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Diagnostic and pathogenetic significance of apolipoprotein disorders in patients with alcoholic fatty liver

Dijagnostički i patogenetski značaj apolipoproteinskih poremećaja kod bolesnika sa alkoholnom masnom jetrom

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Abstract

Background/Aim. Alcohol is the most common cause of fatty liver. Alcohol metabolism takes place in the liver by alcohol dehydrogenase, to toxic acetaldehyde, with fatty acids accumulation in the liver as a consequence. By daily intake of the amount greater than 80 g/day for men and 20 g for women, there is the risk for developing the alcoholic fatty liver (AFLD). The aim of this study was to determine the profile of atherogenic factors in plasma of patients with AFLD compared to patients with non-alcoholic fatty liver (NAFLD) and determine its diagnostic significance. **Methods.** The study included 74 patients with AFLD who consumed alcoholic beverages daily in large quantities and over 80 g [for men: 3–4 units (U) of alcohol and for women 2–3 U]; the control group consisted of 70 patients with NAFLD verified with ultrasound. A total holesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) and apoliporoteins (ApoA1 and ApoB) were determined and the ratios TC/HDL-C, ApoB/ApoA1 and LDL-C/HDL-C were calculated. **Results.** The study included two group: 74 AFLD patients (21% of women and 79% of men), mean age 42.65 ± 9.73 years, who consumed alcoholic beverages daily in the amounts of 80 g, or greter, during the average 2.31 ± 0.96 years and 70 patients with NAFLD (37.5% of women and 63.5% of men) with the average 41.3 ± 4.1 years. There was no significant difference in gender distribution and the average age between the examined groups. Higher values of TG – 9.94 ± 2.94 mmol/L, TC 14.53 ± 2.81 mmol/L, LDL-C 8.57 ± 2.15 mmol/L and ApoB 3.97 ± 0.28 g/L and lower values of HDL-C 0.43 ± 0.11 mmol/L, Apo A1 0.49 ± 0.09 g/L and ApoB/ApoA1 ratio 2.43 ± 1.27 were registered in the AFLD group compared to those registered in the NAFLD group, (TG 8.74 ± 2.54 mmol/L TC 9.87 ± 2.36, LDL-C 6.72 ± 1.98 mmol/L, Apo B 2.38 ± 0.16 g/L, HDL-C 0.78 ± 0.09 mmol/L, Apo A1 0.98 ± 0.04 g/L and ApoB/ApoA1 ratio 7.81±1.42). There were no differences in albumin concentration, international normalized ratio (INR) and values of haemoglobin and haematocrit between the groups. **Conclusion.** Lipids and the ApoB/ApoA1 ratio, besides markers of hepatocelular damage, can serve as a diagnostic criteria for the presence of AFLD, and as a better indicator of atherogenic risk.

Key words:
liver disease, alcoholic; non-alcoholic fatty liver disease; risk factors; apolipoproteins a; apolipoproteins b; lipids; diagnosis; alcohol drinking.

Apstrakt

Uvod/Cilj. Alkohol je najčešći uzrok masne jetre. Metabolizam alkohola odvija se u jetri posredstvom alkoholne dehidrogenaze do toksičnog acetaldehida sa posledičnom akumulacijom masnih kiselina u jetri. Svakodnevnim unosom količina većih od 80 g/dan za muškarce, odnosno 20 g za žene, postoji rizik od razvoja alkoholne masne jetre. Cilj rada bio je da se utvrdi profil aterogenih faktora u plazmi bolesnika sa alkoholnom masnom jetrom (AFLD) u odnosu na bolesnike sa nealkoholnom masnom jetrom (NAFLD) i odredi njegov dijagnostički značaj. **Metode.** Studijom je bilo obuhvaćeno 74 bolesnika sa AFLD koji su konzumirali razno alkoholno piće u dnevnoj količini 80 g i više [za muškarce 3–4 jedinice (U) alkhola dnevno, a za žene 2–3 U]; kontrolnu grupu činilo je 70 bolesnika sa ultrazvučno potvrđenom NAFLD. Praćeni su ukupni holesterol (TC), trigliceridi (TG), lipoproteini velike gustine (HDL-C), lipoproteini male gustine (LDL-C) i apolipoproteini (ApoA1 i ApoB) i izračunavan je odnos TC/HDL-C, ApoB/ApoA1 i LDL-C/HDL-C. **Rezultati.** Studijom su bile obuhvaćene: grupe: I – 74 bolesnika (21% žena i 79% muškaraca) sa AFLD, prosečne starosti 42,65 ± 9,73 godina, koji su svakodnevno konzumirali alkoholne napitke u količini većoj od 80 g, tokom prosečno 2,31 ± 0,96 godina, i grupa II – 70 bolesnika sa NAFLD

