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Correlation of inflammation parameters and biochemical markers of cholestasis with the intensity of lipid peroxidation in patients with choledocholithiasis

Povezanost inflamatornih parametara i biohemijskih markera holestaze sa intenzitetom lipidne peroksidacije kod bolesnika sa holedoholitijazom

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Abstract

Background/Aim. During choledocholitiasis inflammatory oxidant stress involves the promotion of mitochondrial dysfunction through an intracellular oxidant stress in hepatocytes leading mainly to necrosis and less to apoptosis. The product of oxidative stress, malondialdehyde (MDA), is extremely cytotoxic and damages cell membranes and intracellular macromolecules. The toxicity of MDA is based on its ability to act as a mutagenic agent in a cell. Therefore, the aim of this prospective study was to establish correlation of the parameters of inflammation and biochemical markers of cholestasis with the intensity of oxidative stress in pathogenesis of liver function disorders. **Methods.** Seventy adult subjects of either sex included in the study were devided into two groups: I – 40 patients with obstructive icterus caused by choledocholithiasis, and II – 30 healthy individuals. All the participants were subjected to a clinical, laboratory and ultrasonic check-up at the Internal Department of the Military Hospital in Niš. The parameters of oxidative stress: MDA, a measure of lipid peroxidation, and inflammation parameters: C-reactive protein (CRP), fibrinogen, albumins, number of leukocytes (Leu), granulocytes (Gr), lymphocytes (Ly) and monocytes (Mo) and biochemical markers of cholestasis: activity of γ-glutamyltransferase (γ-GT) and alkaline phosphatase (AP) enzymes, the level of total, direct and indirect bilirubin were determined by standard biochemical methods. **Results.** Lower values of albumin ($p < 0.001$), and significantly higher values of fibrinogen ($p < 0.05$) and CRP ($p < 0.001$) were found in the blood of the patients with cholestasis due to choledocholithiasis in relation to the controls. Significantly higher values of Leu ($p < 0.01$) and Gr ($p < 0.001$) with decreasing number of Ly ($p < 0.001$) and Mo ($p < 0.001$) were found in blood of the patients with cholestasis due to choledocholithiasis in relation to the control. Similarly, higher values of γ-GT, and AP ($p < 0.001$), as well as the level of total, direct and indirect bilirubin ($p < 0.001$) were found in blood of the patients with cholestasis due to choledocholithiasis in relation to the controls. The concentration of MDA ($p < 0.001$) was increased in the patients with choledocholithiasis in relation to the controls. There was a significant positive linear correlation of the number of leukocytes ($r = 0.51$, $p < 0.05$) and the concentration of total ($r = 0.87$, $p < 0.01$), direct ($r = 0.85$, $p < 0.01$) and indirect ($r = 0.88$, $p < 0.01$) bilirubin with the concentration of MDA in the group of patients with choledocholithiasis. **Conclusion**. Neutrophils and the levels of total, direct and indirect bilirubin have a significant positive linear correlation with the level of lipid peroxidation in patients with choledocholithiasis. Neutrophilia and hiperbilirubinemia observed in this way represent important parameters in estimating the level of liver tissue damage in choledocholithiasis.

Key words:
choledocholithiasis; oxidative stress; cholestasis; inflammation.

Apstrakt

Uvod/Cilj. U toku holedoholitijaze inflamatorni oksidativni stres uzrokuje mitohondrijalnu disfunkciju kroz intracelularni oksidativni stres u hepatocitima dovodeći uglavnom do nekroze, a ređe i do apoptoze. Produkt oksidativnog stresa, malondialdehid (MDA), izuzetno je citotoksičan i oštećuje ćelijske membrane i intraćelijske makromolekule.

