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# Correlation between leptin level with lipid profile and free fatty acid in liver cirrhosis patients

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## ABSTRACT

Malnutrition is a common condition in liver cirrhotic patients. Leptin regulates body weight physiologically by suppressing appetite and increasing energy expenditure. Leptin is higher in female than male. Studies have shown correlation between leptin with metabolic factors like body mass index (BMI) and lipid profile in cirrhotic patients. This study was conducted to investigate the correlation between serum leptin levels with lipid profile and free fatty acid in male patients with liver cirrhosis. This was a cross sectional study that conducted at Gastroentero-Hepatology Clinic and Internal Ward at Dr. Sardjito General Hospital, Yogyakarta. The inclusion criteria were patients with liver cirrhosis $\geq$ 18 years old, male, with Child-Pugh classification B and C, and provided informed consent. The exclusion criteria were liver cirrhotic patients with comorbidity chronic kidney disease, chronic heart failure, diabetic, cancer, infection/septic, pregnancy, breast feeding, and steroid use. Data collecting was performed by anamnesis, physical examination, abdominal ultrasonography examination, and blood chemistry test. Pearson test was used to evaluate the correlation between the serum leptin level with the lipid profile and free fatty acid. The results showed that no significant negative correlation was observed between the serum leptin level with the total cholesterol ($r = -0.052$; $p = 0.766$), high-density lipoprotein/HDL ($r = -0.078$; $p = 0.658$) and triglyceride ($r = -0.170$; $p = 0.328$) in male patients with liver cirrhosis. Furthermore, no significant positive correlation was observed between the serum leptin levels with the low-density lipoprotein/LDL ($r = -0.013$; $p = 0.942$) and free fatty acid/FFA ($r = 0.007$; $p = 0.968$). In conclusion, there was no correlation between serum leptin levels with lipid profile and FFA in male patients with liver cirrhosis.

## ABSTRAK

Malnutrisi merupakan keadaan yang sering terjadi pada penderita sirosis hati. Leptin secara fisiologi mengatur berat badan dengan cara menekan nafsu makan dan meningkatkan pengeluaran energi. Kadar leptin lebih tinggi pada wanita daripada laki-laki. Penelitian menunjukkan adanya hubungan antara kadar leptin dengan beberapa faktor metabolisme seperti indeks massa tubuh (IMT) dan profil lipid. Penelitian ini bertujuan untuk mengkaji hubungan antara kadar leptin serum dengan profil lipid dan asam lemak bebas pada laki-laki penderita sirosis hati. Penelitian ini merupakan studi cohort yang dilakukan di Poliklinik Gastroentero-Hepatologi dan Bangsal Ilmu Penyakit Dalam RSUP Dr. Sardjito, Yogyakarta. Kriteria inklusi meliputi laki-laki penderita sirosis hati berumur $\geq$ 18 tahun, dengan klasifikasi Child-Pugh B dan C, serta bersedia ikut penelitian dengan menandatangani lembar persetujuan penelitian. Kriteria eksklusi adalah penderita sirosis hati dengan gagal ginjal kronik, gagal jantung kronik, diabetes mellitus, kanker, infeksi/sepsis, hamil, menyusui, dan menggunakan steroid. Seleksi subjek dilakukan melalui anamnesis, pemeriksaan fisik, pemeriksaan ultrasonografi abdomen, dan pemeriksaan kimia darah. Analisis adanya hubungan antara kadar leptin serum dengan profil lipid dan asam lemak bebas menggunakan uji Pearson. Hasil penelitian menunjukkan tidak ada hubungan negatif yang bermakna antara kadar leptin serum dengan kadar kolesterol

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total (r= -0,052; p=0,766), HDL (r= -0,078; p=0,658) dan trigliserida (r= -0,170; p=0,328) pada laki-laki penderita sirosis hati. Selain itu juga terbukti tidak ada hubungan positif yang bermakna antara kadar leptin serum dengan LDL (r=0,013; p=0,942) dan asam lemak bebas (r=0,007; p=0,968). Dari penelitian ini dapat disimpulkan bahwa tidak ada hubungan antara kadar leptin serum dengan profil lipid dan asam lemak bebas pada laki-laki dengan sirosis hati.

