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ORIGINAL ARTICLE

Sterol parameters as markers of liver function in primary biliary cirrhosis before and after liver transplantation

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Summary

Serum cholesterol reflects poorly cholesterol metabolism. From serum noncholesterol sterols cholestanol, campesterol, and sitosterol are surrogate markers of cholesterol absorption, but reflect also cholestasis, while those of lathosterol reflect cholesterol synthesis and hepatic parenchymal function. We investigated these sterols at end-stage of primary biliary cirrhosis (PBC) – prior to liver transplantation and shortly after transplantation in 67 patients to show their role as index of cholestasis and parenchymal liver function. Median preoperative values of cholestanol were increased 7.6 times, those of plant sterols 1.6–3.7 times above and the campesterol/sitosterol ratio was decreased twice below our control values, respective lathosterol levels being mainly subnormal. After transplantation, the proportions to cholesterol of the absorption markers decreased, and those of synthesis markers and the ratios of campesterol/sitosterol increased significantly. Thus, surrogate sterol markers of cholesterol absorption and synthesis in serum are also good clinical markers of chronic cholestasis and degree of hepatic parenchymal cell function in PBC. Postoperative improvement of serum sterol profile indicate clinically good function of the liver graft.

Introduction

The liver and the intestine are essential for the regulation of the cholesterol metabolism. The liver is prime organ of cholesterogenesis [1]. It is the only organ capable of net excretion of cholesterol from the body [2,3]. Bile acids are essential for cholesterol absorption from the intestine [4]. Measurements of serum cholesterol are not helpful in considering of cholesterol metabolism [5]. Instead, three kinds of serum noncholesterol sterols are implying that better. Cholesterol precursor sterol lathosterol reflect cholesterol synthesis [6–10], whereas the diet-originating plant sterols campesterol and sitosterol are markers for cholesterol absorption from the intestine [11–14]. Normally, the intestinal absorption of campesterol is better, its serum concentration is higher and its biliary secretion rate is slower than those of sitosterol [9,11,12]. Recently identified ABCG5/8 transporters are responsible for the different absorption and biliary secretion rate of campesterol, sitosterol, and cholesterol [15–18]. Cholestanol is produced from endogenous cholesterol by enzymatic action in the liver, and it is almost absent from the diet [19]. Its serum levels are normally very constant [12,19] and reflect positively cholesterol absorption efficiency and negatively cholesterol synthesis [12]. We and others have shown that serum cholestanol is a sensitive marker of chronic cholestasis [20–23] and is elevated before serum bilirubin level begins to increase in primary biliary cirrhosis (PBC) [24]. Cholesterol precursor sterols, plant sterols and cholestanol reflect differently liver function (hepatic lipid synthesis, biliary lipid secretion and efficacy of intestinal sterol absorption).

Liver transplantation provides a unique opportunity to investigate the contribution of the liver to cholesterol metabolism. PBC combines the elements of chronic cholestasis and parenchymal liver disease. Because our earlier

studies were small pilots with only 11 patients with end-stage PBC [21–23], we wanted to more accurately evaluate the earlier serum sterol findings just before and shortly after liver transplantation in 67 patients with end-stage PBC to show further their clinical significance and role as index of, cholestasis and parenchymal liver function.

Patients and methods

Patients

The study population included 67 consecutively (between 1987 and 2002) transplanted end-stage PBC patients (female/male: 57/10) at mean age 53 years (range: 36–68) from The University Hospital of Helsinki. Most of the transplanted PBC patients had Child-Pugh C or B cirrhosis. The indication for liver transplantation in these patients was highly increased serum bilirubin, seriously decreased synthesizing capacity and clinical complications of cirrhosis. Most of these patients had Child C or B cirrhosis. Two patients had infernal pruritus as the main indication. Before transplantation, mean serum bilirubin was 260 μmol/l (SD 201). Serum bilirubin was above the normal level in all patients and only slightly increased (less than four times normal level) in 10 of 67 patients. Initial immunosuppression consisted of cyclosporine-based triple therapy with azathioprine and decreasing doses of corticosteroids.

Control population for sterol measurements was healthy consecutive 59 blood donors from Finnish Red Cross Blood Transfusion Service.

