ISSN: 234-5197
Impact Factor: 3.765

INTERNATIONAL JOURNAL OF RESEARCH SCIENCE & MANAGEMENT

ASSOCIATION BETWEEN INTERLEUKIN 6 SERUM LEVEL AND NON-ALCOHOLIC FATTY LIVER DISEASE

Annisa Yuanita Anggreini[1], GontarAlamsyah Siregar[*2] & Leonardo Basa Dairi[3]
Division of Gastroenterology-Hepatology, Department of Internal Medicine, Faculty of Medicine, University of Sumatera Utara/ Adam Malik General Hospital Medan

DOI: 10.5281/zenodo.3342743
Keywords: NAFLD, Interleukin 6.

Abstract
Background. Non-Alcoholic Fatty Liver Disease (NAFLD) emerged as a major health problem throughout the world and is increasing as the prevalence of obesity and diabetes continues to increase and is recognized as a major cause of liver disease-related morbidity and mortality. Adipokine is critically involved in healthy liver physiology and in the pathophysiology of acute and chronic liver disease as an intermediary for liver inflammation, liver cell death, cholestasis, and fibrosis. Interleukin-6 (IL-6) is one of adipokines widely studied recently. IL-6 showed improvement and played an important role in the pathogenesis of NAFLD.
Aim. To determine association of serum Interleukin 6 level with NAFLD and other marker associate with NAFLD.
Methods. Cross-sectional study of 30 consecutive NAFLD patients who came to Adam Malik General Hospital Medan and North Sumatra University Hospital in 2018. NAFLD was diagnosed by abdominal ultrasound. Complete blood count, blood sugar level, parameters of liver function, lipid profile and IL-6 were measured in each subject.
Results. Of the 60 subjects, they are divided into 2 groups; 30 subjects in the NAFLD group and 30 subjects in the NAFLD group. The mean serum IL-6 level in the NAFLD group was 5.1 ± 2.87pg / ml and the control group was 1.9 ± 0.50pg / ml.
Conclusion. There was a significantly higher difference in serum Interleukin 6 (IL-6) levels between the Non Alcoholic Fatty Liver Disease (NAFLD) group and the control group.

Introduction
Non-Alcoholic Fatty Liver Disease (NAFLD) is a manifestation of the metabolic syndrome in the liver which is characterized by excessive deposition of free fatty acids and triglycerides in the liver parenchyma. Obesity, diabetes mellitus hypertension, hypertriglyceridemia and hypercholesterolemia are considered as potential causative factors for NAFLD. NAFLD emerged as a major health problem throughout the world and is increasing as the prevalence of obesity and diabetes continues to increase and is recognized as a major cause of liver disease-related morbidity and mortality. The prevalence of NAFLD worldwide is 10% -24% in the general population.[1]

The pathogenesis of NAFLD / NASH is still not fully understood. The pathogenesis of NAFL is thought to involve a multi-hit process, where the 'first hit' shows that fat accumulation in the liver, associated insulin resistance (IR) and 'second hit' show an increase in beta fatty acid oxidation, adipokine, oxidative stress and endotoxemia. Adipose tissue secretes a number of cytokines called adipokine, which have local, peripheral, and central effects. Adipokine is critically involved in healthy liver physiology and in the pathophysiology of acute and chronic liver disease as an intermediary for liver inflammation, liver cell death, cholestasis, and fibrosis. Adipokine that has been widely studied is interleukin-6 (IL-6), tumor necrosis factor (TNF-α), adiponectin, leptin and resistin. Some studies have concluded that adipokine is involved in the pathogenesis of NAFLD and progression to NASH through pro-or anti-inflammatory activity.[1]

Recent research has focused on adipokine, a bioactive protein secreted by adipose tissue, including adiponectin and IL-6. IL-6 showed improvement and played an important role in the pathogenesis of NASH.[2] IL-6 is produced by adipocytes, immune cells, fibroblasts, endothelial cells and monocytes. IL-6 interfered with insulin receptors in hepatocytes, resulting in an increase in liver gluconeogenesis, followed by hyperglycemia and

hyperinsulinemia. Expression of liver IL 6 increased significantly in NASH patients and positively correlated with inflammation and fibrosis.[3]

From several studies that have been conducted, it was found that there was an association between the incidence of NAFLD and an increase in serum IL-6 levels. This event also correlated with NAFLD fibrosis scores as a parameter to assess the prognosis and severity of fibrosis in patients suffering from NAFLD. It was suspected that there was an increase in systemic IL-6 levels in patients with NAFLD. However, research on this subject is still very limited. From the description above the author is interested in examining more deeply the relationship between serum IL-6 levels in patients with NAFLD. This relationship may be useful for the better management, prevention, diagnosis and treatment in NAFLD patients in the future.

