C-Reactive Protein and Interleukin-6 Correlated with Resistin Level in Liver Cirrhosis

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ABSTRACT

Background: *The incidence of insulin resistance is very high in liver cirrhosis. Resistin is thought to be one of the factors contributing to the occurrence of insulin resistance. Liver Cirrhosis is a chronic inflammatory condition. C-reactive protein (CRP) and interleukin-6 (IL-6) are inflammatory mediators, which are associated with the severity of liver damage. Resistin also increases in liver cirrhosis. This study aimed to determine whether CRP and IL-6 levels were correlated with resistin levels in patients with liver cirrhosis.*

Method: *An analytic cross-sectional study was conducted, in cirrhosis patients, to determine CRP and IL-6 levels as markers of the inflammatory process and blood resistin levels.*

Results: *In this study was found, CRP and IL-6 levels were associated with the severity of liver cirrhosis. Resistin levels are associated with the severity of cirrhosis of the liver. CRP and IL-6 levels are positively correlated with resistin levels and as independent predictors of resistin levels.*

Conclusion: *This study concluded that CRP and IL-26 levels are correlated with resistin levels and as independent predictors of resistin levels in liver cirrhosis patients.*

Keywords: *Resistin, C-reactive protein (CRP), interleukin-6 (IL-6), liver cirrhosis*

ABSTRAK

Latar belakang: *kejadian resistensi insulin sangat tinggi pada sirosis hati. Resistin diduga sebagai salah satu faktor yang berperanan terhadap terjadinya resistensi insulin. Sirosis hati merupakan kondisi inflamasi kronis. C-reactive protein (CRP) dan interleukin-6 (IL-6) merupakan mediator inflamasi, yang berhubungan dengan beratnya derajat kerusakan hati. Resistin juga meningkat pada sirosis hati. Penelitian ini untuk mengetahui apakah kadar CRP dan IL-6 berhubungan dengan kadar resistin pada penderita sirosis hati.*

Metode: *Dilakukan penelitian potong lintang analitik, pada pasien sirosis, untuk mengetahui kadar CRP dan IL-6 sebagai petanda proses inflamasi dan kadar resistin darah.*

Hasil: *Pada penelitian ini didapatkan, kadar CRP dan IL-6 berhubungan dengan beratnya penyakit sirosis hati. Kadar resistin berhubungan dengan beratnya penyakit sirosis hati. Kadar CRP dan IL-6 berkorelasi positif dengan kadar resistin dan merupakan prediktor independen dari kadar resistin.*

Simpulan: *Dari penelitian ini disimpulkan bahwa kadar CRP dan IL-6 berkorelasi positif dengan kadar resistin pada pasien sirosis hati.*

Kata kunci: *Resistin, c-reactive protein (CRP), interleukin-6 (IL-6), sirosis hati*

INTRODUCTION

High insulin resistance is found in liver cirrhosis (LC).[1] Almost all LC patients (96%) have insulin resistance, 60-80% out of this have glucose intolerance, and 20-30% develop diabetes mellitus (DM). One factor that is thought to have a role in insulin resistance is Resistin.[2,3] Resistin reduces insulin sensitivity in adipocytes, muscle cells and hepatocytes by suppressing insulin stimulation uptake glucose.[4] Resistin is known to increase in LC patients compared to healthy controls, and Resistin is thought to play a role in the occurrence of insulin resistance in LC.[5,6,7] Resistin is a 12.5-kd adipokine and belongs to a new family of small, cysteine-rich secretory proteins known as Found in Inflammatory Zone (FIZZ-3) or resistin-like molecules.[3] Resistin, also known as adipocyte secreted factor (ADSF), was discovered during a screen for genes which are downregulated in murine adipocytes by antidiabetic activators of peroxisome proliferator-activated receptor c (PPARc).[8]

There is a chronic inflammatory condition in liver cirrhosis as proved by an increase in various proinflammatory cytokines such as IL-6, tumour necrosis factor alpha (TNF alpha), CRP. This occurs in response to injury to the liver, both due to viral infections and non-viral causes such as drugs, alcohol, toxins, and metabolic. The interaction of cytokines and various types of cells in the liver will trigger fibrogenesis, which will worsen the course of the LC disease itself.[9] CRP and IL-6 levels correlate with the severity of liver damage.[10] The more severe the degree of liver damage based on Child-Turcotte-Pugh (CTP) criteria, the higher levels of CRP and IL-6. Research conducted on peripheral blood monocyte cells found a response to increased production of Resistin after induction by proinflammatory cytokines such as IL-6, TNF alpha.[8] In this study, we want to find out how is the correlation of the inflammatory process with levels of Resistin in patients with liver cirrhosis, whether this inflammatory process is related to blood resistin levels that will cause insulin resistance in patients with liver cirrhosis. These study aimed to find out the correlation between the inflammatory process and the increase in resistin levels in patients with liver cirrhosis.