Methods

Blood samples were obtained immediately before and mean 27 days after liver transplantation. Serum bilirubin was determined by the routine clinical method. Serum cholesterol, lathosterol, cholestanol, campesterol and sitosterol were analyzed by gas–liquid chromatography (GLC) [25,26]. Briefly, 5α-cholestane (50 μg) was added to serum (0.1 ml) as internal standard. After alkaline hydrolysis, extraction, and derivatization to the trimethylsilyl ethers, the sterols were quantified by GLC. Noncholesterols in serum are transported in a mixture with cholesterol by lipoproteins, up to 90% by low-density lipoproteins. The noncholesterol sterol values are expressed as proportions to cholesterol (100 mmol/mol of cholesterol) so as to correct the differences in the serum levels of sterols as a consequence of a different number of lipoprotein particles as a result of the variation of their synthesis and removal.

Statistical analysis

The statistical analysis was performed using STATVIEW software (version 5.0.1 for Windows version; SAS Institute Inc., Cary, NC, USA). Comparison between sterol levels before and after transplantation was performed with paired *t*-test when normally distributed and a nonparametric Wilcoxon signed rank test was used when appropriate. For the correlation coefficients, Spearman rank correlation was used or after logarithmic transformation regression was used.

Results

Serum median bilirubin level was 260 μmol/l (reference 2–20 μmol/l) before transplantation. Median concentration of cholesterol was normal (Table 1) but a significant amount, 39% of patients had pretransplant serum cholesterol level under 3 mmol/l.

Pretransplant median proportion (Table 1) of cholestanol was increased 7.6 times and those of plant sterols 1.6 (campesterol) and 3.7 (sitosterol) times above our control

Table 1. Concentration of cholesterol and proportions of noncholesterol sterols in 67 primary biliary cirrhosis (PBC) patients before and after liver transplantation and in healthy consecutive 59 blood donor controls*.

Variable	Total cholesterol (mmol/l)	Lathosterol (100 mmol/mol of cholesterol)	Campesterol (100 mmol/mol of cholesterol)	Sitosterol (100 mmol/mol of cholesterol)	Campe/sito ratio	Cholestanol (100 mmol/mol of cholesterol)
Pretransplant						
Median	3.70	84	489	542	0.96	972
Range	0.90–21	23–238	13–1786	30–2583	0.28–1.80	453–2459
Post-transplant						
Median	4.40	114	266	221	1.19	363
Range	1.31–13	26–271	62–1329	69–2792	0.44–2	131–952
P-value	0.006	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Reference*	5.01	162.9	299.9	147.3	2.04	128.5
Values	3.66–6.64	104–322	122–592	70–289	1.34–2.85	80–192

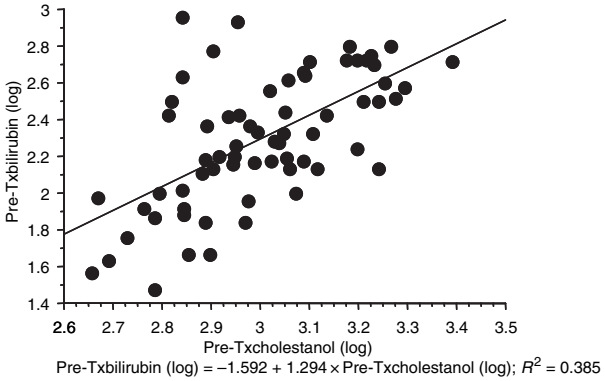

Figure 1 Correlation between cholestanol level before liver transplantation with the total serum bilirubin level.

values, whereas the ratio of campesterol to sitosterol was two times lower than in controls (Table 1). The serum proportions of cholestanol did not overlap in the PBC and control groups (Table 1). The proportion of lathosterol was mainly subnormal. The median ratio of lathosterol to campesterol (also a indicator of cholesterol synthesis [9]) was three times lower than the control value. The ratio of campesterol to sitosterol was two times lower than in controls, mostly below 1. After transplantation (Table 1) the proportions of cholestanol and plant sterols to cholesterol were significantly improved. Cholesterol and lathosterol levels and the ratio of campesterol/sitosterol were significantly increased but the ratio was still far from the level of the controls. A strong positive correlation between the pretransplant bilirubin levels and the proportions of pretransplant cholestanol was observed (Fig. 1) whereas the correlation between the pretransplant bilirubin levels and the pretransplant campe/sitosterol ratio was negative ($r = -0.566$, $P < 0.001$). The pre- and post-transplantation proportions of cholestanol were negatively related to the respective ratio of campesterol/sitosterol ($r = -0.431$, $P = 0.0006$ and $r = -0.359$, $P = 0.0035$, respectively). The pre- and post-transplant proportions of lathosterol and cholestanol were not interrelated. Quartile ranking according to the cholestanol pretransplant values revealed that the post-transplant cholestanol in the highest quartile was decreased but was still markedly higher compared with three other quartiles (Fig. 2a) but the highest post-transplant bilirubin level occurred in a different quartile (Fig. 2b). The ratio of campe/sitosterol was reversed (over 1) in all quartiles.

