

## Correlation of Tumor Necrosis Factor-Alpha Serumlevels in Non-Alcoholic Fatty Liver Disease

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### ABSTRACT

Fatty liver disease is the addition of free fatty acids in liver. This causes an increase in fat oxidation and esterification. When the oxidative stress condition in the liver exceeds the ability of anti-oxidants, it will activate stellate cells and pro-inflammatory cytokines which leads to a progressive inflammatory process. TNF- $\alpha$  is a pro-inflammatory cytokine that experiences a significant increase in NAFLD.

**Method:** This study was a cross-sectional study with a total of 30 NAFLD patients who came to Adam Malik General Hospital Medan, from April to September 2018. Assessment for NAFLD was done by ultrasound imaging. A univariate and bivariate (Chi-square and fisher exact test) analysis were performed using the SPSS version 22.

**Results:** In the NAFLD group, there were 17 males (56.7%) and 13 females (43.3%). Average age of subjects was 44.57 years old. Median value of TNF- $\alpha$  in the NAFLD group was 3.18 pg/ml and 1.72 pg/ml in the control. There is a significant difference of TNF- $\alpha$  levels between the NAFLD and control group ( $p=0.0001$ ). Median value of blood glucose level in NAFLD group is 150 mg/dl and 98 mg/dl in control. In a hypothesis test, there was a significant difference in blood glucose levels between the NAFLD and control groups ( $p=0.0001$ ).

Median value of total cholesterol, HDL, LDL and TG in NAFLD group were 251, 40, 178 and 175 pg/ml while in the control were 174, 60, 88.5, and 123 pg/ml. There is a statically significant difference in lipid profile between the NAFLD and control groups ( $p=0.0001$ ).

For evaluating the levels of liver enzymes, the median value of ALT and AST in the NAFLD group were 31.5 and 27 U/L, while in the control group was similar which was 20 U/L. There is a significant difference in levels of liver enzymes between the NAFLD and control groups ( $p=0.0001$ ).

**Conclusion:** There were significant association between blood glucose levels, lipid profile, liver function test, TNF- $\alpha$  level and NAFLD patient as well as its control.

**Keywords:** NAFLD, Inflammation, TNF- $\alpha$

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### ABSTRAK

Kondisi perlemakan hati terjadi dikarenakan adanya penambahan asam lemak bebas di hati. hal ini akan menimbulkan peningkatan oksidasi dan esterifikasi lemak. Ketika kondisi stres oksidatif di hati melebihi kemampuan perlawanan anti oksidan, maka aktivasi sel stelata dan sitokin pro inflamasi akan berlanjut dengan inflamasi progresif. TNF- $\alpha$  merupakan salah satu sitokin pro-inflamasi yang mengalami peningkatan signifikan pada kondisi NAFLD

**Metode:** Studi potong lintang terhadap 30 pasien NAFLD secara konsekutif yang datang ke Rumah Sakit Umum Adam Malik Medan dari April–September 2018. Penilaian NAFLD menggunakan ultrasonografi. Analisis univariat, bivariat (uji Chi-square dan fisher exact) dengan SPSS versi 22.

**Hasil:** Pada kelompok NAFLD, 17 orang (56,7%) dengan jenis kelamin pria dan 13 orang (43,3%) dengan jenis kelamin perempuan. Umur rerata 44,57. Pada grup NAFLD memiliki nilai median TNF- $\alpha$  3,18 pg/ml dan pada grup kontrol dengan nilai median 1,72 pg/ml. Terdapat perbedaan yang signifikan nilai TNF- $\alpha$  antara grup NAFLD dengan grup kontrol ( $p=0,0001$ ). Nilai median kadar gula darah grup NAFLD 150 mg/dl dan pada grup kontrol 98 mg/dl. Pada uji hipotesis didapati perbedaan signifikan kadar gula darah antara grup NAFLD dan kontrol ( $p=0,0001$ ).

