, and wistar male rats aged 10 weeks, body weight 150-200 grams. The research was carried out in an integrated biomedical laboratory, Medical Faculty, Udayana University.

2.2 Experimental Design

The design of this research is a true laboratory experimental post test control study. Subject were wistar male rats (Rattus norvegicus) aged 10 weeks body weight 150-200 grams were divided into 3 groups, normal, atherogenic (high cholesterol) and atorvastatin group. Rats were acclimatized 7 days before being given treatment

a) Normal group : given standard feed for 90 days
b) Atherogenic group: standard feed: pork fat: yolk (80:15:5), 1% calcium daily and Vitamin D3 10000 IU orally every week for 60 days [12] and CMC-Na 0, 3% for 30 days.
c) Atherogenic group + atorvastatin (7.2 mg/kg): given standard feed: pork fat: yolk (80:15:5) 25 g/head and 1% calcium daily and Vitamin D3 10000 IU orally every week for 60 days and atorvastatin 7.2 mg/kg for 30 days.

After 90 days of treatment, serum lipid profile and IL-1β levels were measured. Ethical clearance was obtained from the Medical Faculty, Udayana University, with certificate number of 924/UN14.2.2.VII.14/LT/2021.

Test of Total Cholesterol and IL-1β Levels
The serum is allowed to clot for 10-20 minutes at room temperature. Centrifuged at 2000-3000 RPM for 15 minutes. Total cholesterol was quantified using an enzymatic-colorimetric quantitative assay. Measurement of IL-1β levels using an enzyme-linked immunosorbet assay (ELISA) kit from the Bioassay Technology Laboratory; Cat.No E0119Ra**.**

2.3 Statistic test

The data obtained were analyzed by SPSS version 22. Data on total cholesterol levels and IL-1β were examined for the homogeneity and the Shapiro-Wilk normality. If the data is normally distributed, then it is continued with the one way ANOVA test and the Least Significant Difference (LSD), with statistical significance of p <0.05. To see the correlation between Lipid Profile and IL-1β levels, Pearson's test with statistical significance of p <0.05 was used.

3. Results

Total cholesterol levels in each group showed a significant difference. The highest average cholesterol level was in the atherogenic group fed with high-cholesterol diet 331.793 ± 19.118 mg/dL. The total cholesterol in the normal group was 113,864±6,615 mg/dL and the total cholesterol in the atorvastatin group was 144.132±35,214 mg/dL. Based on the LSD test, total cholesterol levels in the normal group and atorvastatin were significantly lower (P<0.05) between the atherogenic group. Table 1 shows lipid profile data and serum IL-1β levels

Table 1: Lipid Profile and Il-1β level in both groups

Variables	*Groups*	Mean ± SD	LSD P Value
IL-1β (pg/mL)	Normal	4,348±0,273	<0,001*
	Atherogenic	11,733±2,217	<0,001**
	Atorvastatin (7,2 mg/kg)	4,860±1,766	<0,001*
Total Cholesterol (TC)(mg/dL)	Normal	113,864±6,615	<0,001*
	Atherogenic	331,793 ±19,118	<0,001**
	Atorvastatin (7,2 mg/kg)	144,132±35,214	<0,001*
Triglyceride (TG) (mg/dL)	Normal	65,38475±4,098	<0,001*
	Atherogenic	133,335±20,080	<0,001**
	Atorvastatin (7,2 mg/kg)	73,077±3,854	<0,001*
HDL Cholesterol (mg/dL)	Normal	78,419±10,683	<0,001*
	Atherogenic	41,239±1,894	<0,001**
	Atorvastatin (7,2 mg/kg)	61,324±10,241	<0,001*
LDL Cholesterol (mg/dL)	Normal	22,369±17,1901	<0,001*
	Atherogenic	263,887±19,032	<0,001**
	Atorvastatin (7,2 mg/kg)	68,192±24,947	<0,001*

*Compared to Atherogenic Groups
**Compared to Normal Groups

Triglyceride levels in the normal and atorvastatin groups showed significantly lower levels than the atherogenic group (P<0.05). HDL and LDL levels in the normal group and atorvastatin showed significantly lower levels than the atherogenic group (P<0.05). The highest LDL level was in the atherogenic group with a value of 263.887±19,032 mg/dL.

Table 2: Pearson Correlation IL-1β to Lipid Profile

	Parameters	*TC*	*TG*	*HDL*	*LDL*
IL-1β	Pearson Correlation	0,940	0,910	- 0,749	0,936
	P Value	<0,001*	<0,001*	<0,001*	<0,001**

*Correlation is significant at 0,05

Table 2 showed that IL-1β was significantly positively correlated (P<0.05) on the total cholesterol, LDL cholesterol, triglycerides. Pearson's R value with the highest correlation was total cholesterol with a value of 0.940. Unlike the case with HDL cholesterol which has a negative correlation with IL-1β with a value of -0.749. Based on these correlation results, with increasing levels of total cholesterol, LDL, and triglycerides, it is possible that there is a correlation to the increase in serum IL-1β levels.