METHOD

We performed an analytic cross-sectional study to determine whether CRP and IL-6 levels correlate with serum resistin levels in patients with liver cirrhosis. The research was carried out at Sanglah Hospital Denpasar by examining CRP, IL-6 and resistin levels at the Prodia Clinical Laboratory, Denpasar. The target population is all adult LC patients (18 years or more). The affordable population is all adult LC patients, both inpatient and outpatient polyclinic in Sanglah Hospital Denpasar. The sample selection is done by consecutive sampling, Inclusion Criteria: LC patients, regardless of their aetiology, aged 18 years, both men and women, willing to participate by signing informed consent. Exclusion Criteria: Patients with LC with stage III – V CKD, hypertension, coronary heart disease, hyperthyroidism, malignancy, DM, are taking anti-diabetic drugs, insulin, corticosteroids, and statins in the last two weeks.

The independent variables in this study were CRP and IL-6 levels, and the dependent variable was the serum resistin level. Serum resistin levels were examined using the ELISA (Enzyme-linked immunoabsorbent assay) method and the Quantikine® Human Resistin Immunoassay kit, expressed in units of ng/ml. IL-6 levels were examined by an enzyme immunoassay sandwich technique, using Quantikine HS immunoassay kits manufactured by R&D Systems Inc. 614 McKinley N.E. Minneapolis, MN 55413 USA. CRP levels were examined by immunometric techniques.

This study was approved by the Ethics Commission of the Medical Faculty of Udayana University. Subjects were given an explanation of the benefits, objectives, and risks regarding the study. If subjects voluntarily are willing to participate as a sample, they are asked to sign an informed consent. Data analysis was performed with the Statistics Program for Social Science v.23.0. (SPSS Inc., USA). The p-value < 0.05 with a 95% confidence interval is considered statistically significant.

RESULTS

In this study, 115 cirrhosis patients were examined; only 77 patients fulfilled the inclusion and exclusion criteria. Of these 77 patients, 51 (66.2%) were male, and the remaining 26 (33.8%) were female. The median age of patients participating in this study was 52.0 (55) years. To see the severity of the degree of liver disease, CTP criteria were used. Based on CTP criteria, 11 (14.3%) with CTP A, 32 (41.6%) CTP B, and 34 (44.1%) with CTP C. Complications of liver cirrhosis found in the sample were 27 (35, 1%) had upper gastrointestinal bleeding, ascites 42 (54.5%), Spontaneous Bacterial Peritonitis 4 (5.2%), Hepatorenal syndrome 1 (1.3%), hepatic encephalopathy 14 (18.2%). The complete patient characteristics data can be seen in Table 1.

Table 1. Patients characteristic

Variable	CTP A median (range)	CTP B median (range)	CTP C median (range)
Number, n (%)	11 (14,3)	32 (41,6)	34 (44,2)
Age (year)	53,0 (54)	55,0 (42)	50,0 (55)
Sex			
Male N (%)	6 (54,5)	21 (65,6)	24 (70,6)
Female N (%)	5 (45,5)	11 (34.4)	10 (29,4)
Ascites N (%)	2 (18,2)	15 (46,9)	25 (73,5)
SBP N (%)	0 (0)	1 (3,1)	3 (8,8)
HRS N (%)	0	0	1 (2,9)
UGI bleeding N (%)	6 (54,5)	14 (43,8)	7 (20,6)
SC (mg/dL)	0,78 (0,63)	0,87 (2,16)	1,14 (2,59)
Resistin (range)	13,2 (20,96)	12,6 (60,62)	28,41 (60,29)

CTP: Child-Turcotte-Pugh, SBP: spontaneous bacterial peritonitis, HRS: hepatorenal syndrome, UGI: Upper gastrointestinal, SC: serum creatinine

Table 2. Correlation of inflamation markers with CTP

Variable	CTP A Median (range)	CTP B Median (range)	CTP C Median (range)
WBC	4,8 (9,2)	6,26 (17,43)	8,21 (21,76)
CRP (mg/L)	0,8 (9,7)	7,95 (96,38)	17,35 (88,30)
IL-6 (pg/mL)	1,25 (9,9)	7,95 (288, 34)	17,30 (163,45)

CTP: Child-Turcotte-Pugh, IL-6: interleukin-6, CRP: C-reactive protein, WBC: white blood cell count

In this study examined several markers of inflammation, such as IL-6, CRP and WBC. The median value of IL-6 was 9,29 (288,9) pg/mL. The median value of CRP was 9,8 (96,38) mg/L. The median value of WBC was 7,49 (24,53). A comparison of the values of each marker of inflammation based on the severity of liver damage can be seen in Table 2.

To see the relationship between WBC, IL6 and CRP variables, the Spearman correlation test was performed. Spearman correlation test obtained a strong positive correlation between IL-6 and CRP ($r = 0.75$; $p < 0.001$), poor positive correlation between WBC and IL6, and moderate positive correlation between WBC with CRP ($r = 0.39$; $p < 0.001$ and $r = 0.53$; $p < 0.001$).

Inflammatory marker such as CRP and IL6 correlate with the severity of liver disease determined by the CTP score criteria. Spearman correlation test obtained a significant moderate positive correlation between CRP with CTP scores ($r = 0.46$; $p < 0.001$), and a significant moderate positive correlation between IL-6 with CTP scores ($r = 0.46$; $p < 0.001$).

