Scholarly Research Exchange

Volume 2009 • Article ID 123481 • doi:10.3814/2009/123481

Clinical Study

Corticosteroids and Cholelithiasis in Systemic Lupus Erythematosus

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Received 11 December 2008; Revised 5 March 2009; Accepted 9 March 2009

The aim. To evaluate the frequency of gallstone formation and alteration of plasma lipid profiles in SLE patients long treated with prednisolone. *Material and methods*. Sixty patients with SLE were divided into 2 groups: (1) 38 SLE patients without gallstones; (2) 22 SLE patients with gallstones. Gallbladder ultrasonography was performed in all the patients, and the serum lipid profile was determined. To identify the composition and structure of gallstones obtained during cholecystectomy, color cathodoluminescence scanning electromicroscopy was used. *Results*. Gallstone disease was detected in 22 (36.7%) patients of the 60 examinees and in 22 (68.8%) of the 32 SLE patients receiving prednisolone therapy; whereas none of the 28 prednisolone-untreated patients was found to have the disease ($P = .001$). There were the most significant differences between the SLE patients with and without gallstones in the duration of administration of prednisolone and in its mean daily and mean monthly doses, and cumulative ones. *Conclusion*. Age at the onset of the disease, the mean daily dose of corticosteroids, and the duration of therapy with these agents are the most likely factors predisposing to gallstone disease in SLE patients. The CCL-SEM study identified predominantly the protein-cholesterol structure of gallstones.

1. Introduction

Systemic lupus erythematosus (SLE) is a multisystem autoimmune disease characterized by immune dysregulation, which results in the production of autoantibodies, activation of the complement system, and generation of immune complexes [1]. An intrinsic feature of the disease is vascular injury manifesting itself as cutaneous vasculitis, glomerulonephritis, cardiopulmonary, cerebrovascular and, less commonly, gastrointestinal lesions.

In several past decades, the use of corticosteroids and cytotoxics has dramatically improved the prognosis of the disease [2, 3]. Prednisone, prednisolone, methylprednisolone, and hydrocortisone are the most commonly used corticosteroids in the treatment of SLE. The long-term systemic use of corticosteroids is one of the most important issues in the management of SLE due to their potentially serious side effects. Long-term corticosteroid therapy is well known to cause dyslipoproteinemia characterized by elevated plasma total cholesterol (TCh), triglycerides (TGs), and low-density lipoprotein cholesterol (LDL-Ch) [4, 5]. The major catabolic pathway for cholesterol is its transformation into bile acids involving P_{450} cytochrome and subsequent bile excretion from the body. The elevated level of total cholesterol may change a bile acid/cholesterol ratio and lead to the formation of gallstones in patients with SLE.

The aim of this study was to evaluate the frequency of gallstone formation and alteration of plasma lipid profiles in SLE patients treated long with prednisolone.

2. Matherial and Methods

2.1. Subjects. This was a retrospective study covering 60 consecutive SLE patients (45 females and 15 males; Caucasians; mean age 30.5 ± 9.4 years) treated and followed up at the Institute of Rheumatology, Russian Academy of Medical Sciences (Moscow, Russia) with January 2003 to December 2006 years. All patients met four SLE diagnostic criteria or more (American College of Rheumatology, 1982) [6]. This study was approved by the Ethics Committee of Institute of

Table 1: Baseline disease-related features of the SLE patients recruited.

Variable	*n*
≥4 ACR criteria, *n* (%)	60 (100)
Sex (female/male)	44/16
Median age at the time of this study, *n* (range), years	28 (18–44)
Median age at the onset of disease, *n* (range), years	20 (10–36)
Median age at diagnosis, *n* (range), years	21 (15–29)
Median disease duration, *n* (range), years	9.6 (1–21)
Median (IQR) SLEDAI, score	15 (10–22)
Median (IQR) SLICCA damage index, score	1.1 (0.4–2.3)
Current steroid therapy, *n* (%)	32 (53.3)
Median (IQR) current steroid dosage, mg	5.5 (0–15.5)
Median (IQR) steroid therapy duration, years	5 (0–12)
Median (IQR) monthly steroid dose, mg/month	12 (0–16)
Cumulative steroid dose (range), g	107.3 (0–583.7)
Current antimalarial therapy, *n* (%)	24 (40)
Current immunosuppressive therapy, *n* (%)	16 (26.7)
Gallstone disease, *n* (%)	22 (36.7)

IQR: Interquartile range.

Rheumatology, Russian Academy of Medical Sciences, and all the patients gave an informed consent. SLE was first diagnosed in 28 patients and none of them had received corticosteroids. Thirty two SLE patients had been treated with prednisolone. The average statistical interval between the occurrence of the first sign of the disease and the establishment of the diagnosis was one year (Table 1).

The mean monthly and cumulative dose of prednisolone and its treatment duration were recorded during a chart review. The mean monthly dose of prednisolone was defined as its total sum in milligrams divided by the total number of follow-up months, the cumulative dose of the agent was as its total sum in grams taken during the same period, and the duration of treatment with the drug was estimated as the time interval (years) during which a patient had taken it (Table 1).

