

## CORRELATION BETWEEN INFLAMMATORY PARAMETERS AND MARKERS OF CHOLESTASIS IN PATIENTS WITH CHOLEDOCHOLITHIASIS

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The aim of this research was to examine the relationship between inflammatory parameters and markers of cholestasis in patients with choledocholithiasis. All subjects underwent clinical, laboratory and ultrasound examination at the Department of Internal Medicine, Military Hospital Niš, Serbia. Inflammatory parameters and biochemical markers of cholestasis were measured by standard biochemical methods. Most of cholestasis markers showed no significant correlation with inflammatory parameters in blood, except for a weak significant correlation between the number of monocytes with the activity of aspartate aminotransferase (AST) ( $r=0.37$ ,  $p<0.05$ ). Hyperbilirubinemia was significantly correlated with the fibrinogen values ( $r=0.42$ ,  $p<0.05$ ), while albumin positive values were positively associated with alanine aminotransferase (ALT) activity ( $r=0.5$ ,  $p<0.05$ ), and negatively with the alkaline phosphatase (AP) ( $r=-0.43$ ,  $p<0.05$ ). In patients with choledocholithiasis, there was a positive correlation between the number of monocytes and the activities of AST, hyperbilirubinemia, and fibrinogen, albumin and ALT, while a negative correlation between albumin and AP was present. *Acta Medica Medianae* 2014;53(2):28-32.

**Key words:** *cholestasis, inflammation, choledocholithiasis*

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extrahepatic cholestasis caused by choledocholithiasis (4-8). However, there are no published data which show the correlation between inflammatory and cholestasis markers in patients with choledocholithiasis.

Therefore, the aim of this study was to examine the correlation between inflammatory and cholestasis markers in patients with choledocholithiasis.

### Patients and methods

The study included 70 subjects divided into two groups: the choledocholithiasis group (CHDL) - 40 patients with obstructive jaundice caused by choledocholithiasis and the control group - 30 healthy individuals.

The patients with extrahepatic cholestasis due to mechanical obstruction caused by choledocholithiasis were included in the study. The obstruction of biliary ducts caused by other factors was not considered.

The diagnosis of obstructive icterus was made according to anamnestic data, clinical features, and biochemical and ultrasound examination of biliary ducts. For the ultrasound examination of biliary ducts in the supine position, Sono et Medison Co. Ltd ultrasound was used.

All the patients were anaesthetically and clinically observed at the Internal Department of

### Introduction

Choledocholithiasis is the most common cause of abdominal pain. The clinical manifestations of the disease may appear in the form of biliary colic, hepatitis, cholangitis, pancreatitis, and may be asymptomatic. Migration of gallstones from the gallbladder is the leading etiological factor for choledocholithiasis. Choledocholithiasis as a major cause of morbidity requires adequate diagnosis and removal of existing obstructions (1,2).

Cholestasis is the associated pathophysiological syndrome of choledocholithiasis. Cholestasis is a disorder of secretion and excretion of bile, which leads to accumulation of intrahepatic bile acid and other toxic compounds that lead to progressive liver injury (3).

Numerous experimental and clinical studies suggest the existence of inflammatory disorders of

Military Hospital in Niš, Serbia. Basic biochemical indicators were determined in Biochemical Laboratory of Military Hospital Niš.

All the patients with choledocholithiasis were tested in the first three days after the occurrence of cholestasis syndrome and before surgery or endoscopic retrograde cholangiopancreatography (ERCP) with papillotomy.

Participants of both groups did not differ in gender and age structure. Out of the total number of studied subjects, 37 (53%) were men and 33 (47%) women. The average age of the patients was  $58.8 \pm 15.9$  years.

#### Biochemical analysis

Inflammatory and cholestatic parameters: activity of  $\gamma$ -glutamyltransferase ( $\gamma$ -GT), alkaline phosphatase (AP), level of total, direct and indirect bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), sedimentation after the first hour (SE (I h)), albumin (Alb), fibrinogen (Fib), C-reactive protein (CRP), leukocytes (Leu), lymphocytes (Ly), monocytes (Mo) and granulocytes (Gr).

The previously mentioned biochemical parameters were determined by the ready tests produced by Ellitech Company, on the biochemical analyzer BTS-370 (Bio-system).