***Keywords***: cirrhosis - leptin level - lipid profile - free fatty acid – liver disease

## INTRODUCTION

Chronic liver disease is a major health problem in the world. Chronic liver disease such as cirrhosis and hepatocellular carcinoma contributes to an estimated 1.5 million deaths. Patients with chronic liver disease showed some symptoms such as weakness, anorexia, malnutrition and weight loss.[1] Malnutrition in liver cirrhosis is influenced by several factors, namely the existence of a metabolic disorder of carbohydrate, protein, and fat, as well as increased energy expenditure, impaired absorption and digestion, and food intake are not adequate.[2]

Mechanism of malnutrition in liver cirrhosis is not fully understood. Decreased food intake and increasing energy expenditure is estimated to play a role in negative energy balance in liver cirrhotic patient.[3] Several studies have shown that there were increased levels of circulating serum leptin in alcoholic cirrhosis. It provides evidence that leptin is involved in malnutrition in cirrhosis.[4]

Leptin is a family of cytokines, mainly secreted by adipocytes and is known to decrease food intake. Leptin also has an important role in the regulation of body weight in mamalia.[1] Leptin level is higher in female than in male. This occurs because of differences in fat distribution in female who have more subcutaneous fat and the effects of estrogen and progesterone induction on leptin secretion.[5]

Some researchers find that leptin level increases in cirrhosis patients.[6] Cirrhosis is often associated with hypermetabolism, and serum leptin levels are found to increase significantly. Leptin also plays a role in the pathogenesis of symptoms such as body weakness.[7] Moreover, levels of cholesterol and triglycerides decreased in cirrhosis. This is possibly related to hepatic synthesis defects and malnutrition known and reported as a complication in liver cirrhosis patients. Decreasing cholesterol and triglyceride levels in cirrhosis indicate a poor prognosis.[8]

One of the causes of liver cirrhosis is chronic hepatitis C. Liu *et al*.[9] reported that serum leptin levels increase in patients with chronic hepatitis C affecting liver metabolic functions. Furthermore, a close relationship between serum leptin levels and fat metabolism and between hepatic necroinflammation and lipid and fat metabolism in the patients. This relationship may be not only observed in patients with liver cirrhosis caused by hepatitis C virus infection, but also observed in other liver cirrhosis patients. Petrides *et al*.[10] reported that liver cirrhosis is characterized by several abnormalities in the circulation of substances and concentrations of hormones such as free fatty acid (FFA), glucagon, growth hormone, norepineprin, and insulin. There was an increased plasma FFA levels in patients with liver cirrhosis after absorption despite hyperinsulinemia. The increased lipid oxidation observed by some researchers may reflect changes in nutritional intake and catabolic processes.

This study was conducted to investigate the correlation between serum leptin levels with lipid

profile and free fatty acid in liver cirrhotic patients.

## MATERIALS AND METHODS

### Patients

This was an observational study using cross sectional design to evaluate the correlation between serum leptin levels with lipid profile and free fatty acids in patients with liver cirrhosis. Thirty five patietns with liver cirrhosis in the outpatient of Gastro-Hepatology Clinic admitted to the Internal Medicine ward of Dr. Sardjito General Hospital, Yogyakarta who met inclusion and exclusion criteria were studied. The inclusion criteria were liver cirrhosis Child B & C based on clinical criteria, laboratory and ultrasonography, age of e" 18 years, male, has a complete medical record and agreed to join this study by signing an informed consent. The exclusion criteria were cirrhosis patients coincident with renal failure, sepsis, hepatocellular carcinoma, chronic heart failure, diabetes mellitus and using steroid. This study was approved by the Health Research Ethics Committee of Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta.

### Blood sample analysis

Fifteen mL of blood samples were obtained from all patients after overnight fasting. Samples were then centrifuged and serum were collected and stored at -20 $^{o}$C until analyzed. Serum leptin levels were assayed using a commercial ELISA kits (Human Leptin Quantikine ELISA Kit). Serum total cholesterol, HDL cholesterol, LDL cholesterol and triglyceride as well as FFA were determined on an automatic analyzer (Hitachi 7170A, Hitachi Koki Co., Ltd).

### Statistical analysis

Data characteristics of subjects were presented as mean ± standard deviations (SD). Normality distribution of variables was determined by the Shapiro-Wilk test. Correlation between serum leptin levels with lipid profiles and FFA were analyzed by Spearman if the data distribution was not normal, and by Pearson analysis when data distribution was normal. Statistical analysis was considered significant when p value was < 0.05 with 95% confidence interval.

## RESULTS

A total of 42 male patients with liver cirrhosis were recruited by sequential sampling from October 2009 to October 2010. However, only 35 of these patients met the inclusion and exclusion criteria. The characteristic of patients is presented in TABLE 1.

