Original article

BIOCHEMICAL MARKERS IN PATIENTS WITH EXTRAHEPATIC CHOLESTASIS

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Cholestasis is characterized by regurgitation of bile and its constituents to liver and circulation. Obstruction of ductus hepaticus, choledochus or papilla Vateri are the causes of extrahepatic cholestasis and jaundice. The most important toxic constituents are hydrophobic bile salts and unconjugated bilirubin.

The aim of this work was to analyse biochemical markers such as activity of GGT and AF, bilirubin concentration, total proteins and albumin concentration in patients with different kinds of extrahepatic cholestasis.

Total number of 90 patients were examined and divided into four groups. Control group (20 healthy volunteers), I group (20 patients with intraluminal extrahepatic cholestasis -ILH), II group (20 patients with intramural obstruction-IMH), III group (30 patients with extraluminal extrahepatic cholestasis-ELH).

There are significant increases in GGT and AF activity in blood of cholestatic patients compared to control ($p<0,001$). In group III, GGT activity was increased compared to I group ($p<0,05$), while AF activity was reduced in I group compared to II ($p<0,05$) and III group ($p<0,01$). Total and conjugated bilirubin concentrations were higher in patients with cholestasis ($p<0,001$) compared to control. Concentration of conjugated and unconjugated bilirubin in blood of patients with cholestasis from II and III were significantly higher compared to I group. Albumin concentration was significantly lower compared to control. In patients with IMH and ELH, there was an inverse correlation of moderate intensity between conjugated bilirubin and albumin concentration.

In patients with extrahepatic cholestasis there is a significant increase in cholestatic markers (GGT and AF activity) and total bilirubin concentration. The most prominent changes are in patients with ELH. Meanwhile, cholestasis induces reduction of albumin concentration most prominently in patients with intramural and extraluminal cholestasis. Acta Medica Medianae 2007;47(1):5-12.

Key words: cholestasis, GGT, alkaline phosphatase, bilirubin, albumin

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Introduction

Cholestasis is a disturbance of liver function which occurs when the bile can not flow through the bile ducts into small intestine, as a consequence of which the bile constituents are retained in the liver and regurgitate into the blood. It can be intrahepatic or extrahepatic.

Extrahepatic cholestasis occurs as a consequence of mechanical obstacle to the flow of bile through ductus hepaticus, choledochus or papilla Vateri. Extrahepatic cholestasis is a clinical syndrome caused by obstruction of extrahepatic bile ducts and increase of the bilirubin level in plasma to the values of more than 30 μmol/l, which leads to manifest icterus or jaundice (1).

In obstructive cholestasis, there are adaptive mechanisms of resorption of bile salts from the obliterated lumen of bile duct. This has been confirmed by the studies of Trauner et al. (2), which provided clear molecular and functional proofs of increased expression of Na^+-dependent transporters of bile salts (Isbt) on the luminal membrane of cholangiocyte, which provide the transport mechanisms for removal of bile salts from obstructed bile duct through proliferated bile epithelium. Two transporters on basolateral membrane of cholangiocyte t-Asbt and MRP-3, provide additoinal mechanisms for removal of reapsorbed bile salts from cholangiocyte back to the system circulation. This is an additional channel for bile salts from cholestatic hepatocytes and cholangiocyte (2) (Figure 1). Isbt membranic proteins are also found in apical membrane of terminal ileum, where they participate in enterohepatic circulation of bile salts, and therefore is their activity in cholestasis diminished (3). These adaptive molecular expressions Isbt and

MRP-2, facilitate the urinal loss of bile salts in obstructive cholestasis (2), which is also confirmed in rats with underbound ductus choledochus, in whose urine the quantity of bile salts progressively grows (4). Trauner et al. came to the conclusion that during the obstructive liver disease the body reacts with adaptive and regulative responses, increased expression of transportive proteins of bile salts in kidney and cholangiocytes, which facilitate excretion of bile salts through alternative channels (2).

The available data suggest that hydrophobic bile acids have toxic role in the cholestatic liver diseases. Research of Schmucker et al. have shown that hydrophobic bile acids damage hepatocelular membrane due to their detergent-like influence (5). However, this research was done with the concentration of bile acids expressed in milimols, so that even hydrophile bile acids (which show no detergent-like properties) caused damage of hepatocytes. New studies have shown that micromolar concentrations of bile acids damage hepatocytes through non-detergent mechanisms. These mechanisms include oxidative stress and increased lipid peroxidation, toxic damage of mitochondria and apoptosis of hepatocytes, all these caused by the hydrophobic bile acids in the liver cells (6).

