

INHIBITION OF BILE ACID ACCUMULATION DECREASED THE EXCESSIVE HEPATOCYTE APOPTOSIS AND IMPROVED THE LIVER SECRETION FUNCTIONS ON OBSTRUCTIVE JAUNDICE PATIENTS

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Abstract

Excessive hepatocyte apoptosis induced by bile acid accumulation occurred in severe obstructive jaundice, and impair the liver secretion function. The objective of this study is to determine whether the inhibition of bile acid accumulation through bile duct decompression affect the excessive hepatocyte apoptosis and caused improvement the liver secretion functions on human model. In this study we use a before and after study on severe obstructive jaundice patients due to extra hepatic bile duct tumor was decompressed. Bile duct decompression was performed as a model of the role of inhibition of bile acid accumulation inhibition bile acid accumulation and excessive hepatocyte apoptosis. Bile acid and marker of liver secretion functions were serially measured. Liver biopsy pre and post decompression was performed for Hepatocyte apoptosis pathologic examination by TUNEL fluorescing, which measured by 2 people in double blinded system. Total bile acid, and liver secretion functions were measured by automated chemistry analyzer. The result of this study shows that twenty one severe obstructive jaundice patients were included. After decompression the hepatocyte apoptosis index decreased from an average of 53.1 (SD 105) to 11.7 (SD 13.6) ($p < 0.05$). Average of bile acid serum decreased from 96.4 (SD 53.8) to 19.9 (SD 39.5) until 13.0 (SD 12.6) $\mu\text{mol/L}$ ($p < 0.05$) Total bilirubin decreased from 20.0 (SD 8.9) to 13.3 (SD 5.0) until 6.2 (SD 4.0) mg/dL ($p < 0.05$), while the phosphates alkaline (ALP) and γ -glutamyl transpeptidase (γ -GT) activities also decreased significantly. In conclusion, bile acids accumulation and excessive hepatocyte apoptosis through bile duct decompression improve the liver secretion functions by inhibition mechanism.

Keywords: bile acids, hepatocyte apoptosis, liver secretion

Introduction

Inhibition or induction apoptosis pathways has been shown to be a promising targeted therapy in many diseases including liver diseases.¹⁻⁴ Excessive hepatocyte apoptosis promoted by accumulation of bile acid will lead to hepatocellular damage, either fibrosis or atrophy with liver failure as its end point.⁵⁻⁹ Obstructive jaundice is a human model of excessive hepatocyte apoptosis induced by accumulation of bile acid via extrinsic apoptosis pathway.¹⁰⁻¹² Biliary duct decompression in obstructive jaundice animal study shows reduction of bile acid accumulation, hepatocyte apoptosis index, and stimulate hepatocytes regeneration.¹²⁻¹⁴

To date, no study has reported the role of inhibition of bile acid accumulation and excessive hepatocyte apoptosis and its subsequent effect on liver secretion functions in human. Obstructive jaundice model in animal study differs from that in human because in animal study obstructive jaundice occurs acutely in a short period.^{15,16} This study in human model should prove this differences.

Methods

The study was carried out at Department of Surgery, Cipto Mangunkusumo Hospital and Eijkman Institute of Molecular Biology, Jakarta. A before and after study in severe obstructive jaundice patients with bile duct

decompression as a model of bile acid accumulation and excessive hepatocyte apoptosis inhibition. The inclusion criteria for this study were adult obstructive jaundice due to extra hepatic bile duct tumor (Klatskin and Periapillary tumor), with total bilirubin level of more than 10 mg/dL, Karnofsky score of more than 70 and no major hematological problem. Patient was excluded if he/she had active hepatitis A, B or C, malignant liver tumor, severe liver dysfunction with massive ascetic, or already had bile duct decompression, and in late stage malignancy.