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Cilj ovog istraživanja bio je utvrđivanje povezanosti parametara inflamacije i biohemijskih markera holestaze sa intenzitetom oksidativnog stresa u patogenezi poremećaja funkcije jetre. **Metode.** Ukupno 70 odraslih osoba oba pola uključenih u ispitavanje bilo je podeljeno u dve grupe: grupa I – 40 bolesnika sa opstruktivnom žuticom izazvanom holedoholitijazom i grupa II – 30 zdravih ispitanika. Svi učesnici ispitani su klinički, laboratorijski i ultrazvučno na Internom odeljenju Vojne bolnice u Nišu. Parametri oksidativnog stresa: MDA, mera lipidne peroksidacije, inflamatorni parametri: C-reaktivni protein (CRP), fibrinogen, albumini, broj leukocita (Leu), granulocita (Gr), limfocita (Ly) i monocita (Mo); i biohemijski markeri holestaze: aktivnosti enzima γ-glutamiltransferaze (γ-GT) i alkalne fosfataze (AP), nivo ukupnog, direktnog i indirektnog bilirubina, određivani su standardnim biohemijskim metodama. **Rezultati.** Niže vrednosti albumina ($p < 0,001$) i značajno veće vrednosti fibrinogena ($p < 0,05$) i CRP ($p < 0,001$) nađene su kod bolesnika sa holestazom izazvanom holedoholitijazom u odnosu na kontrolu. Značajno veća vrednost Leu ($p < 0,01$) i Gr ($p < 0,001$), uz opadanje broja Ly ($p < 0,001$) i Mo ($p < 0,001$), uočena je u grupi bolesnika sa holestazom izazvanom holedoholitijazom u odnosu na kontrolnu grupu. Povećane vrednosti enzima γ-glutamiltransferaze (γ-GT) i alkalne fosfataze (AP) ($p < 0,001$), kao i nivo ukupnog, direktnog i indirektnog bilirubina ($p < 0,001$) nađene su u krvi bolesnika sa holedoholitijazom u odnosu na kontrolu. Koncentracija MDA bila je povišena kod bolesnika sa holedoholitijazom u odnosu na kontrolnu grupu. Ustanovljena je značajna pozitivna linearna povezanost između broja leukocita ($r = 0,51$, $p < 0,05$) i koncentracije ukupnog ($r = 0,87$, $p < 0,01$), direktnog ($r = 0,85$, $p < 0,01$) i indirektnog ($r = 0,88$, $p < 0,01$) bilirubina sa koncentracijom MDA u grupi bolesnika sa holedoholitijazom. **Zaključak.** Neutrofili i nivo ukupnog, direktnog i indirektnog bilirubina pokazuju značajnu pozitivnu linearnu povezanost sa stepenom lipidne peroksidacije kod bolesnika sa holedoholitijazom. U skladu sa tim, neutrofilija i hiperbilirubinemija predstavljaju značajne parametre u proceni stepena oštećenja jetrinog tkiva kod holedoholitijaze.

Ključne reči:
holedoholitijaza; stres, oksidativni; holestaza; zapaljenje.

Introduction

The biochemical syndrome occurring in patients with choledocholithiasis is called cholestasis [1]. Hyperbilirubinaemia, which is the integral part of cholestasis syndrome, leads to liver function damage, dysfunction of gastrointestinal barrier, immunodeficiency, coagulation disorders and disorders in detoxification, accompanied by impeded wound healing [2].

Choledocholitiasis (CHDL) also initiates an inflammatory response [3]. The mechanism by which cholestasis initiates an inflammatory response in the liver, however, is not known. In the study of Allen et al. [4] two mechanisms of inflammation were examined. Firstly, activation of Toll-like receptor 4 (TLR4), either by bacterial lipopolysaccharide or by damage-associated molecular pattern molecules released from dead hepatocytes, triggers an inflammatory response. Secondly, bile acids act as inflammagens, and directly activate signaling pathways in hepatocytes that stimulate production of proinflammatory mediators. Koutelidakis et al. [5] have reported that the development of inflammatory response in the liver is a very complex process, involving a coaction of numerous pro- and anti-inflammatory mediators and immune cells.

Numerous experimental studies proved more intense oxidative stress and increased intensity of lipid peroxidation in the plasma and liver tissue in animals with experimentally induced cholestasis [6–7].

Studies have demonstrated that oxidative stress occurs in human livers with choledocholitiasis [3, 5, 8]. Jaeschke [8] has reported that bile duct obstruction is associated with hepatocellular injury, cholangiocyte proliferation, stellate cell activation, Kupffer cell activation, oxidative stress, inflammation and fibrosis.

Oxidative stress is a process of tissue injury due to the free radicals effect. They can damage almost all important biomolecules and cells in an organism [9]. Inflammatory oxidant stress insufficient to directly cause cell damage can induce transcription of stress defense genes including antioxidant genes [8].