Apoptosis of hepatocytes in the condition of cholestasis can be induced by: mitochondrial disfunction, caused by bile acids (7). Toxic, hydrophobic bile acids cause the transfer of pro-apoptosis Bax protein from cytosol into mitochondria, which in turn leads to releasing of cytochrome C which activates caspase and causes apoptosis (8).

Patients with cholestatic liver disease mostly show extrahepatic manifestations of this disease. These include itching, tiredness and symptoms connected with metabolic disorder in bones (9).

In clinical presentation, depending on the cause of the extrahepatic cholestasis, the colic type pain occurs, but this pain can sometimes be blunt, localised in the upper right part of abdomen and in the area of epigastrium, accompanied by discomfort and pressure, or the pain can be belt-like. The pain is accompanied by sickness and vomiting. The character of pain can oscillate depending on the type of obstruction, as attacks and remissions, and there can also occur depression. Sometimes, pain does not accompany icterus as the consequence of extrahepatic cholestasis, for example in the case of malignant causes. If besides obstruction, presence of foreign body and bacteria there also appears cholangitis, then the patient develops Charcot´s trias pain, high body temperature (chill, shivering) and jaundice (10).

Biochemical disorders are numerous. They are mostly the consequence of the increased level of conjugated bilirubin. In the case of incomplete and segmented obstruction it can happen that hyperbilirubinaemia does not appear. In long partial obstruction because of the damage of hepatocytes, hyperbilirubinaemia is mixed, conjugated and unconjugated. In cholistasis, the activity of the membranic enzymes in plasma (γ-glutamyltransferase, alkaline phosphatase, 5′-nucleotidase) increases due to retention of bile acids which solubilitate the membrane, which leads to the passing of the abovementioned enzymes into circulation. Much more reliable indicator of biliary obstruction is the increased activity of AF, especially in incomplete and segmented obstruction where the bilirubin values remain normal. Alkaline phosphatase takes much longer to return to normal values, and sometimes remains permanently increased despite the clinically successful biliary reconstruction.

Longlasting obstruction leads to reduced synthesis of albumin, especially in the case of malignant obstruction. Hypoalbuminaemia points to the liver damage and its reduced capability of synthetising proteins. In the cases of heavily damaged liver hypoglycemia occurs as a result of reduced glycogenolysis. Damaged central nervous system (CNS) in the condition of cholestasis is a consequence of hiperbilirubinaemia and hypoxia. During the obstruction of bile ducts there can also be noticed the presence of surplus of free cholesterol in serum and formation of ksantoma. Enterohepatic circulation of bile salts can be completely interrupted or damaged depending on the degree of obstruction. Lack of bile salts in intestines results in proliferation of intestinal bacteria and increased resorption of endotoxins. Reduced quantity of bile salts in small intestine leads to disturbances in apsorption of lipids and liposoluble vitamins, and thus to steatorrea. Hypovitaminosis of vitamin K leads to disorder of blood coagulation due to the reduced synthesis of coagulation factors – protrombin and factors VII, IX and X. Longlasting cholestasis leads also to osteomalatia due to hypovitaminosis of vitamin D. Accumulation of bile salts in the skin causes itching. Also, in plasma, the activity of enzymes from hepatocytes (aspartate and alanin–aminotransferase) is increased because of the increased permeability of cell membranas or necrosis (7,8,11).

Aims

Aims of this study arise from the attempt to analyse the differences in metabolic occurences in the cases of liver damage in patients with intraluminal, intramural or extraluminal obstruction of extrahepatic bile ducts.

- Analysing the liver damages during extrahepatic cholestasis, through monitoring the activity of enzyme Gamma-glutamyltransferase (γ-GT) and alkaline phosphatase (AF) in blood plasma.
- Analysing the disorders of hepatobiliar function of liver through monitoring the variation of bilirubin concentration in blood plasma of the patients with different kinds of extrahepatic cholestasis.
- Estimation of synthetic function of cholestatic liver, based on the concentration of total protein and albumin in blood plasma.

Material and methods

This study comprised the total of 90 subjects, out of which 70 were patients with diagnosed extrahepatic cholestasis and 20 were healthy subjects. Anamnesis was taken and clinical examinations were performed, after which the

patients were treated at the Surgery Clinic. The research and clinical testing of patients were undertaken in the period between June 2006 and October 2007. Clinically-diagnostic methods for establishing the causes and estimating the degree of obstruction of bile ducts were performed at the Surgery Clinic. Biochemical parameters were determined in the Biochemical laboratory of the Clinical Centre Nis (Pediatric Department) and in the laboratory of the Institute for Biochemistry of the Medical University in Nis. All subjects were divided into four groups. The first was the control group, and the three clinical groups (I, II and III) were formed based on the type of of extrahepatic obstruction.

