INTERNATIONAL JOURNAL OF RESEARCH SCIENCE & MANAGEMENT

INTERLEUKIN-8 SERUM LEVEL AND NON-ALCOHOLIC FATTY LIVER DISEASE

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Abstract

Backgrounds: Non-alcoholic fatty liver disease (NAFLD) is a condition of fat accumulation in the liver without excessive alcohol consumption (<20 grams per week). Known risk factors for NAFLD are obesity, hyperglycemia and hypertriglyceridemia. Excess fat deposits in hepatocyte cells trigger acute injury causing liver cells to release acute phase proteins in the form of IL-6 and alpha TNF which are potent stimuli for the synthesis of IL-8. IL-8 recruits neutrophils to release various types of enzymes and cause more severe damage to liver tissue.

Aim : To determine association of serum Interleukin 8 level with NAFLD and other marker associate with NAFLD.

Method: This study was a cross-sectional study aimed at assessing differences in serum IL-8 levels between groups of NAFLD patients and the control group. Study samples are patients diagnosed with NAFLD by an ultrasound examination aged ≥ 18 years at Adam Malik General Hospital Medan & FK USU Hospital started from April to July 2018.

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-8 level in the NAFLD group was 45.3 ± 17.66 pg / ml and the control group was 17.6 ± 5.03 pg / ml.

Conclusion: There were statistically significant (p<0,01) differences in serum IL-8 levels between the case group (NAFLD patients) and the control group (healthy subjects).

Introduction

Non-Alcoholic Fatty Liver Disease (NAFLD) is a term used to refer to a condition of fat accumulation in the liver without the consumption of excessive alcohol (less than 20 grams per week). The prevalence of NAFLD in Western countries in the adult population is around 20-40%. The severe spectrum of NAFLD, steatohepatitis (NASH), is estimated to be present in 2-3% of the general population, and increases to 37% in obesity. Likewise there is an increase in the prevalence of NAFLD in children and adolescents, estimated at around 3% and increasing by 53% in obese children.

Known risk factors of NAFLD in adults and children are obesity, hyperglycemia and hypertriglyceridemia. From various prognosis studies of long-term mortality in NAFLD patients, the results showed that compared to the control population, there was an increase in mortality in NAFLD patients.

It has been agreed that excess fat deposits in hepatocyte cells trigger acute injury to liver cells which initiates the pathogenesis of NAFLD. Acute injury to the liver tissue will result in the release of acute phase proteins by the liver, including IL-6 and alpha TNF. These two cytokines are potent stimuli for the synthesis of IL-8 by inflammatory cells. There are evident from various studies that the higher the levels of TNF alpha and IL-6, the higher the level of IL-8.

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IL-8 is chemokine produced by several types of cells, including inflammatory and endothelial cells. The main role of this chemokine is to regulate the recruitment of neutrophils in inflamed tissue. Neutrophils that have infiltrated liver tissue then release various types of protease enzymes such as matrix metalloproteinase, elastase, cathepsin-G, and myeloperoksidase and Reactive Oxygen Species (ROS) which cause even more damage to liver tissue. Several studies have found that serum IL-8 levels correlate with the occurrence of fibrosis in the liver and are closely related to the Child-Pugh score. Patients with alcoholic liver disease had the highest IL-8 levels compared to NAFLD, while normal liver tissue had the lowest IL-8 levels. Thus, it is indicated that there is a relationship between IL-8 levels and the incidence of NAFLD.

Material and methods

The present study was a cross sectional study on 30 consecutive NAFLD diagnosed by an ultrasound examination at Adam Malik General Hospital Medan & FK USU Hospital started from April to July 2018. 30 patients without NAFLD were taken as controls.

Inclusion criteria:
1. Men and women 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

Exclusion criteria:
1. Patients consuming long-term hepatotoxic drugs Kidney failure (LFG <60 ml / min / 1.73 m2)\
2. Patients consuming alcohol > 30 g / day for men and> 20 gr / day for women
3. Patients consuming long-term corticosteroid
4. Patients suffering from Hepatitis B and Hepatitis C infections
5. patients who do not participate cooperatively

Table 1.Basic characteristics of the subjects

	Case		Control		p value
	n	%	n	%	
Male	17	56,7	14	46,7	0.43
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	0.93
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	< 0.01*
Overweight	0	0,0	5	16,7	
Gr 1 Obesity	16	53,3	8	26,7	
Gr2 Obesity	14	46,7	1	3,3	
TOTAL	30	100	30	100	

All patients getting tested for routine blood tests, PT, APTT, INR, HBsAg, Anti HCV, SGOT, SGPT, Alkaline phosphatase, Gamma GT, Total Bilirubin, Ureum, Creatinine, Albumin, and Blood Sugar during, Abdomen ultrasound examination, and venous blood collection will be carried out for serum IL-8 examination as much as 5 mL taken 1-2 hours after the patient enters the emergency department before the patient gets additional resveratrol therapy.