The mechanism of reactive oxigen species (ROS)-induced cell killing during inflammation involves the promotion of mitochondrial dysfunction through an intracellular oxidant stress in hepatocytes leading mainly to necrosis and less to apoptosis [10]. The products of oxidative stress, such as malondialdehyde (MDA), have been found in in the blood of patients with cholestasis. These products are extremely cytotoxic and damage cell membranes and intracellular macromolecules [9]. MDA is an end product of lipid peroxidation and is a good indicator of oxidative stress. Consistent with previously reported findings from studies of obstructive jaundice in rodents [11,12], the toxicity of MDA is based on its ability to act as a mutagenic agent in a cell [13].

There is a lack of information about the correlation between the inflammatory parameters and markers of cholestasis with intensity of lipid peroxidation in the patients with choledocholitiasis.

Therefore, the aim of this study was to establish the correlation of parameters of inflammatory disorders and characteristic biochemical markers of cholestasis with the intensity of lipid peroxidation in pathogenesis of liver function disorders. Such knowledge in patients with choledocholithiasis may contribute to better understanding of the disease and possibly to its more rational treatment.

Methods

The study included 70 subjects divided into two groups: the group I – 40 patients with obstructive jaundice caused by choledocholithiasis and the group II (control) – 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 a Sono et Medison Co. Ltd ultrasound was used.

All the patients were anamnaestically and clinically observed at the Internal Department of Military Hospital in Niš. Basic biochemical indicators and parameters of oxidative stress were determined in Biochemical Laboratory of Military Hospital in Niš and the Laboratory of the Biochemistry Institute at the Faculty of Medicine in Niš.

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

Biochemical analysis

Inflammatory and cholestasis parameters: C-reactive protein (CRP), fibrinogen, albumins, sedimentation (SE) rate, number of leukocytes (Leu), granulocytes (Gr), lymphocytes (Ly), monocytes (Mo), activity of γ-glutamyltransferase (γ-GT) and alkaline phosphatase (AP) and the level of bilirubin were determined.

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

The intensity of lipid peroxidation in plasma was measured spectrophotometrically, and based on the thiobarbituric response products as described by Ohkawa et al. [14]. Malondialdehyde (MDA – lipid peroxidation end-product) concentration was expressed as μmol/L, using the MDA molecular absorbance coefficient (1.56×10^{-5} mol cm^{-1}).

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 χ^2 test. The results were presented as means ±/SD. Statistical significance was set to $p < 0.05$. To determine the correlation of the parameters of inflammatory disorders and characteristic biochemical markers of cholestasis with the intensity of lipid peroxidation the Pearson's correlation coefficient (*r*) was used.

Results

The demographic characteristics in the control group and the patients with choledocholitiasis are shown in Table 1.

Table 1

Demographic characteristics in the control group and the patients with choledocholitiasis (CHDL)

Parameter	Control	CHDL	Total
Age (years), $\bar{x} \pm SD$	55.5 ± 18.0	61.4 ± 14.1	58.8 ± 15.9
Gender, n (%)			
male	18 (60)	19 (47.5)	37 (53)
female	12 (40)	21 (52.5)	33 (47)

χ^2 test and Student's *t*-test did not reveal any significant differences in gender distribution and in the average age between the examined groups.

Participants of both groups did not differ in gender and age structure. Out of the total number of studied subjects, there were 40 patients with extrahepatic cholestasis caused by choledocholitiasis and 30 control (healthy) individuals; 37 (53%) were men and 33 (47%) women. The average age of the patients was 58.8 ± 15.9 years.

The clinical characteristics of the patients with choledocholitiasis are shown in Table 2.

Table 2

Clinical characteristics of the patients with choledocholitiasis

Clinical characteristics	Number of patients (%)
Icterus	21 (52.5)
Subicterus	19 (47.5)
Abdominal pain	40 (100)
Nausea and vomiting	23 (57)
Aholic stool	32 (80)

Abdominal pain was presented in all the patients with choledocholitiasis and 89% of the patients had aholic stool. All the patients with choledocholitiasis had icterus or subicterus while nausea and vomiting were registered in about half of the patients.

The level of inflammation and cholestasis, measured *via* biochemical indicators, and the intensity of lipid peroxidation measured in the form of MDA, are shown in Table 3.