TABLE 1. Characteristics of patients with liver cirrhosis (mean ± SD or frequency)

| Variables | Mean ± SD or Frequency |
|---|---|
| Age (years) | 51.09±12.25 |
| Child-pugh (N/%) | |
| • Child B | 16 (45.7) |
| • Child C | 19 (54.3) |
| Leptin (ng/mL) | 12.03±1.25 |
| FFA (µmol.kg.min) | 0.73±0.26 |
| Cholesterol (mg/dL) | 134.90±44.58 |
| HDL (mg/dL) | 31.27±15.05 |
| LDL (mg/dL) | 85.97±34.89 |
| Triglyceride (mg/dL) | 88.28±41.11 |
| Etiology (N/%) | |
| • Hepatitis B | 20 (57.1) |
| • Hepatitis C | 3 (8.7) |
| • Alcoholic | 6 (17.1) |
| • Others | 6 (17.1) |

FFA: free fatty acid, HDL: high-density lipopreotein, DL: low-density lipoprotein

No significant negative correlation was observed between the serum leptin levels with the total cholesterol, HDL and triglyceride (FIGURE 1) in male patients with liver cirrhosis. Furthermore, no significant positive correlation was observed between the serum leptin levels with the LDL and FFA (FIGURE 1 and 2).

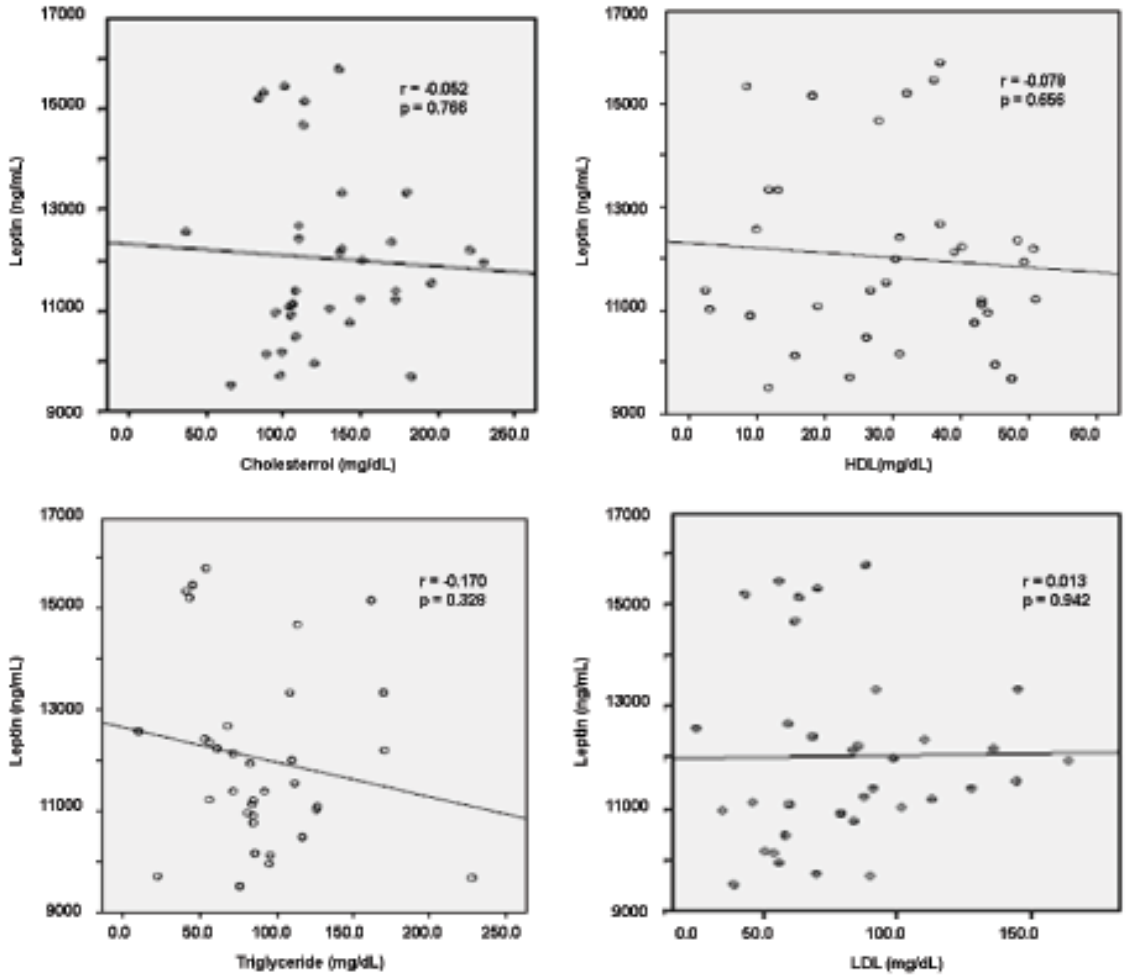


FIGURE 1. Correlation between serum leptin levels and lipid profiles in male patients with liver cirrhosis