Nilai median kadar Kolesterol total, HDL, LDL dan TG pada grup NAFLD adalah 251, 40, 178 dan 175 pg/ml. Pada kelompok kontrol, nilai median Kolesterol total, HDL, LDL dan trigliserida adalah 174, 60, 88,5, dan 123 pg/ml. Terdapat perbedaan yang signifikan secara statistik pada profil lipid antara grup NAFLD dengan kontrol ( $p=0.0001$ ). Untuk penilaian kadar enzim hati, nilai median ALT dan AST pada grup NAFLD adalah

31,5 dan 27U/L, sementara pada grup kontrol nilai median ALT dan AST sama sebesar 20U/L. Terdapat perbedaan signifikan pada kadar enzim hati antara grup NAFLD dan kontrol ( $p=0,0001$ ).

**Kesimpulan:** Terdapat perbedaan yang signifikan kadar TNF- $\alpha$  antara grup NAFLD dengan kontrol. Dimana kadar TNF- $\alpha$  serum lebih tinggi pada grup NAFLD dibandingkan dengan grup kontrol

**Kata kunci :** NAFLD, Inflamasi, TNF- $\alpha$

## I. Introduction

Alcoholic Fatty Liver Disease (NAFLD) is part of the Fatty Liver, whereby there is an accumulation of Triglyceride and free fatty acid in the liver. Wide spectrum NAFLD pathology shows that a simple steatosis up to a Non Alcoholic Steatosis Hepatitis (NASH) has the potential of turning fibrotic and cirrhotic.<sup>1</sup>

Indonesia is a country with high prevalence of NAFLD. At least 30% of its population suffers from NAFLD. This is a matter of consideration for further investigations. Some suggest that lifestyle changes such as inactivity and diets high in calorie may contribute to this.<sup>2</sup>

There is a considerable amount of population with NAFLD and most NAFLD is associated with obesity. NAFLD is even found in children and young. Obesity itself includes fat accumulation not only in the adipose tissue but also in the muscle cells, and this accumulation can cause insulin resistance in adipocytes and muscles. Increased pro-inflammatory cytokines production is also considered a pathogenic factor in the occurrence of NAFLD. Tumor Necrosis Factor-alpha (TNF- $\alpha$ ) is one of the pro-inflammatory factor cytokines found in NAFLD.<sup>3</sup>

Fatty Liver conditions occurs due to the increase free fatty acids in the liver. This causes an increase in oxidation and esterification of fats. When there is an oxidative stress condition in the liver which exceeds the ability of the anti-oxidants to resist it, it would activate stellate cells and pro-inflammatory cytokines which would then lead to a progressive inflammation, swelling of hepatocytes, formation of Mallory Body as well as fibrosis.<sup>4</sup>

Fatty Liver process starts with the accumulation of fats in the hepatocytes. In addition to the fat accumulation, fatty tissue also enlarges and triggers the release of pro-inflammatory cytokines. One of the pro-inflammatory factors released is TNF- $\alpha$ . This causes an inflammation that can lead to the progressivity of NAFLD.

TNF- $\alpha$  serum is also associated with insulin resistance, whereby insulin resistance can be a risk factor in developing NAFLD. A study conducted by Paredes et al. stated the relation between TNF- $\alpha$  serum and the occurrence of NAFLD as well as the severity of NAFLD.<sup>5</sup>

## II. Material And Method

This study was conducted from April to September 2018 with 30 NAFLD patients, 17 males and 13 females, and 30 control. The mean age of patients that were studied was 44,57 years old.

The design of this study was a cross sectional design with the independent variable being NAFLD and the dependent variable being rate of serum TNF- $\alpha$ .

- a. The targeted population of this study were patients with NAFLD, while the approachable patients were patients with NAFLD who came to Haji Adam Malik Hospital and its co-operating hospitals.
- b. The samples of this study were NAFLD patients who met the inclusion criteria and who did not meet the exclusion criteria.