In the patients with choledocholithiasis, statistically significantly lower values of albumins ($p < 0.001$), as well as significantly higher values of fibrinogen ($p < 0.05$) and CRP ($p < 0.001$) compared to the control group were found. Statistically much higher values of SE ($p < 0.05$), Leu ($p < 0.01$), and Gr ($p < 0.001$), with a decreasing number of Ly ($p < 0.001$) and Mo ($p < 0.001$) were found in the group of patients with choledocholithiasis compared to the control group.

The activity of alkaline phosphatase (AP) and γ-GT and the levels of total, direct and indirect bilirubin in the blood plasma of the patients with extrahepatic cholestasis caused by choledocholithiasis showed a significant increase ($p < 0.001$) compared to the control group.

Also, the values of MDA were significantly increased ($p < 0.001$) in the patients with extrahepatic cholestasis caused by choledocholithiasis, compared to the control group.

Table 3

The results of laboratory parameters in the control group and the patients with choledocholitiasis (CHDL)

Parameter	Control	CHDL
Albumin (g/L)	46.1 ± 4.3	36.7 ± 6.6***
Fibrinogen (g/L)	3.5 ± 1.1	5.1 ± 1.2*
CRP (mg/dL)	4.7 ± 1.3	11.2 ± 7.1***
Leu (G/L)	6.1 ± 1.4	9.9 ± 6.3**
Ly (%)	28.8 ± 9.4	15.3 ± 8.2***
Mo (%)	8.5 ± 3.0	5.2 ± 3.9***
Gr (%)	62.2 ± 8.9	79.4 ± 10.6***
AP (U/L)	81.4 ± 37.7	385.0 ± 459.0***
γ-GT (U/L)	24.1 ± 6	364.0 ± 382.0***
Bil – total (mmol/L)[1]	9.5 ± 2.8	123.2 ± 101.1***
Bil – direct (mmol/L)[1]	3.01 ± 1.09	55.1 ± 39.4***
Bil – indirect (mmol/L)[1]	6.6 ± 2.4	73.6 ± 61.8***
MDA (μmol/L)	21.6 ± 2.	51.2 ± 8.6***

CRP – C-reactive protein; Leu – leukocytes; Ly – lymphocytes; Mo – monocytes; Gr – granulocytes; AP – alkaline phosphatase; γ-GT – γ-glutamyl transferase; Bil-bilirubin; MDA – malondialdehyde.
***p* < 0.05; ***p* < 0.01; ****p* < 0.001 compared to the control.**

The correlation of biochemical parameters of inflammation and cholestasis with the intensity of lipid peroxidation in the patients with choledocholithiasis is shown in Table 4.

Table 4

Correlation between biochemical parameters of inflammation and cholestasis with the intensity of lipid peroxidation in the control group and the patients with choledocholitiasis (CHDL)

Parameter	Correlation with MDA (*r*) Control CHDL	CHDL (*r*)
Albumin (g/L)	-0.05	-0.11
Fibrinogen (g/L)	0.53*	-0.23
CRP	0.55*	-0.24
Leu (G/L)[3]	0.15	0.51*
Ly (%)[4]	0.34	0.02
Mo (%)[5]	-0.10	0.32
Gr (%)[6]	-0.33	-0.13
AP (U/l)	0.12	0.01
γ-GT (U/L)	0.15	-0.03
Bil – total (μmol/L)	-0.05	0.87**
Bil – direct (μmol/L)	0.05	0.85**
Bil – idirect (μmol/L)	0.15	0.88**

CRP – C-reactive protein; Leu – leukocytes; Ly – limphocytes; Mo – monocytes; Gr – granulocytes; AP – alkaline phosphatase; γ-GT – γ-glutamyl transferase; Bil-bilirubin; MDA – malonidialdehyde.
***r* – Pearson's correlation coefficient**
***p* < 0.05; ***p* < 0.01 compared to the control**

The concentration of fibrinogen ($r = 0.53$, $p < 0.05$) and CRP values ($r = 0.55$, $p < 0.05$) were in a direct positive linear correlation with the intensity of lipid peroxidation in healthy individuals, but the correlation was not present in the patients with choledocholithiasis There was a significant positive linear correlation between the number of leukocytes ($r = 0.51$, $p < 0.05$) and the concentration of MDA in the group of patients with choledocholithiasis, while the other inflammation parameters in this group did not show such correlation.