- **Control group** - 20 healthy subjects
- **I group** - 20 patients with *intraluminal* extrahepatic obstruction
- **II group** - 20 patients with *intramural* extrahepatic obstruction
- **III group** - 30 patients with *extraluminally* caused extrahepatic obstruction

Control group comprised 20 healthy subjects with age and sex distribution similar to the clinical groups. All of them were blood donors.

The first clinical group consisted of the patients with intraluminal obstruction caused by calculus (bilirubin, choesterin, mixed) which was formed during the primary or secundary choledocholythiasis.

The second clinical group consisted of the patients with intramural obstruction of lumen of extrahepatic bile ducts caused by tumourous infiltration (Klatskin tumour), benignant stenosis, inflamations-cholangitis.

The third clinical group consisted of the patients with extraluminal obstruction of extrahepatic bile channels caused by tumours of ampular region (distal part of ductus choledochus, papilla Vateri and head of pancreas), inflammation of gall-bladder (Mirizzi syndromes), pancreatitis and enlarged lymph glands.

All biochemical methods were determined with the tests produced by company Ellitech, on biochemical analyser BTS-370 (BioSystems).

Gamma-glutamyltransferase **(γ-GT)** was determined through 4-nitroaniline in alkaline environment. Activity of the enzymes in plasma was expressed in the units per litre of plasma (U/L).

Determining the activity of **alkaline phosphatase (AF)** in plasma was done with the colorimetric measuring of the level of freed p-nitrophenol, by application of this enzyme on p-nitrophenilphosphat at 405 nm. Activity of enzymes in plasma was expressed in units per litre of plasma (U/L).

Level of **bilirubin** in plasma was determined using the colorimetric method at 540 nm. Concentration of the total, conjugated and unconjugated bilirubin in plasma was expressed in μmol/L.

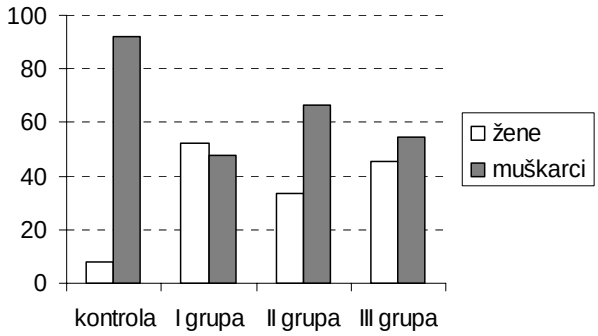
Quantity of **total proteins** in plasma was determined according to biuret-method. Concentration of total proteins in plasma was expressed in g/L.

Quantity of **albumin** in blood plasma was determined by reaction with BCG, bromcresol green. Concentration of albumin in blood plasma was expressed in g/L.

The data are analysed by using standard descriptive statistical methods (average value and standard deviation). The results were analysed using the appropriate tests depending on the size of the group, kind of characteristcs and type of distribution.

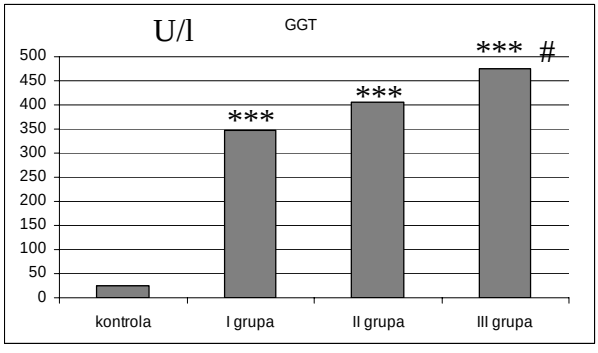
Results

The groups have equal distribution of male and female patients, and no statistically important difference was established regarding the presence of the two sexes in clinical groups. In the control group, however, the percentage of male participants was significantly higher than of female ones (Graph 1).



Graph 1. Number of female and male patients in the clinical groups

Activity of enzyme γ-GT in the plasma of patients with all three types of cholestasis is shown in Graph 2. The results show that there is a significant increase in the activity of this enzyme in the plasma of cholestatic patients, compared to the control group (I group 347,89±271,17 U/l, II group 405,25±296,76 U/l, III group 474,95±358,87 U/l compared to the control 24,84±5,85 U/l; p<0,001). It can be noticed that in the plasma of the patients in group III the activity of γ-GT increased compared to the group I of the patients (p<0,05).