SLE activity was quantified, by using the SLE disease activity index (SLEDAI) [7]. None of patients had underlying conditions (nephrotic syndrome or renal insufficiency) or concomitant diseases (diabetes mellitus) that could potentially cause dyslipidemia. The study excluded pregnant women, 6-month nursing mothers, women who had used oral contraceptives or estrogen-containing agents for 6 months before the study, and patients who had used statins.

The patients were clinically interviewed and examined according to the standard protocol comprising demographic characteristics, family history, and lifestyle (Table 2). To evaluate the effect of corticosteroids on gallstone formation, all the SLE patients were divided into 2 groups: (1) 22 patients with gallstones (a gallstone group); (2) 38 patients without gallstones (a nongallstone group).

2.2. Laboratory Studies

2.2.1. Routine Clinical Samples. All samples were collected after a written informed consent had been given under institutional review board-approved protocols in accordance with the Helsinki Declaration. Hemoglobin, leukocyte and platelet counts, fasting plasma glucose, serum creatinine, serum liver functional tests (bilirubin, cholesterol, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, total protein, albumin, and globulin levels), urine microscopy, DNA antibodies, complement profile (C_3, C_4, C_{50}), and antinuclear antibodies were measured as a part of the routine laboratory assessment.

2.2.2. Ultrasound Study. Ultrasonography was performed with a Combison 530 with 3.5 MHz convex (Kretz, Austria). Gel was applied to the skin to provide an acoustic interface.

2.2.3. Lipids. Blood was sampled after a 14-hour of fasting. On the day of sampling, plasma and serum were immediately separated, centrifuged, and stored at −20°C before testing the lipid profile. TCh, TG, and HDL-Ch were measured, by employing commercially available assays (Biocon, Germany) on an Airone-200 analyzer (Crony, Italy). HDL-Ch was measured after precipitation of LDL and very low-density lipoprotein (VLDL), by using sodium 12-tungstophosphate (1.1 mmol/L) and magnesium chloride (45 mmol/L) (a final concentration) [8]. The level of LDL-Ch was calculated using the Friedewald formula [9]. Apolipoprotein AI (Apo-AI) and apolipoprotein B (Apo-B) were measured by radial immunodiffusion. All assays were repeated.

2.2.4. Composition and Structure of Gallstones. Color cathodoluminescence scanning electron microscopy (CCL-SEM) was employed to study the composition and structure of gallstones. A "Stereoscan MK-IIA" SEM (Cambridge Instruments, UK) was equipped with a CCL-attachment (developed by GVS, SKO, and PVI at the Department of Physics, Moscow State University, Russia) and operated in the "real-color mode" at an electron energy of under 20 keV and a beam current of 10 nA. As a result, color images of the samples under examination were electron-induced luminescence of major chemical gallstone components located onto the section surface. "Adobe Photoshop" software was used to separate colors and to have intensity histograms. The method and its possible fields of application were characterized in detail in our earlier publications [10–12]. Ten gallstones obtained from 4 SLE patients on 10- to 20-year prednisolone treatment at cholecystectomy were selected for CCL-SEM evaluation of their composition, microarchitectonics, and microstructure. All samples of the gallstones were cut in half through their centers.

Table 2: Comparisons of baseline demographic characteristics, prednisolone therapy, SLE-related features, and laboratory data in SLE patients with/without gallstones.

Variable	SLE patients with gallstones ($n = 22$)	SLE patients without gallstones ($n = 38$)	P-value
Sex (female/male)	17/5	27/11	NS (χ^2)
Median age at the time of this study (range), years	33 (22–44)	24 (18–40)	.0002
Median age at the onset of the disease (range), years	23 (10–35)	19 (8–36)	.015
Median age at diagnosis (range), years	25 (18–35)	21 (17–37)	NS
Median disease duration (range), years	11.8 (8–15)	1.8 (0–8.0)	.000001
Median (IQR) SLEDAI, score	7 (6–10)	12 (10–20)	.000145
Current steroid therapy			
Median (IQR) current steroid dosage, mg	16.0 (10.0–18.5)	2.1 (0–5.0)	.0001
Median (IQR) steroid therapy duration, years	12 (8.0–15.0)	0 (0–5.0)	.0001
Median (IQR) monthly steroid dose, mg/month	16.1 (15.0–16.5)	0 (0–8.0)	.0001
Cumulative steroid dose (range), g	848.0 (502.0–972.5)	0 (0–65.7)	.0001
Laboratory results			
TCh (range), mg/dL	220.0 (172.0–251.0)	177.0 (142.0–190.0)	.0004
HDL-Ch (range), mg/dL	46.0 (40.0–56.0)	38.0 (34.0–42.0)	.008
Ratio of TCh and HDL-Ch	4.9 (4.1–5.5)	4.3 (3.6–4.8)	.05
LDL-Ch (range), mg/dL	135.0 (103.0–173.0)	111.2 (84.6–136.0)	.006
ApoA1-HDL (range), mg/dL	130.0 (115.0–151.0)	112.0 (103.0–141.0)	.05
ApoB-LDL (range), mg/dL	108.0 (89.0–136.0)	97.0 (77.0–119.0)	.005
Fibrinogen (range), g/L	3.8 (3.1–4.2)	3.4 (2.6–4.2)	NS

TCh: Total cholesterol; HDL-Ch: HDL cholesterol; LDL-Ch: LDL cholesterol; IQR: Interquartile range; NS: Nonsignificant. All comparisons are Mann-Whitney U-test unless where a chi-squared test is indicated.