### Inclusion Criteria

1. Above 18 years of age
2. Patients diagnosed with Fatty Liver from a Ultrasonography Examination
3. Not a regular alcohol consumer, < 30 gr/day for males and < 20 gr/day for females
4. Cooperative and willing to participate in this study till completion

### Exclusion Criteria

1. Alcohol consumption of > 30 gr/day for males and > 20 gr/day for females
2. Patients with systemic diseases and malignancies
3. Patients suffering from Hepatitis B or Hepatitis C
4. Uncooperative patients

### Controlled Criteria

1. Males and non-pregnant female above 18 years of age
2. Not a regular alcohol consumer, < 30 gr/day for males and < 20 gr/day for females

3. Not suffering from Hepatitis B or Hepatitis C, kidney failure (LFG < 60 ml/minute/1,73m<sup>2</sup>) malignancies or autoimmune diseases
4. Not diagnosed with Non Alcoholic Liver Disease clinically or with other investigations. Diagnosis is confirmed with an Abdominal Ultrasonography.

### **Working Procedure**

#### **Examination of Patient's Characteristics**

Patients were interviewed on their demographic and clinical characteristics with a questionnaire which consisted of the patients age, race, marital status, education level, occupation, alcohol consumption habits, smoking, physical activity, measurement of body weight and height to determine nutritional status, complaints experienced, comorbidities, family history and physical examination.

#### **Examination of Ultrasonography Imaging**

In order to diagnose NAFLD, The Abdominal USG is performed by operator. The USG procedure is done by an experienced operator. The procedure was done after the patient has been fasted overnight, for about 6 to 12 hours. Respondents suspected of Fatty Liver underwent an USG examination. Patients with fatty liver will show a 'bright echo' and a 'deep attenuation' on a USG examination. Abdominal USG was conducted and examined by two different operators. If there happened to be a difference between the two operators, a third operator was called in to confirm it.

#### **Laboratory Examinations**

Laboratory examinations were done to classify the patients according to the NAFLD Fibrosis Score. The NAFLD Fibrosis Score comprises of patient's characteristic examination (age, sex, body weight and height), levels of haemoglobin, leucocytes, platelet count, fasting glucose levels and ad random glucose levels, AST, ALT, GGT. Alkaline phosphatase, albumin, total bilirubin, indirect bilirubin, total cholesterol, LDL, LDH, Triglyceride.

#### **Examination of Serum TNF- $\alpha$ level**

The examination of serum TNF- $\alpha$  is using Quantikine Human Immunoassay TNF- $\alpha$

### **III. Results**

This study was participated by 60 subjects who met the inclusion criteria. The subjects were divided into two groups which were the NAFLD group (n=30) and the control group (n=30). In the NAFLD group, 17 subjects (56,7%) were males and 13 subjects (43,3%) were females. Average age of the subjects was 44,57 years old, with the majority of the subjects working as an entrepreneur.

The comorbid disease factor in the NAFLD group was mostly caused by Diabetes Mellitus which accounted for 16 subjects (53,3%).

Average BMI for the NAFLD group was 29,03 kg/m<sup>2</sup>.

<b>Karakteristik</b>	<b>NAFLD (n = 30)</b>	<b>Control</b>	<b>P value</b>
Sex, n (%)			0,605
Male	17 (56,7)	15 (50)	
Female	13 (43,3)	15 (50)	
Race, n (%)			
Batak	17 (56,7)	19 (63,3)	N/A
Jawa	7 (23,3)	10 (33,3)	
Melayu	2 (6,7)	1 (3,3)	
Others	4 (13,3)	-	
Occupation, n (%)			N/A
Entrepreneur	14 (46,7)	6 (20)	
Housewife	3 (10,0)	15 (50)	
Employee	6 (20)	6 (20)	
Farmer	2 (6,7)	2 (6,7)	
Others	5 (16,7)	1 (3,3)	
Age, average (SB), years	44,57 (11,915)	43,27 (13,261)	0,691

*Correlation of Tumor Necrosis Factor-Alpha Serumlevels in Non-Alcoholic Fatty Liver Disease*

BMI, average (SB), kg/m <sup>2</sup>	29,030(2,636)	24,27 (3,58)	0,0001
Comorbid disease, n (%)		-	N/A
DM			
Dyslipidemia	16 (53,3)		
Hypertension	12 (40,0)		
	2 (6,7)		

In his study, both, NAFLD and control groups, were measured using three variables which were the blood glucose levels, lipid profile and liver enzymes. The lipid profile measured were total cholesterol, HDL, LDL and TG while the liver enzymes included the AST and ALT.