Material And Methods

The present study was a cross sectional study on 30 consecutive NAFLD patients who were admitted to Adam Malik General Hospital Medan and North Sumatra University Hospital, Indonesia. 30 patients without NAFLD were taken as controls.

Inclusion criteria:

1. Patient diagnosed with fatty liver by abdominal ultrasound examination
2. Had agreed in writing to participate voluntarily in this study included conducting a physical examination, laboratory and abdominal ultrasound that has been approved by the Health Ethics Research Committee

Control Criteria:

1. Patients aged ≥ 18 years
2. Had agreed in writing to participate voluntarily in this study included conducting a physical examination, laboratory and abdominal ultrasound that has been approved by the Health Ethics Research Committee
3. Not diagnosed with NAFLD by abdominal ultrasound examination.

Exclusion criteria:

1. Patients aged < 18 years
2. Patients consuming alcohol > 30 g / day for men and> 20 gr / day for women
3. Patients with autoimmune or malignancies disease
4. Patients with Hepatitis B or Hepatitis C
5. Pregnant woman

Patients were interviewed about demographic and clinical characteristics. An ultrasound was conducted after the subject had fasted for 6-12 hours a night. Then the examination of levels of hemoglobin, leukocytes, platelet counts, fasting blood sugar, adrandom blood sugar, AST, ALT, GGT, Alkaline phosphatase, albumin, total bilirubin, indirect bilirubin, total cholesterol, LDL, LDH, and triglycerides to assess NAFLD Fibrosis Score and the last was examination to determine the level of serum IL-6.

Statistical analysis

Analysis of the data used was univariate analysis to determine the basic and clinical characteristics of the research subjects, while bivariate analysis to analyze the independent and dependent variable relationship. Independent T-test was used to compare the serum IL6 levels between NAFLD patients and the control group if the data was normally distributed or with the Mann Whitney test if the data distribution was not normal. The T-independent test was used as comparative test in 2 groups with a numerical dependent variable scale. The independent variables were categorized into NAFLD and control groups, while the dependent variable was serum IL-6.

Result

Basic characteristics of the subjects

Table 1 shows that the case group is dominated by men (56.7%), and in the control group is dominated by women (53.3%). In both the case and control groups, the age most often found was the age range of 50-59 years (36.7% in the case group and 33.3% in the control). In this study it was found that all subjects in the case group belonged to obesity, namely 53.3% obesity 1, and 46.7% obesity 2. Conversely, only 30% of subjects in the control group were classified as obese.

Table 1. Basic Characteristics of Subjects

	Casw		Control	
	n	**%**	**N**	**%**
Male	17	56,7	14	46,7
Female	13	43,3	16	53,3
< 30 years	4	13,3	3	10,0
30 - 39 years	6	20,0	5	16,7
40 - 49 years	8	26,7	10	33,3
50 - 59 years	11	36,7	10	33,3
>= 60 years	1	3,3	2	6,7
Underweight	0	0,0	3	10,0
Normoweight	0	0,0	13	43,3
Overweight	0	0,0	5	16,7
Obesity gr 1	16	53,3	8	26,7
Obesity gr 2	14	46,7	1	3,3
TOTAL	**30**	**100**	**30**	**100**

Characteristics of subject laboratories results

NAFLD group had worse lipid profile; higher total cholesterol, triglycerides and LDL and lower HDL levels compared to the control group. There were no significant differences in the levels of SGPT enzymes between the two groups. On the other hand, SGOT enzyme levels were found to be higher in the control group compared to the NAFLD group.(Table 2)

Table 2. Characteristics of Laboratory Results of the Subjects

Laboratory Examination Results	Case	Control	p value
Fasting Glucose Level, median (min-max), mg/dL	150 (87-240)	98 (79-112)	< 0.001*
SGOT, median (min-max), U/l	28 (15-33)	33 (23-41)	< 0.001*
SGPT, median (min-max), U/l	31.5 (11-49)	32 (20-42)	0.22
Total Cholesterol, median (min-max), mg/dl	259 (171-282)	134.5 (14-166)	< 0.001*
Triglyceride, median (min-max), mg/dl	175.5 (155-223)	123 (109-146)	< 0.001*
HDL, median (min-max), mg/dl	39.5 (35-56)	43 (40-49)	< 0.001*
LDL, median (min-max), mg/dl	180 (87-209)	133.5 (119-145)	< 0.001*