Resistin levels are correlated to the severity of the degree of liver disease. From the Spearman test obtained a positive moderate correlation between resistin with CTP scores ($r = 0.51$; $p < 0.001$). These results indicate the more severe the degree of liver disease, the higher the resistin level.

To see the correlation between the inflammatory process and resistin levels, a Spearman correlation test was performed. Spearman test obtained both positive moderate correlation between IL-6 and CRP with Resistin. ($r = 0.51$; $p < 0.001$ and $r = 0.51$; $p < 0.001$).

DISCUSSION

The distribution of patients who participated in this study was not evenly distributed based on CTP criteria, where based on CTP criteria there were 11 (14.3%) with CTP A, 32 (41.6%) CTP B, and 34 (44.1%) with CTP C. The proportion of CTP A patients is the least because this study was conducted at a referral hospital. So patients who come for treatment are those with symptoms or complications. While CTP A patients are usually still in a compensating condition, so there are rarely complaints and have complications, so they did not come for treatment. In contrast to CTP B and C patients, who usually have complications, so they will be more easily obtained when collecting samples. In this study, the proportion of patients with CTP B and C did not differ significantly.

In this study, we found a significant positive correlation between IL-6 and CRP levels and the severity of liver cirrhosis determined by CTP score. The results of this study are in accordance with previous studies conducted by Mariadi, 2008.[10] Which found an association between inflammatory markers IL-6 and CRP with the severity of the degree of liver damage in liver cirrhosis patients with upper gastrointestinal bleeding. Where the heavier the degree of liver damage, the higher levels of CRP and IL-6. These results indicate the most severe inflammatory process was in LC patients with a CTP score of C. This is in accordance with the literature which mentions an increased risk for bacterial infection related to the severity of liver function disorders, the more severe the degree of cirrhosis the higher the risk of getting an infection. This risk arises because of abnormalities in

the body's defence mechanisms, which increase the risk for infection, such as lack of bactericidal and opsonizing activity, impaired monocyte function, suppression of the phagocytic activity of the RES system, lack of chemotaxis, and low serum complement.[11]

Increased concentrations of IL-6 in CTP C are followed by an increase in CRP concentrations. These results indicate that there is no interruption in CRP production in the liver, along with the severity of the degree of liver damage, which means that hepatocytes in cirrhosis patients still respond to IL-6 stimulation to produce CRP. The results of this study are consistent with the results of research from Bota et al who examined serum CRP and Procalcitonin levels in cirrhosis patients with a critical illness. In that study, no significant difference was found in CRP production in cirrhosis and non-cirrhosis patients.[12]

Previous studies have found high plasma resistin in cirrhosis patients compared with healthy controls, and resistin levels increase according to the severity of the degree of liver damage.[5,13] These are consistent with the results in this study, where there was a significant positive correlation between the levels of Resistin and the severity of the degree of liver damage, determined by CTP criteria ($r = 0.51$; $p < 0.001$).

High levels of Resistin in patients with liver cirrhosis are often associated with insulin resistance. However, it is unclear whether this high level of Resistin is a cause of insulin resistance or a result of impaired liver function. Resistin induced by proinflammatory cytokines may contribute to insulin resistance in inflammatory conditions.[3]

The basis of metabolism for increased Resistin in cirrhosis is not due to decreased liver clearance.[3] Because 10% of the total liver cell mass consists of kupffer cells, a significant increase in resistin levels may be due to an increase in the production of Resistin by these cells in response to inflammation. Other liver cells do not appear to be a source of Resistin, because hepatocytes and endothelial do not express Resistin.[14]

In this study we found a significant correlation between IL-6 and CRP with serum resistin levels. The results of this study are in accordance with previous studies which found an association between levels of Resistin with the severity of the liver inflammatory process in patients with Non-Alcoholic Steato Hepatitis.[15] And also other studies in patients with chronic liver disease and in liver cirrhosis.[4,7,13,16] High serum resistin was associated with intrahepatic inflammation in liver cirrhosis related to Hepatitis B virus.[7] Other studies have found that resistin mRNA is controlled by proinflammatory cytokines in peripheral blood monocyte cells, which suggests that peripheral blood monocyte cells may have a very important role to play in resistin-mediated insulin resistance. Increased levels of resistin mRNA by IL-1, IL-6 and TNF alpha support the hypothesis that Resistin plays a role in linking the sequence between inflammation and insulin resistance associated with obesity.[8]

There is a fundamental difference between tissue synthesizing resistin in mice and humans. Where in almost all Resistin mice produced in adipose tissue, whereas in humans, the role of adipocytes in resistin synthesis is very small.[17] In humans, monocytes or macrophages are the main synthesis sites of Resistin compared to adipocytes.[18] The expression of Resistin in these cells increases in response to the inflammatory stimulation of TNF alpha. This result reinforces the previous hypothesis, which states that resistin levels are influenced by the inflammatory process.[18]

CONCLUSION

Serum CRP and IL-6 level are positively correlated with resistin serum levels in liver cirrhosis. This result indicate that inflamation proccess correlated with resistin serum level in liver cirrhosis.

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