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(37% žena i 63% muškaraca) prosečne starosti 41,3 ± 4,1 godina. Nije registrovana značajnija razlika u starosti i polu između ispitivanih grupa. Registrovane su značajno više vrednosti TG 9,94 ± 2,94 mmol/L, TC 14,53 ± 2,81 mmol/L, LDL-C 8,57 ± 2,15 mmol/L i Apo B 3,97 ± 0.28 g/L a niže vrednosti HDL-C 0,43 ± 0,11 mmol/L, Apo A1 0,49 ± 0,09 g/L i odnosa ApoB/ApoA1 2,43 ± 1,27, u grupi AFLD u odnosu na grupu NAFLD, (TG 8,74 ± 2,54 mmol/L, TC 9,87 ± 2,36 mmol/L, LDL-C 6,72 ± 1,98 mmol/L, Apo B 2,38 ± 0,16 g/L, HDL-C 0,78 ± 0,09 mmol/L, Apo A1 0,98 ± 0.04 g/L i ApoB/ApoA1 7,81 ± 1,42). Nije nađena značajna razlika u koncentraciji albumina, vrednostima *international normalized ratio* (INR), hemoglobina i hemotokrita između grupa. **Zaključak.** Vrednosti lipidnih frakcija i odnosa ApoB/ApoA1 uz markere hepatocelularnog oštećenja, mogu poslužiti kao dijagnostički kriterijum prisustva AFLD i biti dobar pokazatelj aterogenog rizika.

Ključne reči:
jetra, bolesti izazvane alkoholom; jetra, masna, infiltracija, nealkoholna; faktori rizika; apolipoproteini a; apolipoproteini b; lipidi; dijagnoza; alkohol, pijenje.

Introduction

Alcohol is the most common cause of liver disease in 40–80% of cases. The most common form of alcoholic liver disease is the fatty liver, and the most difficult one, cirrhosis of the liver. Cirrhosis is the 9th cause of death, and in the population aged 45–64 years, it is the 6th one [1–3]. The amount and length of alcohol consumption are related to the manifestation of alcoholic liver disease. The risk of alcoholism is higher with the transition of the threshold of 80 g of alcohol a day for men, and 20 g for women.

A diet deficient in protein and antioxidant vitamins, increases the hepatotoxicity of ethanol. Hepatotoxic effects of ethanol are more noticeable with an increase in intake of unsaturated fatty acids. Ethanol increases the absorption of iron from the intestine and increases its disposal in the liver [4–9].

The clinical picture of alcoholic liver disease varies from asymptomatic disease, fatty liver, alcoholic hepatitis, to heavy cirrhosis of liver with complications [10–12]. The overlapping of more than one form of alcoholic liver disease in the same patient is often present. Alcoholic fatty liver disease (AFLD) is often not recognized. The classic clinical picture is presented from asymptomatic state to the manifestation of weakness, fatigue, nausea, and rarely anorexia, vomiting and diarrhea. In laboratory the cytolysis of hepatocytes and retention of bilirubin are present, and rarely reduced synthetic liver function.

The diagnosis is based on properly taken history, including the data on alcoholism, elevated values of aspartate transaminase (AST) and alanine transaminase (ALT) [13]. Alkaline phosphatase (ALP) may be slightly elevated in contrast to cirrhosis, which is up to 4 times higher. Gamma glutamyl transferase (GGT) is induced by alcohol, and quickly returns to normal after stopping the use of alcohol [14–16]. Hyperbilirubinemia and prolonged prothrombin time is more common in more severe forms of alcoholic liver disease. Hyperuricemia and dyslipidemia, follow the chronic alcoholism [4–6]. More severe forms of alcoholic liver disease are compained by leukocytosis with neutrophilia and anemic syndrome as well as hypoalbuminemia with hypergammaglobulinemia. In chronic consumption of alcohol the values of IgA are elevated [17–21]. In the diagnosis of alcoholic liver disease, the biopsy of liver occupies an important place [22–24].