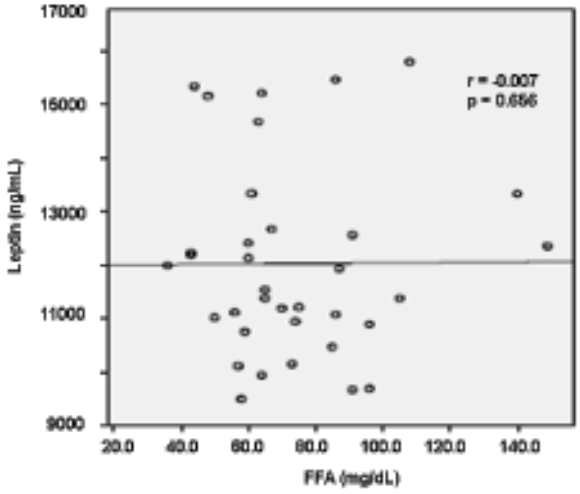


FIGURE 2. Correlation between leptin levels and lipid profiles in male patients with liver cirrhosis

## DISCUSSION

Studies concerning the incidence of liver cirrhosis were reported by some authors. Henriksen *et al.*[6] reported that liver cirrhosis was observed in 62.5% male patients. This result was suported by study conducted by Ockenga *et al.*[11] which found 65% in male patients and by Jian *et al*.[12] which found in 63.7% in male patients. A study in the United Kingdom showed that the incidence of liver cirrhosis in men were 50% higher than in female (relative risk 1.52%, 95% CI 1.42-1.63).[13] It might due to the higher alcohol cinsumption in male than in female.[13] Prevalence of liver cirrhosis in Indonesia has not been reported, yet. However, the incidence of liver cirrhosis in some academic hospital has been reported.[14]

Mean age of patients with liver cirrhosis in this study was 51.09 ± 12.25 years old. The mean age of patients with cirrhosis of the study by Henriksen *et al*.[6] was 53 ± 3 years, Orlando *et al.*[14] was 55 ± 10 years, and Ockenga *et al.*[11] was 48 ± 9 years. Study by Kusumobroto[15] had age range of 13-88 years old with liver cirrhosis largest group between 40-50 years. The most common causes of liver cirrhosis in this study were hepatitis B (57.1%), followed by alcoholic hepatitis (17.1%), hepatitis C (8.7%), and others (non B non C) (17.1%). This is consistent with the study conducted by Gadhir *et al*.[16] which showed that cirrhosis was mostly caused by hepatitis B virus (53%) and hepatitis C virus (8%).

The prevalence of hepatitis B in Indonesia in 1997 was 9.7% for male and 9.3% for female with the highest frequency in the age group 45-49 years of 11.9%, while hepatitis C was 0.8% with the highest frequency in the age 55-59 years of 2.12%.[17] This study showed the frequency of patients with Child C was higher than those of with Child B categories (54.3% vs 45.7%). Meanwhile, Henriksen *et al*,[6] found that the frequencies of patients with Child A, Child B, and Child C categories were 18.75%, 37.5% and 43.75%, respectively. Ockenga *et al.*[11] found that the frequency of patients with Child A, Child B, and Child C categories were 12.5%, 40%, and 47.5%, respectively. Child-Pugh classification was used to assess the degree of liver disease severity based on the degree of ascites, albumin, bilirubin, prothrombin time, and the degree of encephalopathy. The category was related with 1 and 2 years survival as follows: Child A of 85-100%, child B of 60-80%, and child C of 35-45%.[18] Early stage of cirrhosis is often asymptomatic so frequently discovered accidentally during patient's routine medical examination or often found coincidentally in the course of work-up for another illness.[19]