The concentration of total ($r = 0.87$, $p < 0.01$), direct ($r = 0.85$, $p < 0.01$) and indirect ($r = 0.88$, $p < 0.01$) bilirubin was in statistically significant positive linear correlation with the level of lipid peroxidation, while the other biochemical markers of cholestasis do not show such correlation.

Discussion

In this study the patients with choledocholitiasis were examined in the first three days since the occurrence of cholestasis syndrome. Abdominal pain, icterus or subicterus were presented in all the patients with choledocholitiasis. Nausea and vomiting were registered in about half of the patients while 89% of the patients had aholic stool.

Cholestasis syndrome includes liver function disorder due to the obstruction of bile drainage into the intestine, with the consequent retention of bile constituents in liver and their regurgitation in the blood [15, 16].

The intensity of oxidative stress, measured as MDA values, was significantly increased in patients with cholestasis caused by choledocholithiasis ($p < 0.001$). This is in accordance with numerous experimental and clinical studies [3, 5–8]. Obstructive jaundice points to intestinal oxidative stress, which can be the key factor in the loss of intestinal barrier and development of septic complications in these patients [13].

Correlation of the inflammation parameters and the intensity of lipid peroxidation

Albumins as negative inflammatory reactants and markers of impaired synthetic liver function were significantly lower in patients with extrahepatic cholestasis. A decrease in the concentration of albumin indicates liver disorder lasting longer than three weeks, but in rapidly progressive diseases, the decrease can occur even sooner [17].

In this study, the values of both fibrinogen (positive inflammatory reactant) and CRP were significantly higher in the patients with cholestasis. A significant increase in fibrinogen concentrations, as an inflammatory parameter in patients with obstructive icterus, is in accordance with the results of other authors [18]. Inflammatory cytokines inducing fibrinogen transcription in the liver, tissue factor in monocytes, endothelial and muscle cells, VIII factor in the liver, PAI-I in the liver and adipocytes, lead to procoagulation states. There was also a positive correlation between fibrinogen and C-reactive protein in healthy common population [19].

The results of this study show that choledocholithiasis leads to increased number of leukocytes, with the decrease in lymphocyte and monocyte percentage.

The increase in the number of granulocytes is in accordance with the results of other authors stating that the clinical problem in patients with obstructive icterus is mainly the consequence of disorders in neutrophil function [20]. The experimental study by Gujarol et al. [21] shows that activated neutrophils take part in the liver parenchymal damage during a short period of obstructive icterus manifestation (during 5 days from the obstruction of mutual biliary duct). Increased number of leukocytes, neutrophils, hemotaxic neutrophils, increased production of superoxide radicals and increased expression of adhesive molecules, damage endothelial cells of liver sinusoids and apoptosis, playing thus the key role in organic dysfunction [22, 23].

Monocytes start to accumulate in three days from the beginning of obstruction and their number increases synchronously with the increase of concentration of intercellular adhesion molecule (ICAM) – 1 during obstructive jaundice development. Simultaneously, their number returns to normal on the 14th day form the obstruction of ductus choledochus, which indicates the time trend of change in cholestatic organism [24]. These results are not in accordance with the changes in the number of monocytes registered in our study. One of the possible explanations is that all the patients were tested in the first three days since the occurrence of cholestasis syndrome during which there was no significant increase in the number of monocytes.

There is a positive linear correlation of fibrinogen concentration ($r = 0.53$, $p < 0.05$) and CRP values ($r = 0.55$, $p < 0.05$) with the intensity of lipid peroxidation in the control group of the patients. The correlation of inflammatory markers synthesized in the liver, such as albumins, fibrinogen and CRP, with the level of oxidative stress, has not been proven in the patients with extrahepatic cholestasis. This is in accordance with the results of other authors stating that dysfunction of hepatocytes and damaged synthetic liver function in the states of cholestasis make the significance of these markers quite uncertain. Therefore, extrahepatic cholestasis should involve analysis of other markers of inflammation, such as tumor necrosis factor alpha (TNF-a), interleukin (IL)-1, IL-6, number of leukocytes and neutrophils [5].

There was a significant positive linear correlation of the number of leukocytes ($r = 0.51$, $p < 0.05$) with the intensity of lipid peroxidation. Namely, this knowledge in patients with choledocholithiasis might not only contribute to better understanding of pathophysiology of the disease, but also to a possible improvement of its treatment. Accumulated neutrophils are adhered to vascular endothelium due to increased expression of adhesive molecule ICAM-1 and they penetrate into mucosa of involved organs and hepatic tissue, causing oxidative stress and tissue damage [25, 26]. These neutrophiles represent the source of large quantity of free radicals that damage the mentioned structures and increase the content of carbonyl groups [27, 28].