Non-alcoholic fatty liver disease (NAFLD) is a disease of the liver which has histologic features of alcoholic liver disease, and wherein the persons do not consume alcohol. The development of NAFLD can be influenced by drugs or toxins. It can be hereditary and acquired disorders of metabolism. The clinical picture of NAFLD is rarely characterized by nonspecific signs such as fatigue, malaise, mild pain under the right rib arch. This is mostly asymptomatic disease. In the clinical findings of 75% of patients hepatomegaly is registered, and in 25% splenomegaly. It rarely goes into the fulminant form with rapid development of cirrhosis and death.

The diagnosis is set by the history of significant absence of alcohol consumption. In laboratory the moderate rise in aminotransferases, and AST/ALT ratio is less than 1. The disorders of synthetic function of the liver and retention of bilirubin are rare. In a number of patients hyperglycemia, hypertriglyceridemia and hypercholesterolemia are present. Liver biopsy is the gold standard for diagnosis.

The aim of this study was to evaluate the profile of atherogenic factors in plasma in patients with AFLD and patients with NAFLD and determine its diagnostic significance.

Methods

The study included 74 patients with AFLD who consumed alcoholic beverages daily in large quantities and over 80 g; for men it is the amount of 3–4 units (U) of alcohol, and for women 2–3 (one alcoholic U corresponds to approximately single spirit, or 0.5 L of beer or 2 dL wine spritzer or 4 oz) [6]. The control group consisted of 70 patients with verified NAFLD. The diagnosis of NAFLD is set by ultrasonography of abdomen. All the patients were submitted to laboratory processing, AST and ALT, total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and apolipoproteins (Apo) – ApoA1 and ApoB and blood cells count. The ratios of TC/HDL-C, ApoB/ApoA1 and LDL-C/HDL-C were calculated.

The diagnosis of NAFLD was carried out with the ultrasound apparatus Siemens X 300 in a supine position with the World Health Organization (WHO) diagnostic criteria applied from 2005. On that occasion, the so-called phenomena was compared: brightness, deep beam attenuation, liver-kidney contrast, gallbladder wall definition.

By blood tests, from the studies were excluded the patients with viral hepatitis and autoimmune disorders, and by ultrasound examination, the patients with tumor processes and obstruction of the biliary tree.

In this paper, we used the standard descriptive methods, mean values, standard deviation and percentage distribution, and the data between the groups were analyzed by appropriate statistical tests depending on the type and distribution of features (Man Whitney U test, Student's *t*-test, χ^2 test). The significance level of $p < 0.05$ was taken as significant. Data are presented as tabulated.

Results

The study included 144 patients, of whom 37.5% were women and 62.5% men, the mean age of 42.05 ± 8.56 years. The patients were divided into two groups, with AFLD and NAFLD.

The first group included 74 patients with AFLD, aged 22–67 years, the average age of 42.65 ± 9.73 years. They consumed alcoholic drinks in greater quantity than 80 g for 1 to 4 years on the average 2.31 ± 0.96 years. Among the respondents, there was 21% of women and 79% of men. In the second group of patients, there were 70 subjects with NAFLD, aged 24–65 years, mean age 41.31 ± 4.1 years, who were not consuming alcoholic beverages. Among them, there was 37.5% of women and 63.5% of men. There was no significant difference in gender distribution and the average age between the examined groups (Table 1).

The average values of AST, ALT, GGT and AST/ALT ratio in the AFLD group were significantly higher than in the NAFLD group ($p < 0.01$) prospectively. There were no differences in albumin concentration, international normalized ratio (INR) and parameters of anemia between the groups. The number of leukocytes was significantly higher and platelets lower in the AFLD group ($p < 0.05$) (Table 2).