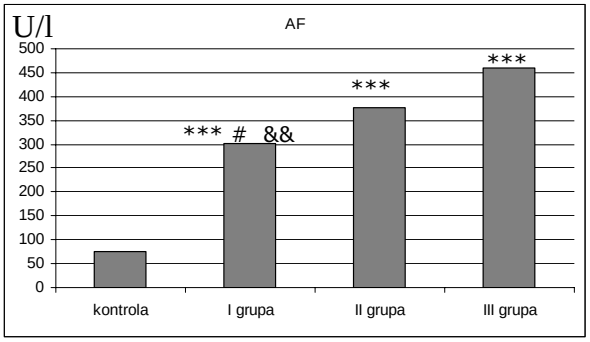


***p<0.001 (compared to control), #p<0.05 (compared to group I);

Graph 2. Activity of enzyme γ-GT in the plasma of patients with extrahepatic cholestasis

Graph 3 shows the activity of AF in the plasma of patients with cholestasis. The patients with cholestasis (in all three clinical groups) showed significant increase of the activity of this enzyme compared to control group (I group 301,5±141,46 U/l, II group 377,41±236,26 U/l,

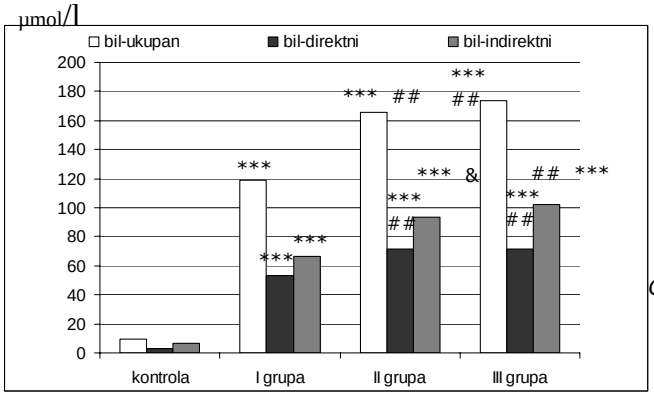
III group 458,79±371,81 U/l compared to control 74,76±15,76 U/l; p<0,001). It can be noticed that in the plasma of the patients in the group I the activity of AF was significantly lower than in group II (p<0,05) and group III (p<0,01) of the patients with extrahepatic cholestasis.



***p<0.001 (compared to control), #p<0.05 (compared to group II), &&p<0.01 (compared to group III)

Graph 3. Activity of enzyme AF in the plasma of patients with extrahepatic cholestasis

Levels of total, direct and indirect bilirubin in the blood plasma of patients with cholestasis in group I were statistically significantly increased (p<0,001) compared to the values of the control group (total - 119,27±75,90 μmol/l compared to 9,61±2,94 μmol/l, direct - 53,11±31,34 μmol/l compared to 2,92±1,03 μmol/l, indirect - 66,15±32,43 μmol/l compared to 6,69±1,43 μmol/l) (Graph 4). The same trend in changes (significant increase) was also noted in the plasma in groups II and III with cholestasis, compared to control (total - 165,35±84,84 μmol/l II group, 173,5±119,17 μmol/l III group, direct - 71,88±37,2 μmol/l II group, 71,36±48,46 μmol/l III group, indirect - 93,47±43,87 μmol/l II group, 102,14±57,98 μmol/l III group; p<0,001) (Graph 4). Concentration of total, direct and indirect bilirubin in blood plasma of the patients with cholestasis in groups II and III were statistically significantly increased compared to the same values of the patients with extrahepatic cholestasis from group I (Graph 4).

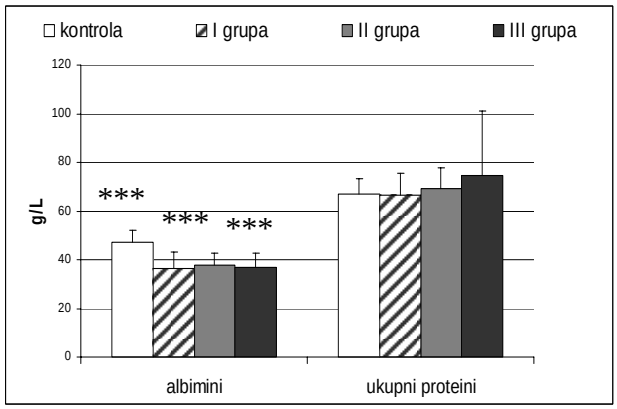


***p<0.001 (compared to control), ##p<0.01 (compared to group I); &p<0.05 (compared to group I)

Graph 4. Level of bilirubin in the plasma of patients with extrahepatic cholestasis

Level of albumin in blood plasma of patients with cholestasis (groups I, II and III) was statistically significantly reduced compared to control (I group -36,22±6,83 g/l, II group 37,63±5,15 g/l, III group - 37,04±5,59 g/l compared to control 47,39±4,65 g/l; p<0,01) (Graph 5).