The mean serum leptin levels in this study was 12.03 ± 1.92 ng/mL. The results are consistent with the results of Henriksen *et al*.[6] Which found that serum leptin levels of cirrhotic group was 7.3 ± 1.6 ng/mL and of control group was 2.6 ± 0.6 ng/mL, whereas Bolukbas *et al*.[4] showed mean serum leptin levels of cirrhotic gorup was 13.5 ng/mL and control group was 6.4 ng/mL. The normal range of serum leptin levels in healthy subjects is 3-5 ng/mL.[20] Based on these results, it is known that serum leptin levels in patients with liver cirrhosis was higher than normal subjects. The significant increase in serum leptin levels in patients with liver cirrhosis can be caused by liver stellate cells which changed in liver cirrhosis that lead to increase leptin expression. Moreover, the increase in cytokines levels such as interleuki-6 (IL-6) and tumor necrosis factor α (TNF-α) can induce the production of leptin by adipocytes cells and decrease the leptin renal excretion.[6,21,22]

The mean levels of lipid profiles in this study were 134.90 ± 44.58 mg/dL for cholesterol, 31.27 ± 15.05 mg/dL for HDL, 85.97 ± 34.89 mg/dL for LDL, and 88.28 ± 41.11 mg/dL for triglyceride. The results are consistent with the results of the study by Ghadir

*et al*.[16] which found that level of lipid profile in cirrhosis patients was lower than controls (cholesterol: 138.9 vs 184.6 mg/dL; HDL: 40.7 vs 44.5 mg/dL; LDL: 80.5 vs 137.2 mg/dL; triglyceride: 82.2 vs 187.8 mg/dL).

The mean level of FFA in this study was 0.73 ± 0.26 µmol.kg$^{-1}$.min$^{-1}$.This results is consistent with the results of study conducted by Shangraw and Jahoor[23] which found an increased FFA in cirrhosis patients compared to controls (1.45 ± 0.18 vs 0.85± 0.17 ìmol.kg$^{-1}$.min$^{-1}$). Furthermore, the study conducted by Riggio *et al*.[24] also found a higher FFA in patients with liver cirrhosis compared to controls (746.6 ± 46.29 vs 359.22 ± 40.82 µmol/L).

Shangraw and Jahoor[23] reported that the increase in FFA in cirrhosis patients is not only caused by acceleration of lipolysis, but also due to impairment of FFA into triglycerides in adipocytes. However, the underlying mechanisms of the impairment of reesterification of FFA in adipocytes of cirrhosis patients have not understood, yet. The increase in plasma FFA levels come from lipolysis of triacylglycerol in adipose tissue or as result of lipoprotein lipase activity during tha plasma triacylglycerols uptake from adipose tissue. It is found in combination with albumin, with varying concentrations between 0.1 to 2.0 ìeq/mL in plasma.[25]

This study showed no significant negative correlation between the serum leptin levels with the lipid profile and FFA in male patients with liver cirrhosis. Several studies concerning the correlation between serum leptin levels with anthropometric and metabolic parameters in patients with cirrhosis have been reported with different results. Liu *et al*.[9] reported strong positive correlation between serum leptin levels and body fat in patients with chronic hepatitis C. Futhermore, Myers *et al*.[26] showed weak positive correlation between serum leptin levels and triglycerides in patients with hepatitis C.

Wongjitrat *et al*.[27] reported no correlation between serum leptin levels and degree of hepatic fibrosis i.e. fibrosis, necroinflammation and steatosis in patients with chronic hepatitis C. Whereas Gadhir *et al*.[16] showed correlation between degree of liver damage and total cholesterol, HDL, and LDL in patients with cirrhosis, however, there was no correlation with triglycerides. Watanabe *et al*.[28] reported strong positive correlation between leptin with BMI and total body fat. Furthermore, these studies conclude that increased serum leptin levels are useful as a biomarker to predict recurrence of hepatocellular carcinoma (HCC) in patients with high risk of the male sex, cirrhosis, high AFP, large tumor size, Hepatitis B and C infection, and alcohol consumption.

Our results showed different results from other studies. It might be caused by several limitations in our study. First, our study was cross-sectional design. Second, the limitation number of the cirrhotic subjects caused by hepatitis C. Several studies have shown the involvement of hepatitis C on lipid metabolism. In this study, we only have 3 cirrhosis patients with hepatitis C.

## CONCLUSION

In conclusion, there is no significant correlation between leptin level and total cholesterol, HDL, triglyceride, LDL and FFA in male patients with lever cirrhosis in Yogyakarta, Indonesia. In addition, further multicenter study with a larger number of subject is necessary to clarify this results.

## ACKNOWLEDGEMENTS

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