Table 1

General characteristics of the studied group of patients

Fatty liver groups	Women / men	Age (years)	Length of alcohol consumption (years)
	n (%)	x̄ ± SD	x̄ ± SD
Alcoholic	21/79 (28/46)	42.65 ± 9.73	2.31 ± 0.96
Non-alcoholic	37.5/63.5 (26/44)	41.31 ± 4.1	-
Total	54/90 (37.5/62.5)	42.05 ± 4.2	-

x̄ – mean values; SD – standard deviation.

Table 2

Biochemical and haemathological parameters in hepatocellular injury

Parameters	Alcoholic fatty liver (x̄ ± SD)	Non-alcoholic fatty liver (x̄ ± SD)
Liver function tests		
AST (U/L)	61.26 ± 8.45	45.37 ± 6.23**
ALT (U/L)	73.69 ± 12.69	59.76 ± 10.31**
AST/ALT	0.83	0.76**
GGT (U/L)	96.7 ± 12.42	83.2 ± 9.56**
Albuminical (g/L)	41.04 ± 5.54	41.03 ± 8.43
INR	1.3 ± 0.09	1.3 ± 0.08
Haemathological parameters		
leukocytes ($\times 10^9$/L)	8.43 ± 0.88	7.96 ± 0.79*
Hb (g/L)	144.4 ± 12.49	145.2 ± 12.31
Hct (%)	42.39 ± 3.17	44.27 ± 3.73
PLT ($\times 10^9$/L)	207.12 ± 39.11	221.09 ± 36.34*
Atherogenic indicators		
TC (mmol/L)	14.53 ± 2.81	9.87 ± 2.36**
LDL-C (mmol/L)	8.57 ± 2.15	6.72 ± 1.98**
HDL-C (mmol/L)	0.43 ± 0.11	0.78 ± 0.09**
TG (mmol/L)	9.94 ± 2.94	8.74 ± 2.54 **
TC/HDL-C	32.61 ± 9.21	12.65 ± 7.25**
LDL-C/HDL-C	17.39 ± 5.01	8.62 ± 3.25**
ApoB (g/L)	3.97 ± 0.28	2.38 ± 0.16**
ApoA1 (g/L)	0.49 ± 0.09l	0.98 ± 0.04**

Hb – haemoglobin; Hct – haematocrit; PLT – platelets; INR – international normalized ratio; GGT – gamma glutamyl tranferase; AST – aspartate aminotransferase; ALT – alanine aminotransferase; TC – total cholesterol; TG – triglycerides; ApoA1 – apolipoprotein A1; ApoB – apolipoprotein B; LDL-C low density lipoprotein cholesterol; HDL-C – high density lipoprotein cholesterol.

x̄ – mean value; SD – standard deviation; *$p < 0.05$, **$p < 0.01$ *vs* alcoholic fatty liver.

All the examined patients had highly elevated TC (more than 6.2 mmol/L) and TG (more than 5.65 mmol/L) as well as highly reduced HDL-C (less than 1 mmol/L) in both AFLD and NAFLD group. All the patients with AFLD had values of LDL-C greater than 4.9 mmol/L while this was not the case in the NAFLD group.

The index TC/HDL-C, was elevated and at high-risk (more than 4.5) with the average value of 32.61 ± 9.21 in the patients with AFLD and 12.65 ± 7.25 in the NAFLD group. The ApoB/ApoA1 index was calculated, and all the values in both groups were in over 1.1 which also represents high risk. The index LDL-C/HDL-C was greater than 3.5 which represents a very high atherogenic risk in all patients.

There were significant differences in lipid parameters and apolipoproteins between the groups. Higher values of TG, TC, LDL-C, ApoB and ApoB/ApoA1 ratio, and lower values of HDL-C, ApoA1 were registered in the AFLD compared to the NAFLD group (Table 2).

Discussion

The examined groups of patients with AFLD and NAFLD were predominantly male with similar average age. During the relatively short period of alchocol consumption in AFLD there was a manifestation of the most lenient alcoholic liver damage, fatty liver. All the patients had elevated transaminases, with ALT predominance, so the AST/ALT was 0.83. The values of GGT were also elevated. In the NAFLD group of not consumers of alcoholic beverages, the transaminases and GGT were increased, with the predominance of ALT. The AST/ALT ratio was lower (0.76) compared with the patients with AFLD (Tables 1 and 2).