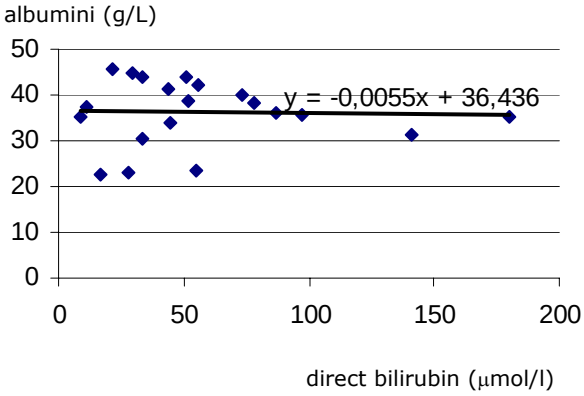
Results showing the quantity of total proteins in blood plasma of the patients indicate that there is no significant statistical difference between all four groups (Graph 5).



***p<0.001 (compared to control)

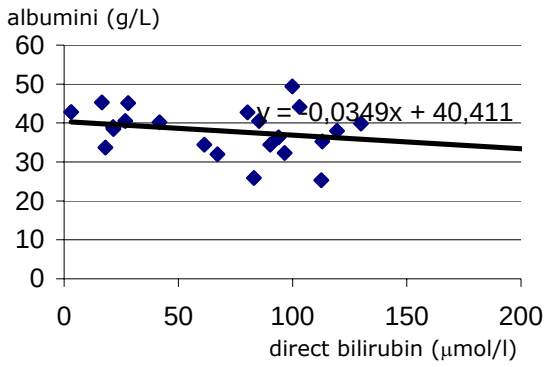
Graph 5. Level of total proteins and albumin in the blood plasma of patients with cholestasis

The group of patients with intraluminal obstruction showed mild inverse correlation between concentration of albumin and values of direct bilirubin C=-0,03, but this correlation was weak and in the linear regression model it did not show significant incline of regression line (Graph 6).



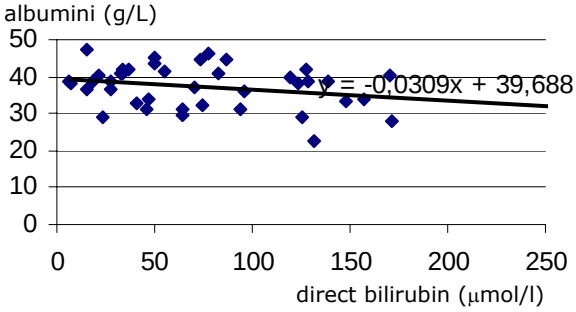
Graph 6. Correlation between concentration of albumin and values of direct bilirubin in patients with intraluminal cholestasis

The group of patients with intramural obstruction shows clear inverse correlation between the concentration of albumin and the values of direct bilirubin C=-0,38. This correlation shown in linear regression model shows significant incline of regression line (Graph 7).



Graph 7. Correlation between concentration of albumin and values of direct bilirubin in patients with intramural cholestasis

The group of patients with extraluminal obstruction shows clear inverse correlation between concentration of albumins and values of direct bilirubin C=-0,35. This correlation shown in linear regression model showed significant incline of the regression line (Graph 8).



Graph 8. Correlation between concentration of albumin and values of direct bilirubin in patients with extraluminal cholestasis

Discussion

In the conditions of cholestasis that lead to damage of cells, primarilly hydrophobic bile salts and unconjugated bilirubin have the most significant toxic effects. These toxic metabolites have dramatical influence on numerous cell functions: processes of transport, signal transudation, genetic expression, protein synhtesis (12). Longlasting, chronic cholestasis leads to irreversible destruction of liver tissue and development of secondary cirrhosis.

The most important and most reliable biochemical indicators of bile flow interruption are increased values of bilirubin, cholesterol and bile salts in blood plasma, as well as an increased activity of „cholestasis enzymes": γ-GT, AF and 5'- nucleotidase.

γ-GT is localised on plasma membranes, and only small part is in cytosol and microsomi of hepatocytes. Activity of this transpeptidase is the highest in epithelium cells where there is intensive active transport (especially in epithelium of bile ducts). From the clinical point of view, activity of γ-GT in serum is the most sensitive indicator of the damage of hepatobiliary system. Very high values of γ-GT follow extrahepatic as well as intrahepatic cholestasis of various etiology.