*) significant with Mann Whitney Test

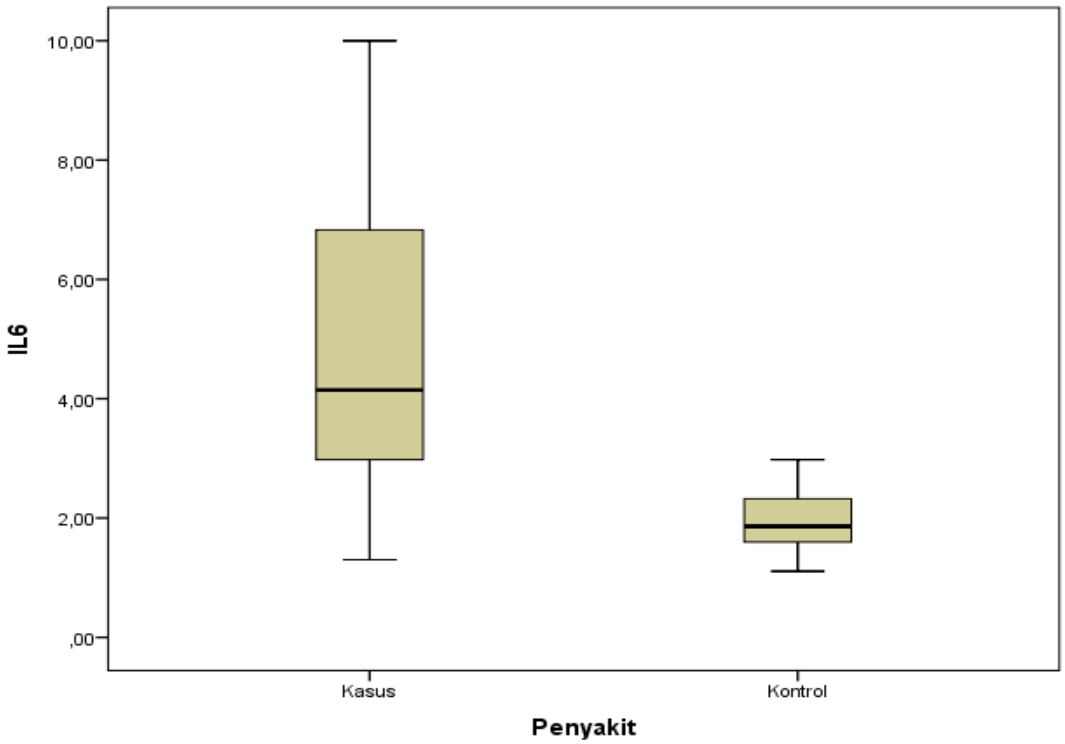
Relationship between Interleukin - 6 Serum with NAFLD Events

Based on Table 3 and Figure 1, it can be seen that the mean serum IL-6 level of NAFLD patients was 5.1 ± 2.87pg / ml. This value was 3 times higher compared to the control group, which was 1.9 ± 0.50pg / ml. It can be seen clearly that there were significant differences ($p < 0.01$) in serum IL-6 levels between the case group (NAFLD patients) and the control group (healthy subjects). Thus it was concluded that serum IL-6 levels were significantly associated with NAFLD events.

Tabel 3 Serum IL-6 Level between Case Group and Control Group

	Case (n=30)	**Control (n=30)**	**p value**
Mean ± Deviation Standard	5.1 ± 2.87	1.9 ± 0.50	< 0.01*
Median (Min - Max)	4.1 (1.3 - 10.0)	1.8 (1.11 – 2.98)	

* *Mann Whitney Test*
In pg/ml



Boxplot diagram of comparison between serum IL-6 level in case group and control group

Factors Affecting Serum Levels of IL-6

Table 4 shows that based on bivariate analysis, it was found that the body mass index was significantly associated with an increase in serum IL-6 levels (p = 0.05). Table showed that subjects with an increase in serum IL-6 levels included in obesity body mass index, both obesity grade 1 and grade 2. Thus, it can be concluded that obesity can increase serum IL-6 levels and along with it will also increase the risk of NAFLD. On the other hand, gender and age were not associated with elevated serum IL-6 levels.

Table 4 Factors Affecting Serum IL-6 Level

		Normal IL-6		**Increasing IL-6**		**p value**
		n	**%**	**N**	**%**	
Sex	Male	24	50,0	7	58,3	0.605
	Female	24	50,0	5	41,7	
Age	< 30 years	5	10,4	2	16,7	0.304
	30 - 39 years	11	22,9	0	0,0	
	40 - 49 years	14	29,2	4	33,3	
	50 - 59 years	15	31,3	6	50,0	
	>= 60 years	3	6,3	0	0,0	

	Underweight	3	6,3	0	0,0	
	Normoweight	13	27,1	0	0,0	
Body Mass Index	*Overweight*	5	10,4	0	0,0	0.05*
	Obesity grade 1	18	37,5	6	50,0	
	Obesity grade 2	9	18,8	6	50,0	
TOTAL		48	100	12	100	