4. Discussion

The results showed, generally the lipid profile of the atorvastatin group was significantly different (P<0.05) compared to the normal group and the atherogenic group given atorvastatin. The high levels of total and LDL cholesterol were triggered by the pork fat and yolks. Giving vitamin D3 and calcium also aims to accelerate the process of atheroma plaque formation [12]. In normal control, the level of IL-1β showed the lowest value, namely 4.348±0.273 pg/mL. The normal group was only given standard feed so that it showed a low level value. Atorvastatin significantly (P<0.05) showed lower IL-1β levels than the atherogenic group. Increased levels of total cholesterol, LDL and triglycerides will degenerated the condition of atherosclerosis. Hypercholesterolemia was considered as one of the main triggers of atherosclerosis. Increased plasma cholesterol affect on arterial endothelial permeability that allow migration of lipids, especially LDL cholesterol, so that in Table 1. LDL levels were high in the atherogenic group. LDL enters the arterial wall and circulating monocytes attach to endothelial cells that express adhesion molecules, such as vascular adhesion molecule -1 (VCAM-1) and selectins, and migrate subendothelially. Monocytes are transformed into macrophages, phosphorylated LDL to be converted to foam cells. LDL in the subendothelial was oxidized and becomes a potent chemoattractants. At this stage, the pro-inflammatory cytokines TNF-, IL-1β, IL-6, IL-8 and granulocyte macrophage colony stimulating factor (GM-CSF) are secreted from macrophages [13]. This process can sooner or later lead to erosion of the arterial wall, formation of thrombus and platelet aggregation referred to as atherothrombosis, which results in occlusion of blood flow and cellular damage [14]. The role of IL-1β in the cardiovascular system is induce inflammatory function of endothelial cells. IL-1β stimulates adhesion molecules to recruit leukocytes including ICAM-1 and VCAM-1. IL-1β also induces chemokines such as monocyte chemoattractant protein (MCP)-1. Interleukin-1 and IL-1ra have an important role in the pathogenesis of atherosclerosis. Binding of IL-1β to the IL-1 type I receptor produces an inflammatory response that is implicated in the development and progression of endothelial dysfunction, atherogenesis and instability of atheromatous plaques [15]. IL-1β requires a series of processes to produce biological effects. The secretion of the IL-1β cytokine and its receptor expression were increased in the atherosclerotic aorta. The inflammatory process and the release of interleukin IL-1β, further increase the inflammatory response (autoinflammatory). IL-1β activation is very important in mediating the proinflammatory response resulting in secondary activation of inflammatory mediators, including IL-6[16].]. Thus, the proinflammatory cytokine IL-1β has a key role in the development of atherosclerosis. Table 1 also shows that the levels of IL-1β, total cholesterol, LDL and triglycerides in the atorvastatin group were significantly lower than in the atherogenic group. This is consistent with the mechanism of statins on activity against oxidized LDL and influences several mechanisms in the formation of IL-1β, L-6, IL-8, TNF- and MCP-1[17]. Atorvastatin has activity on cholesterol and LDL regulation through inhibition of the enzyme HMG-CoA reductase. Thus reducing cholesterol crystals as an endogenous ligand to activate the NF-kβ pathway to form the caspase-1 pathway. Atorvastatin molecular activity reduces LDL circulating in the blood by increasing LDL receptors in hepatocyte cells so that bind to receptors for damage-associated molecular pattern molecules (DAMPs) thereby reducing their binding to macrophage cells. As a result, caspase-1 was not formed and IL-1β levels in the atorvastati group were lower than atherogenic group [17][18]. Table 2. Shows a significant positive correlation (P<0.05) between levels of IL-1β, total cholesterol, LDL, and triglycerides. Cholesterol crystals, LDL cholesterol and triglycerides are able to induce the NLRP3 inflammasome which activates caspase-1 and then breaks down pro-IL-1β into IL-1β [19]. Unlike the case with HDL which is negatively correlated with IL-1β. Cholesterol in arterial walls, especially in macrophages and foam cells, will be removed by HDL particles. Cholesterol in peripheral cells is transported from peripheral cell membranes to the liver and intestines through a reverse cholesterol transport process facilitated by HDL. Decreased HDL plasma levels can accelerate the progression of atherosclerosis by interfering with the clearance of cholesterol from the arterial walls [20]. So when HDL levels are high, a small amount of LDL and cholesterol crystals causes IL-1β levels in the blood decrease.

5. Conclusion

The lipid profile in the atherogenic group showed a significant difference (P<0.05) against the normal control. The administration of atorvastatin showed significantly lower levels of total cholesterol, triglycerides, LDL cholesterol, HDL cholesterol (P<0.05) compared to the atherogenic group. IL-1β as a proinflammatory cytokine in atherosclerosis showed high values and was significantly different in the atherogenic group. Atorvastatin showed significantly lower IL-1β levels (P<0.05) compared to the atherogenic group. Based on the correlation test, total cholesterol, LDL, and triglyceride levels were significantly positively correlated with IL-1β secretion (P<0.05). So that in the future lipid profile and IL-1β can be used as markers of the severity of atherosclerosis.

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