Laboratory analysis verified the elevation of the values of TC, TG and LDL-C, as atherogenic factors, and decreased values of antiatherogenic HDL-C. The lipid disorders were more prominent in the AFLD group than in the NAFLD group (Table 3).

Alcohol in the body is the subject of oxidation, mainly in the liver. Ethanol is metabolized in acetate by using three enzymatic systems: alcohol dehydrogenase (ADH), the microsomal oxidation system (MEOS) and catalase. Ethanol is oxidized, 80–85%, to highly toxic acetaldehyde which damages the cell membrane, leading to cell necrosis. Ethanol oxidation produces the accumulation of fatty acid and triglycerides in the liver and increased lipoprotein synthesis. The increased concentration of NADH changes the redox potential of hepatocytes, leading to the inhibition of protein synthesis, rise of lactate and urate levels in serum. The MEOS is responsible for the metabolism of 10–15% of ethanol. Thus, the consumption of oxygen and the production of acetaldehyde is increased, and also the lipid peroxidation. Catalase system is poorly active in the metabolism of alcohol [4–7].

The values of ApoA1 and ApoB were also determined. Among the patients with AFLD, ApoA1, ApoB/ApoA1 ratio and HDL-C were lower compared to the NAFLD group. ApoA1 is a component of an antiatherogenic lipoprotein and was decreased in AFLD (Table 3). Low values of ApoA1 created conditions for the development of atherogenic effect. ApoB as atherogenic component was extremely increased in AFLD. Due to the reduced ApoA1 and increased ApoB, in patients with chronic consumption of ethanol and developed AFLD and NAFLD, atherogenic effect in plasma was created [4, 20, 21].

Apo B is the primary Apo of chylomicrons and LDL, and is responsible for transporting of TC to tissues [5, 8]. ApoB in particles of LDL is a ligand for the LDL receptors of the cells, and "unlocks" cells, and transport TC to them. By an unknown mechanism of high value ApoB lead to the formation of plaques in blood vessels and the development of atherosclerosis. Thus, the determination of ApoB is a better and more significant indicator of atherosclerosis risk than analysis of LDL and total TC. As in the patients with AFLD there is a significant elevation of ApoB, there is an increased risk of plaques formation in blood vessels, and so, consequently, for the development of atherosclerosis.

Apo A1 is a major component of plasma HDL-C. It leads to the so-called fat-efflux from tissues to the liver [14]. Thus mobilized fat is than excreted from the liver. ApoA1 is a cofactor of lecithin cholesterol transferase (LCAT), important for the synthesis of TC esters in the plasma. ApoA1 is an ingredient of prostacyclin (PGI2), responsible for the realization of antiagregational effects.

The study Incremental Decrease and Events through Aggressive Lipid Lowering (IDEAL) [25], and INTERHEART study [26], emphasize that the determination of the ratio ApoB/ApoA1 is a significant prognostic factor of atherogenic effects. Individual monitoring of TC, LDL-C or ApoB, as atherogenic factors, and HDL-C, and Apo-A1, as antiatherogenic factors, as well as determining the ratios TC/HDL-C or LDL-C/HDL-C is less significant as compared to ApoB/ApoA1 ratio.

Determining the relationship between ApoB/ApoA1 proved to be the most important and most coherent marker for the existence of atherogenic plasma.

Conclusion

Determination of TC, TG, LDL-C, HDL-C, ApoA1, ApoB1 and ratios ApoB/ApoA1, LDL-C/HDL-C, TC/HDL-C is an important parameter of tracking of atherogenic factors in plasma. Both AFLD and NAFLD lead to increase of ApoB, an indicator of atherogenic status of plasma, and increased risk for the formation of plaques in blood vessels, including atherosclerosis. The values of TC, TG, LDL-C, HDL-C, LDL-C/HDL-C and TC/HDL-C ratios showed great variation from mild to very elevated. Determining the value of the ApoB/Apo A1 ratio is less variable, more coherent parameter for tracking of atherogenic changes in plasma. The lipids and ApoB/ApoA1 ratio besides markers of hepatocelular damage can serve as a diagnostic criteria for the presence of AFLD, and as a better indicator of atherogenic risk.

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