The correlation of the number of leukocytes with the intensity of lipid peroxidation is in accordance with the results of other authors indicating the correlation between the oxidative stress and inflammation *via* cytokine TNF-a, transforming growth factor beta (TGF-β), IL-1b and IL-6 [29–31] and transcription factor, NFkB [32]. NF-kB plays a central role in the transcription of cytokines, adhesion molecules, and other mediators involved in the inflammatory reaction and oxidative damage [33, 34].

The level of inflammation is directly proportional to the level of oxidative stress and capability of antioxidative mechanisms, primarily in hepatocytes, to counteract the increased production of free radicals *via* immune cells, such as neutrophils and monocytal macrophageal cells [23].

Correlation of the biochemical markers of cholestasis and the intensity of lipid peroxidation

The values of alkaline phosphatase (AP) in the patients with extrahepatic cholestasis caused by choledocholithiasis were almost 7 times higher than in the control group ($p < 0.001$). According to the results of other authors, the increase of AP is a reliable indicator of biliary obstruction, especially in incomplete and segment obstructions (obstructive icterus) where the values of bilirubin remain normal, while the values of AP are increased [35].

The increased activity of γ-GT in the plasma of patients with extrahepatic cholestasis found in this study, is in accordance with the results of other authors [36]. This is due to the necrosis of epithelial cells of biliary ducts (rich in γ -GT) and their proliferation leading thus to the significant increase of γ-GT activity in the serum [37].

In the patients with extrahepatic cholestasis, there was a significant increase in the values of total, conjugated and unconjugated bilirubin in the plasma, compared to the control patients ($p < 0.001$). The increased values of direct bilirubin in the plasma in cholestasis are the consequence of the increased concentration gradient between the cells and plasma or of wasting bilirubin due to the damage of the cells caused by the obstructive bile drainage. Unlike the conjugated one which is not toxic, the unconjugated bilirubin in free state is

very toxic to cells. Its effect is explained by the strong detergent effects of biliary salts and their ability to solubilize cell membranes [38, 39].

The concentration of total ($r = 0.87$, $p < 0.01$), direct ($r = 0.85$, $p < 0.01$) and indirect ($r = 0.88$, $p < 0.01$) bilirubin shows a significant positive linear correlation with the level of lipid peroxidation. Based on the above results, we support the idea that bilirubin can act *in vivo* as efficient scavenger of ROS and that bilirubin plays a key physiological role in cytoprotection against an oxidant-mediated damage [40].

The correlation between oxidative stress and disorders of production and secretion of bilirubin is in accordance with the results of other authors. Namely, they have shown that a reduced amount of glutathione and decreased activity of glutathione peroxidase in patients with cholestasis, decrease hepatobiliary transport of toxic organic components [41] leading to the development of complications accompaning cholestasis.

The correlation between hyperbilirubinaemia and oxidative stress is an expected result due to insolubilization of cytoplasmic membrane, dysfunction of mitochondrial membrane and freeing of reactive oxygen radicals [13]. Considering the results of other authors, indicating that the products of hems have a significant anti-inflammatory role and decrease mortality in experimental models, the correlation between hyperbilirubinaemia and oxidative stress can be considered as a form of protective effect. It has been found that the products of hems and bilirubin decrease adhesion of leukocytes for the vascular endothelium as a response to oxidative stress [42]. This is achieved by the inhibition of expression of adhesive molecule (VCAM-1) and the reduction of inflammation, increasing the risk of infection set-on [43]. Hyperbilirubinaemia viewed this way could represent a significant factor that leads to immune system disorder and the development of infection complications.

Conclusion

According to the results of this prospective study, it can be conclude that neutrophils and the level of total, direct and indirect bilirubin show a significant positive correlation with the level of lipid peroxidation, measured by MDA as its final product, while the other determined inflammatory parameters and biochemical markers of cholestasis do not show such correlation. Thus, neutrophilia and hiperbilirubinemia observed in this way, easily established with inexpensive and routine laboratory tests, represent an important parameter in estimating the level of liver tissue damage in choledocholithiasis.

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