Bile duct decompression was performed using a standard open cholecystostomy technique under local or general anesthesia as required. Liver biopsy for hepatocyte apoptosis examination was performed during the bile duct decompression procedure, and the second at the time of definitive operation for the obstructive jaundice causes. Hepatocytes apoptosis was identified using TUNEL fluorescence system and the index was counted on 10 high power fields and was blindly evaluated by two independent investigators. Serum bile acid and bile fluid concentration, and markers of liver secretion functions were examined using *automated chemistry analyzer TRX 7010* and *automated chemistry analyzer Hitachi 911* respectively. All blood variables were serially measured (prior to, 7 days and 14 days, after decompression). Pre-decompression and post-decompression values were then compared and analyzed using student's t-test in SPSS v.16 with $p < 0.05$ is considered significant. This study followed the Helsinki convention and was approved by the Hospital Ethics Committee. This article is a part of a dissertation with the title Effect of the Inhibition of Bile Acid Accumulation and Excessive Hepatocyte Apoptosis Following Biliary Duct Decompression on Liver Functions: Study on Severe Obstructive Jaundice Patients, which being divided into several publications base on the hypothesis different.

Results and Discussion

The basic characteristic of the twenty one patients included in the study are shown in Table 1. Bile acid accumulation and liver functions impairment were identified in all patients (Table 2 and Table 3).

After decompression, excessive hepatocyte apoptosis index decreased significantly from 53.1 (SD105) to 11.7 (SD 13.6) ($p < 0.05$). Serial measurements also revealed significant reduction of serum bile acid from 96.4 (SD53.8) to 19.9 (SD 39.5) to 13.0 (SD 12.6) $\mu\text{mol/L}$ ($p < 0.05$), while bile fluid level increased from 987 (SD 182) to 1400 (SD 2076) to 2087 (SD 2476) $\mu\text{mol/L}$ ($p < 0.05$). The glycochenodeoxycholic (GCDC) level decreased in all patients from an average of 46.5 (SD 63.7) to 6.27 (SD 14.3) and 11.4 (SD 22.0) $\mu\text{mol/L}$ ($p < 0.05$).

Total bilirubin level decreased significantly from 20.0 (SD 8.9) to 13.3 (SD 5.0) to 6.2 (SD 4.0) mg/dL ($p < 0.05$), while phosphates alkaline (ALP) and γ -glutamyl transpeptidase (γ -GT) activities also decreased significantly from 908 (SD 873) to 453 (SD 460) to 298 (SD 198) IU/L ($p < 0.05$) and 431 (SD 421) to 222 (SD 153) to 177 (SD 96.6) IU/L ($p < 0.05$), respectively. Aspartate aminotransferase (AST) activity decreased significantly from 111.8 (SD 60.6) to 110.2 (SD 101.7) to 72.6 (SD 36.7) IU/L ($p < 0.05$) while alanine aminotransferase (ALT) increased in the first 8 days from 66.7 (SD 60.3) to 72.8 (SD 78.9) and later decreased to 54.3 (SD 28.7) after the 21st days. The bile duct decompression averages were 21.3 (14-35) days.

This study used a real model of severe obstructive jaundice patients who had bile duct decompression. It was a clinical study designed to determine the connection between biomolecular markers of the liver to its secretion functions which known as translational research.^{17,18} This study also prove a basic targeted therapy strategy in obstructive jaundice patients

Table 1. Basic Characteristics of Patients

Factor	Value
Sex, female/male	7/14
Age, years, Sd(range)	44±12,7 (25-71)
Jaundice duration, months (range)	1,4(1-4)
Decompression duration, days (range)	21,3(14-35)
Diagnosis	
Malignant	15
Benign	6
Definitive operation therapy	
Curative resections	13
Palliative operations	8

Table 2. Basic Laboratory Findings

Factor	Value
Hb (g/dL)	11.2
Ht (%)	32.7
WBC (1000/uL)	11.3
Platelets (1000/uL)	260
Ureum/Creatinin (mg/dL)	35.5/0.9
Blood glucose (mg/dL)	104

Table 3. Liver Secretion Function Test

Factor	Value
Serum bile Acid ($\mu\text{mol/L}$)	96.4
Serum Bilirubin (mg/dL)	20.3
Serum ALP (IU/L)	908
Serum γ -GT (IU/L)	431