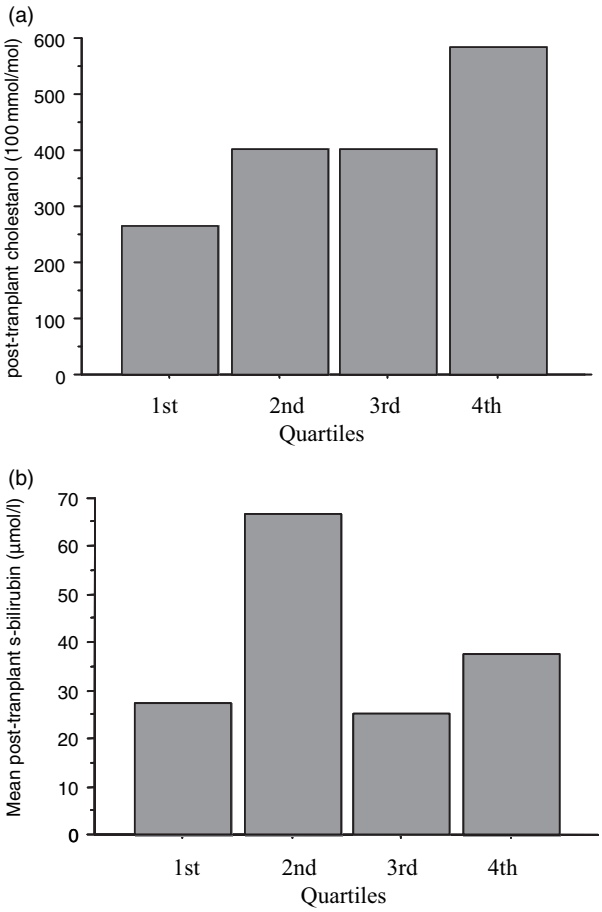

Figure 2 (a) Mean cholestanol (100 mmol/mol) level after transplantation presented in quartiles 25, 50, 75 and 100% of pretransplant cholestanol levels. (b) Mean serum bilirubin (µmol/l) level after transplantation presented in quartiles 25, 50, 75 and 100% of pretransplant cholestanol levels.

Discussion

We have in our earlier pilot studies analyzed, whether a sterol pattern of serum sterols could predict the stage of liver damage in PBC and also in other types of liver diseases, particularly the discriminatory power of noncholesterol sterols for liver damage and timing for liver transplantation in PBC [21–23]. Serum bilirubin and sterol proportions appeared to predict differently the likely outcome of patients with PBC and the results revealed different patterns of serum sterols in different clinical stages of liver damage. Increased serum cholestanol, but normal lathosterol and campesterol/sitosterol ratio was found in early PBC [21]. Furthermore, decreased lathosterol compared with healthy persons seemed to point to intermediate or end-stage PBC reflecting depressed cholesterol synthesis because of more severe cholestasis and/or hepatocellular damage [21]. In an earlier study, a low serum mean proportion of lathosterol, a high mean proportion of cholestanol (>600 × 10^2 mmol/mol cholesterol versus on average 90 in controls) and a low (<1) mean serum campesterol/sitosterol ratio appeared to imply terminal liver damage in PBC patients selected for liver

transplantation [23]. The mean ratio of campesterol to sitosterol was normal and the mean proportions of serum cholestanol only moderately (mean value 306×10^2 mmol/mol cholesterol versus mean value 1081 in end-stage PBC) increased in the acute liver failure [23]. Interestingly, serum cholestanol proportion was high in all PBC patients of varying degree of the disease in a study of Gylling *et al.* [24].