#### Statistical analysis

The data were analyzed by means of the commercially available statistic software package (SPSS® for Windows, v. 9.0, Chicago, USA) using the Student's t-test and Chi-square test. The results were presented as means  $\pm$ SD. Statistical significance was set to  $p < 0.05$ . To determine the

correlation of biochemical markers of cholestasis with the inflammatory parameters, the Pearson's correlation coefficient (r) was used.

### Results

The level of inflammation and cholestasis measured on the basis of biochemical parameters are shown in Table 1.

Statistically significant difference of albumin ( $p < 0.001$ ) and higher values of fibrinogen ( $p < 0.05$ ) and CRP ( $p < 0.001$ ) were in the group of patients with choledocholithiasis compared to the control group. Larger values for SE ( $p < 0.05$ ), leukocyte ( $p < 0.01$ ) and granulocytes ( $p < 0.001$ ), with a decrease in the number of lymphocytes ( $p < 0.001$ ), and monocytes ( $p < 0.001$ ) were found in the group of patients with choledocholithiasis compared to the control group. Activity of alkaline phosphatase,  $\gamma$ -GT enzyme, the level of the total, direct and indirect bilirubin, and the values of the enzymes AST and ALT were significantly increased ( $p < 0.001$ ) in the group of patients with choledocholithiasis compared to the control group.

Correlation between markers of inflammation and cholestasis in patients with choledocholithiasis are shown in Table 2.

Most of cholestasis markers showed no significant correlation with inflammatory parameters, except for a weak significant correlation between the number of monocytes with the activity of AST ( $r = 0.37$ ,  $p < 0.05$ ). Hyperbilirubinemia was significantly correlated with the fibrinogen values ( $r = 0.42$ ,  $p < 0.05$ ), while albumin values were positively correlated with ALT activity ( $r = 0.5$ ,  $p < 0.05$ ), and negatively with the AP ( $r = -0.43$ ,  $p < 0.05$ ).

Table 1. Laboratory parameters in the control group and group of patients with choledocholithiasis

| Parameter                | Control         | CHDL                 |
|--------------------------|-----------------|----------------------|
| SE (I h)                 | 8,0 $\pm$ 4,0   | 26,0 $\pm$ 8,0*      |
| Alb (g/l)                | 46,1 $\pm$ 4,3  | 36,7 $\pm$ 6,6***    |
| Fib (g/l)                | 3,5 $\pm$ 1,1   | 5,1 $\pm$ 1,2*       |
| CRP (mg/dl)              | 4,7 $\pm$ 1,3   | 11,2 $\pm$ 7,1***    |
| Leu (G/l)                | 6,1 $\pm$ 1,4   | 9,9 $\pm$ 6,3**      |
| Ly (%)                   | 28,8 $\pm$ 9,4  | 15,3 $\pm$ 8,2***    |
| Mo (%)                   | 8,5 $\pm$ 3,0   | 5,2 $\pm$ 3,9***     |
| Gr (%)                   | 62,2 $\pm$ 8,9  | 79,4 $\pm$ 10,6***   |
| AP (U/l)                 | 81,4 $\pm$ 37,7 | 385,0 $\pm$ 459,0*** |
| $\gamma$ -GT (U/l)       | 24,1 $\pm$ 6    | 364,0 $\pm$ 382,0*** |
| TB (mmol/l) <sup>1</sup> | 9,5 $\pm$ 2,8   | 123,2 $\pm$ 101,1*** |
| DB (mmol/l) <sup>1</sup> | 3,01 $\pm$ 1,09 | 55,1 $\pm$ 39,4***   |
| IB (mmol/l) <sup>1</sup> | 6,6 $\pm$ 2,4   | 73,6 $\pm$ 61,8***   |
| AST (U/l)                | 30,1 $\pm$ 10,7 | 136,0 $\pm$ 92,0***  |
| ALT (U/l)                | 29,3 $\pm$ 10,5 | 244,0 $\pm$ 207,0*** |

SE (I h) - sedimentation after the first hour; Alb - albumin; Fib - fibrinogen; CRP - C-reactive protein; Leu - leukocytes; Ly - lymphocytes; Mo - monocytes; Gr - granulocytes; AP - alkaline phosphatase;  $\gamma$ -GT -  $\gamma$ -glutamyltransferase; TB - total bilirubin; DB - direct bilirubin; IB - indirect bilirubin; AST - aspartate aminotransferase; ALT - alanine aminotransferase

\* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$  compared to control

Table 2. Correlation between markers of cholestasis and inflammation displayed by using Pearson's correlation coefficient (r)

| Parameter   | SE (I h) | Leu (G/l) | Ly (%) | Mo (%) | Gr (%) | Alb (g/l) | Fib (g/l) | CRP (mg/l) |
|-------------|----------|-----------|--------|--------|--------|-----------|-----------|------------|
| UB (μmol/l) | 0,16     | 0,28      | 0,08   | 0,28   | -0,16  | -0,09     | 0,42*     | -0,24      |
| DB (μmol/l) | 0,29     | 0,24      | 0,09   | 0,13   | -0,11  | -0,06     | 0,38      | -0,27      |
| IB (μmol/l) | 0,17     | 0,24      | 0,12   | 0,32   | -0,2   | -0,1      | 0,36      | -0,22      |
| AST (U/l)   | 0,01     | -0,19     | 0,04   | 0,37*  | -0,17  | 0,35      | -0,34     | 0,07       |
| ALT (U/l)   | 0,2      | -0,22     | -0,08  | 0,23   | -0,02  | 0,5*      | -0,24     | 0,13       |
| AP (U/l)    | 0,15     | -0,11     | 0,22   | 0,27   | -0,27  | -0,43*    | -0,29     | 0,06       |
| γ-GT (U/l)  | 0,07     | -0,03     | 0,08   | 0,08   | -0,09  | 0,27      | -0,22     | 0,06       |

SE (I h)-sedimentation after the first hour; Alb-albumin; Fib-fibrinogen; CRP – C-reactive protein; Leu – leukocytes; Ly – lymphocytes; Mo – monocytes; Gr – granulocytes; AP – alkaline phosphatase; γ-GT – γ-glutamyltransferase; TB – total bilirubin; DB-direct bilirubin; IB – indirect bilirubin; AST – aspartate aminotransferase; ALT – alanine aminotransferase  
\*p<0.05

## Discussion

In this study, markers of inflammation and cholestasis were significantly increased in the group of patients with choledocholithiasis compared to the control group, except for the value of albumin, which was statistically significantly lower in the group with choledocholithiasis. Inflammation plays an important role in occurrence of numerous complications of cholestasis and hepatocyte dysfunction (9,10).

Despite the known facts about the connection between inflammation and cholestasis in patients with choledocholithiasis, most cholestasis markers showed no significant correlation with inflammatory parameters in blood, in addition to the positive correlation of activities of AST and the number of monocytes, the activities of ALT and the values of albumin, total bilirubin values with fibrinogen values and negative correlation with AP values of albumin.

The results obtained in this study showed a statistically significant increase in the activities of ALT and AST in the blood plasma of patients with cholestasis, as compared to the control. In the *in vivo* study, Miyoshi et al. (11) demonstrated that micromolar concentrations of bile acids induce mitochondrial damage to hepatocytes with non-detergent mechanism, by increasing the oxidative stress, lipid peroxidation and initiating apoptosis, which may lead to the release of mitochondrial AST into the blood stream. Decrease in the number of monocytes and monocyte positive correlation with AST can be explained with majority of patients analyzed in the first three days after the cholestasis syndrome, whereas there was no significant increase in monocytes.

The obtained results indicate that during cholestasis, toxic bile acids, accumulated in the liver, damage hepatocellular membrane of hepatocytes and their detergent effects lead to the release of cytosolic ALT into the circulation (12). Albumins as negative reactants and inflammatory markers of synthetic liver function were significantly lower in

patients with extrahepatic cholestasis. The importance of lowering albumins in patients with extrahepatic cholestasis caused by choledocholithiasis lies in the fact that their values fall below 30g/l is a significant predictor of postoperative mortality and morbidity(13).