In this study, patients with extrahepatic cholestasis have shown significantly increased activity of γ-GT in plasma compared to control examinees (Graph 2). This significant increase of activity of γ-GT in plasma of the patients with extrahepatic cholestasis (in all three clinical groups) during this study, can be explained in various ways. First the hydrophobic bile acids, with their detergent-like impact on the cell membrane of hepatocytes, lead to releasing of this enzyme into circulation. In cholestasis, due to regurgitation of bile constituents into blood, activity of γ-GT in serum is increased (the activity of γ-GT in bile of a healthy person is about 10 times higher than in serum). Earlier studies have shown that the patients with cholestasis have increased *de novo* synthesis of γ-GT in hepatocytes. As cholestasis leads to necrosis of epiteluim cells of bile ducts (which have high contents of γ-GT), activity of this enzyme in circulation is increased. And, finally, due to proliferation of epithelium cells of biliary ducts, which have high contents of γ-GT, the activity of this enzyme in serum is significantly increased (13).

Results shown in Graph 2 show that the activity of γ-GT in plasma of patients in group III is increased compared to patients in group I. Most likely, the patients with extraluminally caused extrahepatic obstruction have suffered complete obstruction of bile duct, which lasted for a longer time, and consequently the bile constituents were returned to hepatocytes. Bile acids with their toxic influence on the membrane of hepatocytes, lead to releasing γ-GT into circulation (5,14,15).

Alkaline phosphatase is structurally a glicoproteid and is among the enzymes which are integral parts of cell membrane. Plasma membrane of epithelium cells contain the highest quantity of this enzyme. Liver and bile duct disorders are followed by increased activity of AF, which is especially characteristic of syndrome of cholestasis. Increase of the level of AF in serum is significantly higher in extrahepatic, than in intrahepatic forms of cholestasis. Obstructive icterus caused by malignant processes in the liver usually leads to more significant increase of serum AF than an obstruction caused by benign changes.

In this study, in the blood plasma of patients with extrahepatic cholestasis in all three clinical groups there was a significant increase of the activity of alkaline phosphatase compared to control group (Graph 3). These data are in accordance with the data from literature, which give evidence that in all pathological processes, which lead to obstructing of bile flow, there is a marked solubilisation effect of bile salts, which cause releasing of AF on the outer side of cell membrane without destruction of the cells. Besides, it has already been proved that in cholestasis the bile salts induce synthesis of new

molecules of AF (16). It has been proved that in extrahepatic cholestasis activity of AF can be even 10-12 times higher than normal (by complete obstruction), and in intrahepatic usually 2-3 times (16). Our study showed that the activity of AF in plasma of patients with extrahepatic cholestasis of various etiology was 4-7 times higher than in normal examinees.

The most intensive increase of AF was noticed in the patients from the III clinical group (almost sevenfold increase), then in the II group, and the smallest in the patients from the I clinical group with extrahepatic cholestasis (Graph 3). Enzyme picture sometimes helps to differentiate between the obstructive cholestsis caused by concrements and the one caused by malignant process (most frequently tumour of the head of pancreas), as in the latter case the activity of cholinesterase is usually decreased and that of the AF manyfold increased. If metastasis is present too, great increases of the activities of aldolase and lactate dehydrogenase are reported (LDH) (17) .

Due to the mechanical obstacle in bile ducts, the retention of bile colours into circulation appears. Then cholestasis dominates, and the direction of the flow of bile is changed so that the bile with its constituents (conjugated bilirubin, bile salts) starts flowing from biliar towards sinusoidal part of the hepatocyte. A part of the conjugated bilirubin moves into blood plasma, which leads to hiperbilirubinemia of conjugated type. It has been proved that bile salts increase filtration of conjugated bilirubin through kidney glomerul. This is the reason why in the cases of patients with biliar obstruction the concentration of conjugated bilirubin in blood plasma does not exceed 510-680 μmol/l (1).

Since excretion of bilirubin from hepatocytes is the limiting degree in its metabolism compared to conjugation, at the beginning of cholestasis when the damages are smaller the predominant hiperbilirubinaemia type is always conjugated. Later, as the patological process progresses and the condition deteriorates, the blood plasma shows also increased concentration of unconjugated bilirubin. The explanation is that the increased concentration of conjugated bilirubin in hepatocytes leads to alosterial inhibition of the enzyme UDP glucuronyl transferase (UDPGT), that inhibits the process of conjugation, which in turn results in increased concentration of unconjugated bilirubin in blood. Some studies have shown that greater damages of liver lead to shortening of the life of erithrocytes, when unconjugated hiperbilirubinaemia appears (12).