Median value of blood glucose level of the NAFLD group was 150 mg/dl and the control group was 98 mg/dl. In a hypothesis test, the difference in blood glucose levels between the NAFLD group and the control group was significant (p=0,0001).

Median value of total cholesterol, HDL, LDL and TG in the NAFLD group were 251, 40, 178 and 175 pg/ml. In the control group, the median value of total cholesterol, HDL, LDL and TG were 174, 60, 88,5, and 123pg/ml. There was a significant statistical difference in lipid profile between the NAFLD group and the control group (p=0.0001).

For the assessment of liver enzyme serums, the median value of ALT and AST in the NAFLD group were 31,5 and 27U/L, while the median value of AST and ALT in the control group were equal which was 20U/L. There was a significant difference in the liver enzyme serums between the NAFLD group and the control group (p=0,0001).

<b>Karakteristik</b>	<b>NAFLD (n = 30)</b>	<b>Kontrol (n=30)</b>	<b>P</b>
Fasting blood glucose levels (mg/dl) Median (min- maks)	150 (87-240)	98 (79-112)	0,0001
Lipid profile, (pg/mL) Total Cholesterol Median(min- maks)	251(168-282)	174 (152- 260)	0,0001
HDL, median (min – maks)	40 (29 -60)	60 (44-65)	0,0001
LDL, median (min – maks)	178 (84 – 216)	88,5 (78- 182)	0,0001
Trigliserida, median (min- maks)	175 (85-230)	123(89- 171)	0,0001
Liver Enzymes U/L, ALT median (min-maks)	31,5 (11-53)	20(14-42)	0,0001
AST median (min-maks)	27 (15-33)	20(14-24)	0,0001

The median value of TNF- $\alpha$  in the NAFLD group was 3,18 pg/ while the median value of TNF- $\alpha$  in the control group was 1,72 pg/ml. There was a significant difference in the TNF- $\alpha$  levels between the NAFLD group and the controlgroup (p=0,0001).

<b>Karakteristik</b>	<b>NAFLD (n = 30)</b>	<b>Kontrol (n=30)</b>	<b>P</b>
TNF- $\alpha$ , median (min-maks) (pg/ml)	3,18 (1,28- 15,7)	1,72 (0,93- 2,484)	0,0001

In this study, a correlation between the TNF- $\alpha$  levels and total cholesterol was carried out. Using the Spearman correlation method, it can be concluded that there is a positive correlation between the TNF- $\alpha$  levels and total cholesterol that is statically significant with a medium correlation strength (p=0,001 ; r= 0,55).

Likewise, a correlation between TNF- $\alpha$  and ALT showed a positive correlation with a medium correlation strength between TNF- $\alpha$  and ALT ( $p=0,001$  ;  $r= 0,55$ ).

In this study, the correlation between TNF- $\alpha$  levels and TG was studied using the Spearman correlation method. It can be concluded that there is a positive correlation with a medium correlation strength between TNF- $\alpha$  levels and TG ( $p=0,0001$ ;  $r=0,65$ ).

In this study, the correlation between TNF- $\alpha$  levels and HDL was studied. By using the Spearman correlation method it can be concluded that there is a negative correlation between TNF- $\alpha$  levels and HDL which is statistically significant and has a strong correlation strength ( $p=0,0001$  ;  $r= 0,65$ ).