Based on our results, it can be assumed that in cholestasis damaged liver, synthesis of albumin and other proteins with a longer polypeptide chain is reduced. The explanation for this process is related to protein synthesis and energy metabolism crisis in hepatocytes. Specifically, reduced amounts of ATP in the mitochondria are present in cholestatic syndrome. The process of oxidative phosphorylation is altered due to the structural distortion of the mitochondrial membrane caused by attack of hydrophobic bile salts (14). In this study, the values of AP in patients with extrahepatic cholestasis caused by choledocholithiasis were significantly elevated compared to control subjects. The increase in serum levels of the AP is significantly higher in extrahepatic rather than intrahepatic forms with cholestasis. In all of the pathological processes that lead to disturbances in the flow of bile, salt bilisating effect of bile salts is prominent, which cause the release of AF on the outside of the cell membrane, without lysis of the cells. In addition, it has been previously shown that bile salts induce synthesis of new molecules of AP in cholestasis (15).

Earlier studies have shown that increased levels of conjugated bilirubin in the plasma favor the membrane damage and its solubilisation rather than simple diffusion process. Thus, in the early days of extrahepatic obstruction, increased activity of the enzyme UDP-glucuronyl transferase is present and later, due to hypertrophy and hypoactivity endo-plasmic reticulum in cholestasis due to the toxic effects of bile salts its activity is reduced (16).

In contrast to the conjugated non-conjugated bilirubin in the free state is highly toxic to cells. Therefore, the indirect bilirubin binds albumins from the blood plasma. If it is not bound to plasma

proteins it can pass in to the intracellular space, since it is dissolved in lipids of the cell membrane. Then, free unconjugated bilirubin impairs biological oxidation process, disrupting oxidative phosphorylation and inhibits protein synthesis. It has been shown that most of bile salt is bound to serum albumin, and about 22-34% of lipoproteins. Factors that may also lead to the occurrence of unbound unconjugated bilirubin in blood plasma are increased haemolysis of red blood cells, acidosis and hypoalbuminemia (17).

Fibrinogen as a positive reactant of inflammation and liver function marker was significantly elevated in cholestatic states as compared to the control group. A significant increase in the concentration of fibrinogen and inflammation in patients with obstructive jaundice is consistent with the findings of other authors who state that during cholestasis mean values of fibrinogen amount to  $4.7 \pm 0.9$  g/l and are significantly higher than in

healthy subjects. At the same time the increase of fibrinogen is followed by an increase of the viscosity of the blood which is directly related to the amount of fibrinogen but not the total and conjugated bilirubin (18).

The results of this study confirm that correlation of certain inflammatory parameters and markers of cholestasis are consistent with the findings of other authors on the significant common impact of inflammation and cholestasis in damage and dysfunction of the liver parenchyma (19,20).

## Conclusion

In patients with choledocholithiasis, there is a positive correlation between the number of monocytes and the activities of AST, hyper-bilirubinemia and fibrinogen, albumin and ALT, while the negative correlation was present between of albumin and AP.

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## KORELACIJA INFLAMATORNIH PARAMETARA I MARKERA HOLESTAZE KOD BOLESNIKA SA HOLEDOLITIJAZOM

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Cilj ovog istraživanja bio je ispitati korelaciju inflamatornih parametara i markera holestaze kod bolesnika sa holedoholitijazom. Svi ispitanici sagledani su klinički, laboratorijski i ultrazvučno na Internom odeljenju Vojne bolnice Niš u Srbiji. Inflamatorni parametri i biohemijski markeri holestaze ispitivani su standardnim biohemijskim metodama. Većina markera holestaze nije pokazivala značajniju povezanost sa inflamatornim pokazateljima u krvnoj slici, osim slabe značajne povezanosti broja monocita sa aktivnošću AST ( $r=0,37$ ,  $p<0,05$ ). Hiperbilirubinemija je značajno korelirala sa vrednostima fibrinogena ( $r= 0,42$ ,  $p<0,05$ ), dok su vrednosti albumina pozitivno povezane sa aktivnošću ALT ( $r= 0,5$ ,  $p<0,05$ ), a negativno sa AF ( $r=-0,43$ ,  $p<0,05$ ). Kod bolesnika sa holedoholitijazom postoji pozitivna povezanost broja monocita i aktivnosti AST-a, hiperbilirubinemije i fibrinogena, albumina i ALT-a, dok se negativna povezanost zapaža između albumina i AF. *Acta Medica Medianae 2014;53(2):28-32.*

**Ključne reči:** *holestaza, inflamacija, holedoholitijaza*