As can be seen from the Graph 4, the patients with extrahepatic cholestasis in all three clinical groups had significant increase of the values of total bilirubin in plasma compared to control group, and that from 12 to 18-fold. The increase manifests itself as higher concentration of conjugated as well as unconjugated bilirubin (Graph 4). It is considered that the increased value of direct bilirubin in plasma in cholestsis is the consequence of the increased concentration gradient between the cells and plasma or flowing out of bilirubin due to cell damages caused by obstruction in the bile flow. Earlier studies have shown that increased levels of conjugated bilirubin in plasma can be attributed more to the membrane damage, i.e. its solubilisation, than the simple diffusion process (12,18).

Unlike the conjugated bilirubin which is not toxic, unconjugated bilirubin in free condition is very toxic for the cells. Therefore, the indirect bilirubin binds the complex with albumins from blood plasma. The reason why unconjugated bilirubin can be found in free condition is that it acts as an anion in body liquids, and some other organic anions (e.g. bile acids, fatty acids, caffeine, some medicaments - tetracycline and salicylate), competes with bilirubin for connecting with albumins. Because of this competitive relation, in the presence of larger concentrations of these anions, larger quantity of free (not connected to albumins) bilirubin appears and its toxicity increases. It has been proved that the largest part of bile salts in serum is connected to albumins, and about 22-34% to lipoproteins.

Results achieved through research in this study have shown significant statistical increase on unconjugated bilirubin (Graph 4) and decrease of the level of albumin (Graph 5) in blood plasma of cholestatic patients compared to the same values of control examinees. From all these facts we can suppose and possibly conclude that: due to the reduced level of albumins and large increase of the quantity of total bile acids (which compete with bilirubin for connecting to albumins) in blood plasma, a part of unconjugated bilirubin can be found in free condition and have toxic effects.

Green et al. (1984) have shown that in cholestasis the bile acids are responsible for damaging permeability and the structure of hematoencephalic barrier (19). At the beginning, the transfer of matter is hindered, and then the integrity of this barrier is also damaged in such a way that at first the endothelian cells are destroyed and afterwards also the glia which envelops them. At the beginning, these changes are reversible, and in later phases they get irreversible character, depending on the concentration of bile salts. This effect is explained through strong detergent-like properties of bile salts and their ability to solubilisation cell membranes[20]. One of the toxic substances which can be responsible for brain damages under these conditions is also unconjugated bilirubin, which is increased in cholestsis. At the level of subcellular structures in brain, it has been shown that unconjugated bilirubin causes inhibition of respiratory chain, synthesis of DNK and proteins, it acts as modulator of synthesis, freeing and accepting neurotransmitters, as well as an inhibitor of phosphorylation of proteins.

Not all the areas of the brain are equally sensitive to damage caused by unconjugated hiperbilirubinaemia in cholestasis. It has been shown that neurones are much more vulnerable than astrocyte. This is explained by the fact that neurones show smaller capability of oxidation or active transport of unconjugated bilirubin (21). Data on the toxicity of unconjugated bilirubin are especially well documented in neonatal hiperbiliru-binaemia, particularly if it lasts longer than 5 or 7

days and if indirect bilirubin concentration was higher than 340 μmol/l.

Since recently it is believed that the main factor of the damage of CNS through impact of bilirubin, is via glutamat NMDA receptors. This is in accordance with clinical observations, where encephalopatia caused by bilirubin became worse because of hypoxia/ischemia which also has ekscytotoxic mechanism of neuronal damaging (22).

Results of this research show that the level of albumins in blood plasma of cholestatic patients was lower than that of the control examinees (Graph 5). Our results point out that the patients with extrahepatic cholestasis caused by intramural and extraluminal obstruction, have significants damage of synthetic function of liver resulting in decreased capability of liver to synthetise albumins, which is manifested in inverse correlation between the values of albumins and the increase of direct bilirubin (Graphs 7 and 8). Loss of this inverse correlation in the group with intraluminal obstruction is most probably caused by the fact that this type of obstruction is caused mostly by acute disorders which have not yet led to pronounced insufficientia of the synthetic function of hepatocytes and therefore the increase of direct bilirubin is not followed by reduced concentration of albumins (Graph 6).