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Statistical analysis
Tabulations are used show a descriptive picture of the basic characteristics of the study population. The Shapiro Wilk test is used to determine whether the data is normally distributed or not. To determine the difference between IL-8 levels in NAFLD and non-NAFLD patients, the Independent T test was used if the data were normally distributed and the Mann-Whitney test if the data were abnormally distributed. Data was analyzed using the SPSS statistical program for Windows, version 22.0 (SPSS Inc., Chicago, Illinois, USA); with p value <0.05 considered statistically significant.

Results

Basic Characteristics of the subjects
Of the 60 subjects, they are divided into 2 groups; 30 in NAFLD patients and 30 in control groups. 56.7% of the case group were male, whereas 53.5% of the control group were female. Most subjects were in the age group of 50 - 59 years, 36.7% in case groups and 33.3% in the control group. More than half of the subjects in the case group belonged to grade 1 obesity, and as many as 46.7% of the subjects belonged to grade 2 obesity. In contrast, subjects in the control group, mostly classified as normoweight (43.3%), and only 1 person classified into grade 2 obesity. With the Chi Square test analysis it was found that there was a significant relationship between body mass index and the incidence of NAFLD in the study subjects.

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 - 8 Serum with NAFLD Events
Table 3 and figure 1 showed that there are significant differences in serum IL-8 levels between the case group (NAFLD patients) and the control group (healthy subjects) which are statistically significant (p <0.01). The mean serum IL-8 level of NAFLD patients was 45.3 ± 17.66 pg / ml. which is 2.5 times higher compared to the control group (17.6 ± 5.03 pg / ml). From these results it can be stated that the serum IL-8 level of NAFLD patients is much higher than that of healthy subjects.

Table 3 Comparison of Serum Il-8 Levels between Case and Control Groups

	Case (n=30)	Control (n=30)	p value
Mean ± Standard Deviation	45.3 ± 17.66	17.6 ± 5.03	< 0.01*
Median (Min - Max)	42.5 (14.6 - 64.0)	17.6 (8.4 - 25.0)	

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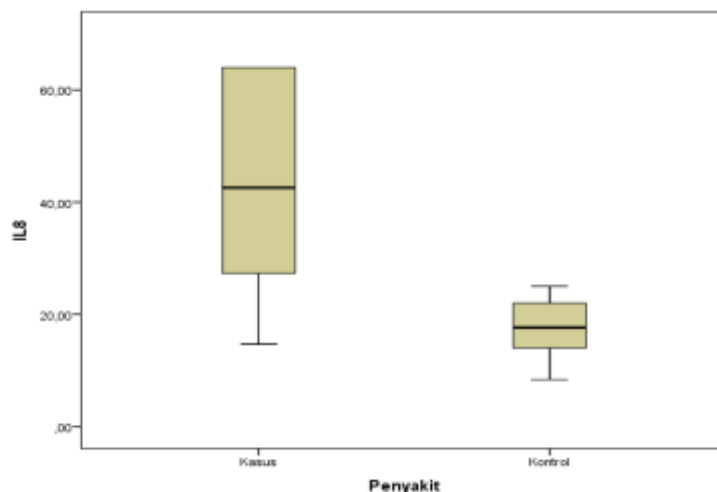*Mann Whitney Test*
In pg/ml

Figure 1 Boxplot Diagram of Comparison of Serum IL – 8 Level in Case Group and Control Group

Factors Affecting Serum Levels of IL-8

Based on table 3 it is known that gender and age do not have an association with serum IL-8 levels. While the body mass index has a significant relationship with an increase in serum IL-8 levels (p <0.01). It can be seen clearly that the subjects who experienced elevated serum IL-8 levels were all classified as obese, both obesity grade 1 and 2. In other words, individuals classified as obese had a greater risk of developing serum IL-8 levels and with thus also has a greater risk of suffering from NAFLD.