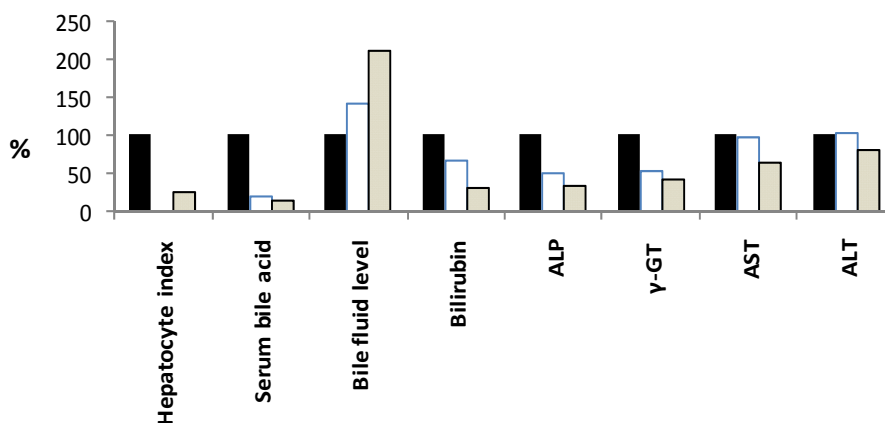


Figure 1. Comparison of the Improvement of Apoptosis Index, to Bile Acid Value, and Liver Secretion Function after Bile Duct Decompression in Percentage, ■ Predecompression, □ D+8, ▒ D+21

The hepatocyte apoptosis index magnitude decreased (Δ 74.2%) Serum bile acid magnitude decreased (Δ 79.4%) and (Δ 86.6%), bile acid in bile fluid increased to 141.8% (Δ 41.8%) and 211.4% (Δ 111.4%). Bilirubin decreased to 66.2% (Δ 33.8%) and 30.9% (Δ 69.1%), ALP to 49.9 (Δ 50.1%) and 32.9% (Δ 67.1%), γ -GT to 51.5 (Δ 48.5%) and 41.1% (Δ 58.9%), AST to 98.6 (Δ 1.4%) and 65.0% (Δ 35.0 %). ALT increased to 109.1% (Δ 9.1%) and later decreased to 81.4% (Δ 18.6%)

management by modulating bile acid and hepatocyte apoptosis as the promoter of liver dysfunction in human.^{4,19} To date, no clinical study had reported the role of bile acid accumulation and excessive hepatocyte apoptosis inhibition in liver secretion functions. Previously, Wang *et al.* and Koyama *et al.* had performed similar studies in animal model.^{13,19} This earlier studies had linked hepatocytes morphological changes (mitochondrial respiration) and hepatocytes destructor bile acid to liver secretion functions in severe obstructive jaundice.

Studies in animals usually use medication to inhibit bile acid accumulation and excessive hepatocyte apoptosis. In contrast, our study had used surgical decompression as a method of inhibition.^{1,4,8,20-23} In animal study, the duration of obstructive jaundice has an acute origin with less prominent apoptosis, compared to necrosis.^{13,24} The advantage of a human model, as was carried out in our study, is that the apoptosis and bile acid process in the model occurred chronically.

Bile duct decompression which lasted for 21.3 days (range of 14-35 days) in our study has shown to be effective to influence liver secretion functions. Effective duration of decompression has been reported for 10-32 days by Aronson *et al.*¹⁴, while Koyama *et al.*¹⁹ reported a duration of 4-6 weeks to achieve normal liver functions based on mitochondrial respiration. The purpose of bile duct decompression in obstructive patients is to optimize hepatocyte functions by release the bile acid accumulation which act as a excessive heaptocyte apoptosis inductor.

Bilirubin, alkaline phosphate (ALP) and gamma glutamil traspeptidase (γ -GT) are the classic liver secretion functions which were used to confirm obstructive origin in jaundice patients. All of these markers were increase in all patients in our study and were considered severe according to criteria discribed by Pitt.^{25,26} The increase of these markers support the fact that the liver secretion functions is impaired and confirmed the existence of cholestasis syndrome. The extra-hepatic cholestasis was confirmed by the increased of direct bilirubin level more than indirect bilirubin in all patients. The variance of bilirubin level was similar to what was introduced by Pitt et al in 1990 but much higher than the report in 2000 (average 14.9 vs. 5 mg/dL) even in the era of a widespread diagnostic support tool.²⁷ In obstructive jaundice due to extra hepatic bile duct malignancy, patient tolerance for radical operation should be optimized while tumor extension and metastasis should also come into consideration.^{27,28}