On the basis of our results it can be supposed that in the liver damaged by cholestasis, the synthesis of albumins and other proteins with longer polypeptid chain is significantly reduced. Explanation of this process is related to synthesis of proteins and crisis of energy metabolism in hepatocytes. Namely, in cholestatic syndrome there is a reduced quantity of ATP in mitochondriae. The process of oxidative phosphorylation is changed due to damaging of the structural organisation of mitochondrial membrane by the attack of hydrophobic bile salts (in which case is the strongest toxic effect displayed by desoxyhol and henodesoxyhol acids) (23).

Conclusions

On the basis of the results shown in this study, the following conclusions can be drawn:

1. The patients with extrahepatic cholestasis show significant increase of activity of the cholestatic "enzyme-markers" (γ-GT and AF). The most intensive increase of these enzymes was found in patients with extraluminally caused obstruction, somewhat lower in the patients with intramural extrahepatic obstruction, and the lowest in patients with intraluminal extrahepatic obstruction of bile ducts.
2. Extrahepatic cholestasis causes significant increase of the level of bilirubin (total, direct and indirect) in the plasma of the patients. Concentrations of total, direct and indirect bilirubin in the plasma of patients with intramural and extraluminal cholestasis are significantly higher compared to the same values of the patients with intraluminal cholestasis.
3. Cholestasis leads to significant disturbances of the synthetic functon of the liver, which are manifested through reduced concentration of albumins in blood plasma. Disturbances of synthetical function are the most pronounced in patients with intramular and extraluminal cholestasis.

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PRAĆENJE BIOHEMIJSKIH PARAMETARA KOD BOLESNIKA SA EKSTAHEPATIČNOM HOLESTAZOM

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U holestazi, zbog nemogućnosti oticanja žuči iz jetre u duodenum, dolazi do posledičnog vraćanja konstituenata žuči u hepatocite i cirkulaciju. Ekstrahepatična holestaza je posledica mehaničke prepreke u oticanju žuči kroz *ductus hepaticus*, *choledochus* ili *papilu Vateri*, što dovodi do pojave manifestnog ikterusa. U događajima koji dovode do oštećenja ćelija, pre svih, hidrofobne žučne soli i nekonjugovani bilirubin imaju najznačajnije toksične efekte.

Ciljevi ovog rada bili su: praćenje biohemijskih parametara, aktivnost enzima *-GT i AF, koncentracija bilirubina, ukupnih proteina i albumina u krvnoj plazmi bolesnika sa različitim vrstama ekstrahepatične holestaze.

U ispitivanje je bilo uključeno 90 ispitanika podeljenih u četiri grupe. Prva je kontrolna grupa, dok su tri kliničke grupe oformljene na osnovu tipa ekstrahepatične opstrukcije: kontrolna (20 zdravih ispitanika), I grupa (20 bolesnika sa intraluminalnom ekstrahepatičnom opstrukcijom-ILH), II grupa (20 bolesnika sa intramuralnom opstrukcijom-IMH), III grupa (30 bolesnika sa ekstraluminalno izazvanom ekstrahepatičnom opstrukcijom-ELH).

Došlo je do značajnog porasta aktivnosti *-GT i AF u plazmi holestaznih bolesnika u odnosu na kontrolnu grupu ($p<0,001$). U plazmi holestaznih bolesnika u III grupi, aktivnost *-GT je povišena u odnosu na I grupu bolesnika ($p<0,05$), dok je u I grupi aktivnost AF snižena u odnosu na II ($p<0,05$) i III grupu ($p<0,01$) bolesnika sa ekstrahepatičnom holestazom. Nivoi ukupnog, direktnog i indirektnog bilirubina u plazmi bolesnika sa holestazom su povišeni ($p<0,001$) u odnosu na kontrolu. Koncentracija direktnog i indirektnog bilirubina u plazmi bolesnika sa holestazom iz II i III grupe su značajno povišeni u odnosu na iste vrednosti kod I grupe bolesnika sa holestazom. Kod bolesnika sa holestazom značajno je snižen nivo albumina u plazmi u odnosu na kontrolu. Kod bolesnika sa IMH i ELH, postoji negativna korelacija umerenog intenziteta između vrednosti direktnog bilirubina i albumina.

Kod bolesnika sa ekstrahepatičnom holestazom primećeno je značajno povišenje markera holestaze (*-GT i AF) i nivoa bilirubina u krvnoj plazmi. Najintenzivniji porast postoji kod bolesnika sa ELH. Holestaza dovodi do značajnih poremećaja sintetske funkcije jetre koji se manifestuju smanjenjem koncentracije albumina u plazmi i to najizrazitije kod bolesnika sa intramuralnom i ekstraluminalnom holestazom. Acta Medica Medianae 2008;47(1): *5-12.*

Ključne reči: *holestaza, *-GT, alkalna fosfataza, bilirubin, albumini*