Table 4 Factors Affecting Serum IL-8 Level

		Normal IL-8		Increased IL-8		p value
		n	%	n	%	
Sex	Male	17	44,7	14	36,4	0.158
	Female	21	55,3	8	18,2	
Age	< 30 years	3	7,9	4	22,7	0.67
	30 - 39 years	6	15,8	5	27,3	
	40 - 49 years	12	31,6	6	27,3	
	50 - 59 years	15	39,5	6	4,5	
	>= 60 years	2	5,3	1	0,0	
Body Mass Index	*Underweight*	3	7,9	0	0,0	< 0.01*
	Normoweight	13	34,2	0	0,0	
	Overweight	5	13,2	0	54,5	
	Gr 1 Obesity	12	31,6	12	45,5	
	Gr2 Obesity	5	13,2	10	100,0	
Total		**38**	**100,0**	**22**	**0,0**	

Chi Square Test

Discussion

Characteristics of Study Subjects

In this study, it was found that most NAFLD group were males (56.7%). This finding is in line with Borai et al's study which found that the percentage of NAFLD sufferers was higher in men of 57.4%.[1] Kojima et al. also

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found that the percentage of NAFLD incidence in men was 26% while in women only 12%.[2] A possible scientific explanation is that higher estrogen hormones in women have a protective effect and inhibit the progress of NAFLD, so the incidence of NAFLD tends to be less in women, although this finding still requires further study.[3]

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.[4,5] Prasetya et al in Cipto Mangunkusumo Hospital found that more than 93% of NAFLD patients were over 40 years old.[6] This can be explained through the mechanism of the pathogenesis of NAFLD; NAFLD is a chronic process where liver cells will experience functional and structural decline as we age, especially in terms of metabolic ability and detoxification. In addition, age at 40 years is also a risk factor for other metabolic diseases such as obesity and diabetes mellitus.[7]

The process of NAFLD is closely related to the presence of metabolic syndrome, in which about 90% of patients with NAFLD meet obesity. One study found that the average body mass index of NAFLD patients ranged from 23.7 - 31.1 kg / m2 (Zahran et al, 2013). Whereas Das et al found that the BMI range of NAFLD patients was 23.1 - 27.6 kg / m2.[5]

In line with the results of the two studies, this study found that the average body mass index of the study subjects was 29.2 ± 2.48 kg / m2, which was classified as obese. Satapathy et al reported that obesity is closely related to NAFLD where the prevalence of NAFLD in obesity is 80%. Crespo et al. found that obese patients with Non-Alcoholic Steatohepatitis (NASH) significantly increased more liver expression of TNF-α and P55 receptors, and increased more expression of TNF-α in adipose tissue compared with those who were not obese. This increase in expression is related to the degree of liver fibrosis. The accumulation of FFA in liver cells stimulates the expression of NF-κB-dependent inflammatory cytokines (TNF-α, IL-6, IL-1β). Kupffer cells as specific liver macrophages are also a potential source for proinflammatory cytokines.

Serum IL-8 levels and their relationship with NAFLD

There were statistically significant ($p < 0.01$) differences in serum IL-8 levels between the case group (NAFLD patients) and the control group (healthy subjects). It was seen that the mean serum IL-8 level of NAFLD patients was 45.3 ± 17.66 pg / ml which is 2.5 times higher compared to the control group (17.6 ± 5.03 pg / ml). Another study by Torer et al and Jarrar et al found that mean IL-8 levels in the NAFLD group and the control group had statistically significant differences.[8,9]

However, Abiru et al found slightly different results where serum IL-8 levels in the control group was 13 pg / ml and was not significantly different from the NAFLD group, which was 13.5 pg / ml.[10]

Acute injury to the liver tissue will result in the release of acute phase proteins by the liver, including IL-6 and alpha TNF. These two cytokines are potent stimuli for the synthesis of IL-8 by inflammatory cells. There is evident from various studies that state that the higher the levels of TNF alpha and IL-6, the higher the level of IL-8. IL-8 will occupy its receptors namely CXCR1 and CXCR 2 which are mainly found in neutrophils, and play an important role as chemoattractants in attracting polymorphonuclear cells (especially neutrophils) to injured and inflamed tissues. Neutrophils that have infiltrated liver tissue then release matrix metalloproteinase, elastase, cathepsin-G, and myeloperoksidase and Reactive Oxygen Species (ROS) causing even more damage to liver tissue. As a result, the increased levels of IL-8 that in plasma will increase again and lead to more neutrophil withdrawal to liver tissue.[11]

Several studies have found that serum IL-8 levels correlate with the fibrosis in the liver and are closely related to the Child-Pugh score. Patients with alcoholic liver disease had the highest IL-8 levels compared to non-alcoholic liver disease (NAFLD), while normal liver tissue had the lowest IL-8 levels.

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Conclusion

It can be concluded that there is a significant relationship between serum levels of Interleukin-8 (IL-8) and the incidence of Non Alcoholic Fatty Liver Disease (NAFLD).

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