Chi Square Test

Discussion

Various studies found that the incidence of NAFLD was higher in men than women. A study conducted by Borai et al found that about 57% of NAFLD patients were male.[4] This may be explained by the fact that the prevalence of central obesity is mainly found in men. Central obesity has long been associated with risk factors for NAFLD due to the effect of the release of adiponectins and pro-inflammatory cytokines that have led to NAFLD. This also may be due to higher estrogen levels in women than in man that have a protective effect on the incidence of NAFLD.[5]

The majority of study subjects with NAFLD were aged 50-59 years with an average age of 44.6 years, of which this age is still classified as young adults (productive age). These results are relatively identical to the previous study of Zahran et al. and Das et al. where the mean age of NAFLD patients was 48.5 years and 42.1 years respectively.[6,7]

High body mass index and visceral obesity are recognized as risk factors for NAFLD. In patients with severe obesity, the prevalence of NAFLD can exceed 90% and up to 5% of patients may have unexpected cirrhosis. The coexistence of visceral obesity and NAFLD in individuals is likely to improve the advanced form of liver disease. NAFLD appears as a major cause of liver disease-related morbidity and mortality. The pathogenesis of NAFLD is thought to involve a multi-hit process with 'first hit' to accumulate liver fat, related to insulin resistance (IR) and 'second hit' to be an increase in fatty acid beta oxidation, adipokine, oxidative stress and endotoxemia. Adipose tissue secretes a number of cytokines called adipokine, which have local, peripheral, and central effects. Adipokine is critically involved in healthy liver physiology and in the pathophysiology of acute and chronic liver disease as an intermediary for liver inflammation, liver cell death, cholestasis, and fibrosis. This study found that the average body mass index of the study subjects was 29.2 ± 2.48 kg / m2, which means that most subjects belong to obesity. This is in line with Zahran et al study that found a mean body mass index of NAFLD patients were ranging from 23.7 - 31.1 kg / m2.[6]

This study found that there were significant differences ($p < 0.01$) in serum IL-6 levels between the case group (NAFLD patients) and the control group (healthy subjects). This was in line with Jarrar et al study results who found that serum IL-6 levels in NAFLD patients were much lower than in obese group (23.1 ± 72.9 pg / ml, vs 7.6 ± 6.3 pg / ml) and they were statistically significant ($p < 0.01$).[8]

The role of IL-6 in the pathogenesis of NASH still need further research. Previous study showed that IL-6 and IL-6R increased in NASH patients compared to patients with simple steatosis and healthy subjects. One possible explanation for the increase in inflammatory markers in obesity is that adipose tissue secretes a number of pro-inflammatory cytokines, including tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6). Although immune cells, fibroblasts, endothelial cells, and monocytes have long been considered the main sources of circulating IL-6, recent research shows that the proportion of circulating IL-6 can also originate from adipose tissue. Circulating IL-6 levels have been reported to increase in obese people and in men with type 2 diabetes. IL-6 levels also correlate with insulin resistance, waist-hip ratio, and fasting insulin levels. However, research conducted by Barbora (2001) stated that the association of IL-6 levels with obesity and NAFLD was influenced by ethnicity. Ethnic Indians had IL-6 levels that did not have significant relationship with the incidence of obesity and NAFLD.[9]

Conclusion

There was a significantly higher difference in serum Interleukin 6 (IL-6) levels between the Non Alcoholic Fatty Liver Disease (NAFLD) group and the control group.

References

[1] Pallavi, M., Suchitra, M. M., & Rao, P. V. L. N. S. (2015). Review Article : Role of adipokines in the pathogenesis of nonalcoholic fatty liver disease, 4:31-9.
[2] Mohamed, A. Al. (2014) 'Role of Serum Adiponectin , IL-6 and Hs CRP in Nonalcoholic Fatty Liver Egyptian Patients', 4(6), pp. 493–504.
[3] Schmidt-Arras, D., & Rose-John, S. (2016). IL-6 pathway in the liver: From physiopathology to therapy. Journal of Hepatology, 64(6), 1403–1415. https://doi.org/10.1016/j.jhep.2016.02.004
[4] Borai et.al
[5] Ballestri et.al
[6] Zahran WE, Salah El-Dien KA, Kamel PG, El-Sawaby AS. Efficacy of Tumor Necrosis Factor and Interleukin-10 Analysis in the Follow-up of Nonalcoholic Fatty Liver Disease Progression. Indian Journal of Clinical Biochemistry. 2013;28(2):141-146. doi:10.1007/s12291-012-0236-5.
[7] Das SK, Balakrishnan V. Role of Cytokines in the Pathogenesis of Non-Alcoholic Fatty Liver Disease. Indian J Clin Biochem. 2011;26(2):202-209
[8] Jarrar, M. H. et al. (2007) 'Adipokines and cytokines in non-alcoholic fatty liver disease', Alimentary Pharmacology & Therapeutics, 27(5), pp. 412–421. doi: 10.1111/j.1365-2036.2007.03586.x.
[9] Barbora et.al