Correlation test between TNF-  $\alpha$  and LDL in this study was conducted using the Spearman method and it can be concluded that there is a strong positive correlation which is significant between the two ( $p=0,0001$ ,  $r=0,67$ ).

In this study, the correlation between TNF- $\alpha$  and AST was also studied using the Spearman method. There was a positive correlation with moderate strength between TNF- $\alpha$  and AST ( $p=0,011$ ,  $r=0,41$ ).

Laboratorium Variabels	TNF- $\alpha$
Kolesterol total	0,55**
TG	0,65**
HDL	-0,65**
LDL	0,67**
ALT	0,55**
AST	0,41*

#### IV. Discussion

From the results of the study, it was found that more men were found with NAFLD disease than women, namely 17 (56.7%) and 13 (43.3%). In general, NAFLD attacks men more than women with a percentage of 60-70%. The following are other studies that reveal the percentage of men:women with NAFLD according to Goh GB et al 53.8%:46.1% (Singapore). Women The average age of patients with NAFLD in this study was 43.9 years. Likewise with the results of the study of Lazo M, et al., The average age of patients with NAFLD was between 40-50 years. The study of Younossi et al, the age of patients with NAFLD was the highest at the age of 40-49 years.<sup>6,7,8</sup>

The mean BMI of NAFLD patients in this study was 28.46. This value according to the WHO in 2000, included in the class of overweight in the international group, but in the Asian group, already included in obesity class 1. This is also in accordance with research by Jian-Gao Fan et al. The study stated that NAFLD was found in overweight and obese patients with a greater percentage (80%), although they revealed a new shifting trend in which NAFLD patients with normal BMI.<sup>9</sup>

The results of fasting blood sugar level examination, the median value of the examination in the NAFLD group was 150 mg / dl with a range of 87-240 mg / dl, while found in the control group, the median value of fasting blood sugar levels was 98 mg / dl with a range of 79 -112 mg / dl. There were significant differences between the NAFLD group and the control group ( $p = 0,0001$ ). In line with this study, Ratnasari N et al. Found a significant difference in the blood sugar levels of the NAFLD group with the control group ( $p = 0.002$ ).

The level of lipid profile of this study, including total cholesterol, HDL, LDL and triglyceride levels had a significant difference between the NAFLD group and the control group ( $p = 0,0001$ ). In accordance with Ardakani T et al's study in Iran comparing NAFLD groups with controls on lipid profile levels had a significant difference ( $p <0.001$ ).

In conditions of fatty liver, there are lipotoxicity conditions. Lipotoxicity can cause inflammation and insulin resistance which will affect the progression of fatty liver disease. This is like an interconnected vicious circle that affects the patient's lipid profile level.<sup>10</sup>

The results of AST and ALT liver enzyme examination also had a significant difference in the NAFLD group compared to the control group ( $p = 0,0001$ ). This result is in accordance with Sanyal et al's study which suggested that there was an increase in liver enzymes in NAFLD patients ( $p = 0.01$ ). Likewise, the Amir Khalili et al study found a significant difference between the NAFLD group and the control group.<sup>11</sup>

In the study Clark et al showed an increase in aminotransferase associated with central obesity and insulin resistance in non-alcoholic fatty liver disease. In fatty liver the AST: ALT ratio is used mainly in alcoholic fatty liver. In the progression of fatty liver disease both alcoholics and non-alcoholics found an increase in AST and ALT.<sup>12</sup>

In this study, the most comorbid disease in NAFLD subjects was Diabetes Mellitus. This is in line with the value of fasting blood glucose levels which had a significant difference between the NAFLD group and the control group. In a study conducted by Araujo AR et al, dividing the NAFLD classification into NAFLD primary insulin resistance, non-primary NAFLD insulin resistance, secondary NAFLD. Found a close relationship between NAFLD and DM associated with conditions of insulin resistance that occur in NAFLD patients.<sup>13</sup>

The focus of this study was on TNF- $\alpha$  values, where in the NAFLD group the minimum and maximum values of TNF- $\alpha$  were 1.28-15.70 pg / ml. The mean and median values of TNF- $\alpha$  in the NAFLD group were 4.90 pg / ml and 3.19 pg / ml. Compared to the control group, the mean and median values of TNF- $\alpha$  were 1.80 and 1.72. The results of the median and mean TNF- $\alpha$  in the NAFLD group were higher than the control group.