Bilirubin level fastly decreased in the first week after decompression, and then slowdown until it reached the normal value. The slowdown period is due to the presence of delta bilirubin which tightly binds to albumin and disappear following the albumin half time (21 days). Due to the excretion of direct bilirubin was also through the urine and sweat gland, so the decrease of its serum value cannot directly demonstrate the hepatocyte functions. The direct bilirubin value does not correlate to the grade and period of obstruction.^{29,30} It means that prognostic value of total bilirubin was not a true value as we know there was no destructive effect of

direct bilirubin. Granato et.al proved that bilirubin role as a bile acid inhibitor and lead lowering of hepatocyte apoptosis on animal study.³¹

Bile acid concentration decreased 59% on the second measurement and 87% on the third measurement. After bile duct decompression the total bilirubin, ALP and the γ -GT decreased as also shown in other report (Figure 1).²⁸ Not all patients reached normalization of ALP and γ -GT level because of a less decompression period and ongoing metabolic process of ALP and γ -GT activities at the canaliculi and hepatocyte. The halftime of γ -GT (26 days) which made the contribute to its activity and a much longer detectibility period should also taken into consideration, the bile duct decompression in this report has improve the severe impairment of liver secretion functions to mild grade, based on the criteria of Keeffe *et al.*³²

After decompression, the reduction of bile substrate accumulation had result in improvement of the hepatocyte apoptosis index 74.2% and was followed by the improvement of liver secretion functions capacity. The improvement was marked significantly by 42% increment of bile fluid level on the second and 111% on the third measurement. Improvement of liver secretion functions were marked by the total bilirubin level decrement of 34 to 69%, activity reduction of alkaline phosphates and γ -glutamyltranspeptidase of 50 to 67% and 48 to 59% respectively (Figure 1).

Decreasing pattern of liver secretion functions marker in this study was consistent with that found in animal study. Rapid reduction in the first week after decompression was due to reversible hepatocyte impairment, thus release of obstruction allowed hepatocytes to function properly. Slower reduction occurred because new hepatocytes are required for its proper function. These findings showed that liver functions improvement not only required decompression but was also influenced by the inhibition of bile acid accumulation.^{33,34} Reduction of AST and ALT activities explained that liver destruction due to apoptosis had been diminished after bile duct decompression.³⁵

Bile duct decompression in severe obstructive jaundice serves as a model of bile acid accumulation and excessive hepatocyte apoptosis inhibition as seen in the improvement of liver secretion functions. Better liver secretion functions after bile duct decompression were supported by the increase of bile acid value in bile fluid from an average 987 (SD 182) to 1400 (SD 2076) to 2087 (SD 2476) $\mu\text{mol/L}$ ($p < 0.05$). Serum bile acid also decreased significantly after decompression. This condition confirmed that lowering the bile acid as a hepatocyte apoptosis inductor will adequately increased the functional hepatocyte count to overcome the liver secretion functions impairment.

Bile duct decompression in severe obstructive jaundice inhibit the bile acid accumulation including the primary bile acid such Glycochenodeoxycholate (GCDC) accumulation. The reduction of GCDC led to diminution of apoptosis index as be known that GCDC was the inductor hepatocyte apoptosis via the extrinsic pathway (Figure 1). The lowering of bile acid was similar to the classic value of liver secretion functions improvement after bile duct decompression in this study and appropriate with other report.³³

Liver dysfunction is one of the surgical risk factors in severe obstructive jaundice that increase morbidity and mortality. This study found that liver dysfunction due to accumulation of bile acid and excessive hepatocyte apoptosis could be inhibited by decompression of bile duct. Therefore, inhibition of accumulation of bile acid and excessive hepatocyte apoptosis by bile duct decompression might lower risk, morbidity, and mortality of patients presenting with severe obstructive jaundice.

Conclusions

Inhibition of bile acids accumulation and excessive hepatocyte apoptosis through bile duct decompression improved the liver secretion functions. Recovery of liver secretion functions were adequately achieved in three weeks period of bile duct decompression, so it is accepted as a treatment option in obstructive jaundice patient.

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