The results of this study are in line with previous findings from our pilot study [21–23]. The serum median pretransplant proportions of cholestanol and plant sterols were markedly high and the ratio of campesterol to sitosterol as well as the ratio of lathosterol to campesterol were low. The new and interesting finding was that the highest bilirubin and cholestanol post-transplant levels were not in the same quartile when quartile ranked was by pretransplant cholestanol proportions.

The concentration of serum cholestanol is increased in cholestatic liver diseases proportionally to impaired liver function shown especially in PBC so that serum mean cholestanol levels in serum are 12 times normal at end-stage patients [21,22]. Cholestanol differs from cholesterol by the reduced delta-5 double bond to 5-α position [4]. Its serum level is regulated by cholestanol intake, absorption, synthesis, and biliary secretion. Cholestanol is found in small amounts in most mammalian tissues [19]. In the baseline control diet, cholestanol accounted for 1.5% (=6 mg) of the total neutral sterols in a study [27]. In the Finnish diet, the amount of cholestanol is <2 mg/day [12]. Using balance methods, the absorption of cholestanol in normal humans has not been demonstrated [28]. So virtually all body cholestanol is produced endogenously by enzymatic action in the liver [19]. Normally, the serum cholestanol proportions are positively related to cholesterol absorption efficiency and negatively to cholesterol synthesis [12]. It is rapidly secreted from serum in bile [19]. A study of Gylling *et al.* on patients with varying severity of PBC showed that the mean proportions of serum cholestanol were positively related to serum bilirubin and bile acid concentrations and inversely to biliary cholesterol secretion, synthesis of bile acids and cholesterol precursor sterols, and cholesterol absorption efficacy [24]. Based on these results it seems that an enhanced cholestanol synthesis and a cholestasis-induced decrease in biliary clearance of serum cholestanol contributed to the excessively high serum cholestanol level in PBC. An accumulation of cholestanol to tissues, in the liver at least [23], appears to relate to hypercholestanolemia, which in turn, seems to develop gradually during worsening state of PBC [21]. Whether this cholestanol loading could cause some functional disability in tissues, is not known. In this study, after transplantation the serum proportions of cholestanol were still increased also in those with good liver graft function, possibly implying discharge of cholestanol from tissues.

Plant sterols, campesterol and sitosterol are solely of exogenous origin. Normally, our diets contain almost equal amounts of cholesterol and plant sterols. However, normal individuals retain on average approximately 40–50% of dietary cholesterol, but <1% of the dietary plant sterols [13,14]. The contents of major plant sterols in the consumed food are estimated to be higher for sitosterol than for campesterol [29]. Sitosterol makes up about 65%, campesterol about 30% and stigmasterol 5% of the plant sterols, confirmed in the Finnish diet as well [11]. The biliary secretion of sitosterol is shown to be better than that of campesterol in healthy subjects [14]. In a recent study, sitosterol showed a significantly higher biliary secretion rate than campesterol, but both plant sterols had significantly lower biliary secretion rates compared with cholesterol [14]. Hepatic clearance of cholesterol was significantly lower compared with campesterol and sitosterol, and the clearance of campesterol was significantly lower compared with sitosterol.

In PBC, the gradual increase in serum sitosterol suggests that biliary secretion of sitosterol is more impaired than dietary sitosterol absorption and more sensitively disturbed than that of campesterol in PBC [30]. Normally, the serum proportions of campesterol are constantly higher than the ones of sitosterol [11]. Intestinal absorption and biliary secretion are regulated by expression of ABCG5/8 genes [15,16]. Identification of these two genes has opened a new field for investigation of sterol regulation, presumably in liver as well [14]. As these ABCG transporters are also expressed in the liver they might regulate biliary output of sterols.

It can be concluded that before liver transplantation at end-stage PBC sterol markers of cholesterol homeostasis are also good markers of chronic severe cholestasis and associate to the degree of liver damage in PBC. Cholestanol, i.e. usually synthesized from cholesterol shows extremely high serum and hepatic levels in circumstances with low cholesterol synthesis and cholestasis. The ratios of cholestanol to cholesterol and campesterol/sitosterol ratios could also be used to evaluate timing for liver transplantation. In this study, good liver graft improved serum sterol profile and cholesterol synthesis markedly in PBC patients early after liver transplantation.

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