Before the hypothesis test, the data carried out by the Shapiro Wilk normality test found  $p < 0.05$ . This shows that the data is not normally distributed, so to test the hypothesis using the non-parametric test with the Mann-Whitney test. From the results of the bivariate analysis using Mann-Whitney test, it was found that there were significant differences in serum TNF- $\alpha$  levels between the NAFLD group and the Control group ( $p = 0,0001$ ). It could be concluded that there were significant differences in serum TNF levels between NAFLD groups. with Control.

## V. Conclusion

There were significant associations between NAFLD, Nuchter Glucose level, Lipid profile, Liver Function Test with TNF- $\alpha$

## References

- [1]. Matteoni CA, Younossi ZM, Gramlich T, Boparai N, Liu YC, McCullough AJ. Non Alcoholic fatty liver disease :a spectrum of clinical and pathological severity. *Gastroenterology* 1999;116:1413-9
- [2]. Lesmana Cosmas RA, Pakasi Levina S, Ingriani S, Aidawati ML, Lesmana LA. Development of non-alcoholic fatty liver disease scoring among adult medical check-up patients: a large cross-sectional and prospective validation study. *Dove Medical Press Limited*. 2015;8:213-218
- [3]. Hirosumi J, Tuncman G, Chang L et al. A central role for JNK in obesity and insulin resistance. *Nature* 2002; 420: 333-336
- [4]. Setiati S, Alwi Idrus, Sudoy Aru W, et al. *Buku Ajar Ilmu Penyakit Dalam Jilid II*. Edisi VI. Jakarta: Interna Publishing; 2016,(24):260-264
- [5]. Paredes AH, Torres DM, Harrison SA. Nonalcoholic fatty liver disease. *Clin Liver Dis*. 2012.5;16(2):397-419
- [6]. Lazo M, Clark JM. The Epidemiology of nonalcoholic fatty liver disease:a global perspective. *Semin Liver Dis*. 2008;28:339-50
- [7]. Younossi ZM, Stepanova M, Afendy M, et al. Changes in the prevalence of the most common causes of chronic liver diseases in the United States from 1988 to 2008. *Clin GastroenterolHepatol*. 2011; 9:524-530.e60
- [8]. Gao GB, Tsukamoto H. Inflammation in Alcoholic and Nonalcoholic Fatty Liver Disease: Friend or Foe? *Gastroenterology*. 2016;150(8):1704-9
- [9]. Fan JG, Kim SU, Wong VW. New Trends on Obesity and NAFLD in Asia. *Journal of Hepatology*. Elsevier. 2017.06;10:250-292
- [10]. Taghavi AA, Sharif MR, Kheirkhah D. Fatty liver disease in obese children in Kashan, Iran. *Caspian J of Pediatr*, March 2015;1(1):17-21
- [11]. Sanyal D, Mukherjee P, Raychaudhuri M, Ghosh S, et al. Profile of Liver Enzymes in Non-alcoholic Fatty Liver Disease in Patients with Impaired Glucose Tolerance and Newly Detected Untreated type 2 Diabetes. *India J Endocrino Metab*. 2015; 19(5):597-601
- [12]. Clark JM. The Epidemiology of nonalcoholic fatty liver disease in adults. *J Clin Gastroenterol*. 2006.06 Suppl 1:S5-10
- [13]. Araujo AR, Rosso N, Bedogni G, Tiribelli C, Bellentani S. Global epidemiology of non alcoholic fatty liver disease/non alcoholic steatohepatitis:What we need in future. *Liver International*. 2018. 38:liv.13643

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