The standard commercial unconjugated bilirubin ($C_{33}H_{36}N_4O_6$, Merck, Germany), unesterified cholesterol ($C_{22}H_{46}O$ Serva, Germany), high molecular-weight protein, the prototype of a bile mucine component (Serva, Germany) were used to obtain control CCL-SEM images of major bile constituents. The color images of the control samples were as follows: the unconjugated bilirubin luminiscented under electronic rays was colored reddish-orange (Figure 1(a)), the crystals of unesterified cholesterol were blue (Figure 1(b)), and high molecular weight protein was yellowish-green (Figure 1(c)). The color micrographs of gallstone samples were compared with those of the control ones. The application of the computer program (software) and color contrast by the CCL-SEM technique permitted the determination of cholesterol, bilirubin, protein, and their relationships within the stone [12].

2.2.5. Statistical Analysis. The results for continuous variables were expressed as mean ± SD. Nonparametric tests were carried out due to the skewed distribution of the Gaussian curve and the small number of samples. Differences between groups were examined by the Mann-Whitney U-test. Proportions were determined using the standard chi-square test (χ^2) or the Fisher exact test. The values of $P < .05$ were considered significant. Univariate analysis using the individual factors was carried out for the association with gallstones. It was used to compare all clinical and lipid profile variables between 2 groups (the gallstone group and the nongallstone group). The variables found significant by univariate analysis were considered to be predictors of gallstone formation in SLE patients. All baseline variables that have found on univariate analysis significant at $P < .05$ were entered into a backward elimination variable selection procedure by the multivariate logistic regression analysis. A multivariate logistic regression analysis was used to assess the relationship between the predictor variables and the presence of gallstones (present or not). The results were expressed as odds ratio (95% confidence interval) (Table 3). The predictors with a $P < .05$ were considered as independent predictors of gallstone formation in SLE patients. Discrimination analysis was used to detect the signs predicting the development of gallstone disease in SLE patients.

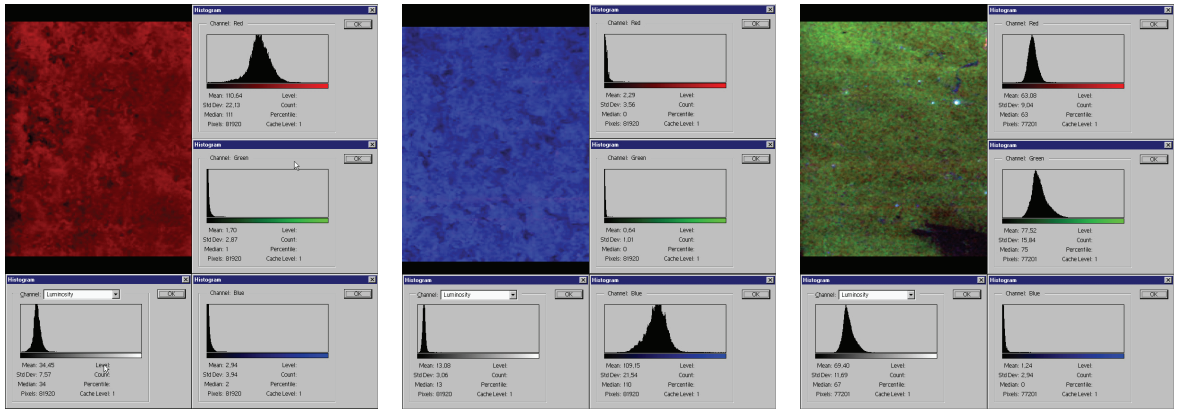
3. Results

3.1. Gallstone Disease in SLE Patients. Table 1 gives the data characterizing the SLE patients being examined, gallstone disease was found in 22 (36.7%) patients of the 60 examinees, by ultrasonography. The size of gallstones varied from 2 to 8 mm in diameter. Gallstone disease was found only in patients on prednisolone therapy. Twenty two (68.8%) out of the 32 SLE patients on prednisolone therapy had cholelithiasis. The later was absent in 28 prednisolone-untreated patients (SLE had been first diagnosed at the examination) ($\chi^2 = 20.6$; $P < .001$). Further analysis

Table 3: Univariable association of predisposing factors to gallstone formations in the study SLE patients.

Variable	SLE patients with gallstone ($n = 22$)		SLE patients without gallstone ($n = 38$)		OR	Ln OR	SE ln OR	95% CI	P-value (χ^2)
	present, n	not, n	present, n	not, n					
Demographic characteristics									
Age at the onset of the disease (≥25.1 years)	11	11	7	31	4.43	1.477	0.562	1.49–15.67	.02 (5.20)
Age at the time of this study (≥30.3 years)	13	9	9	29	4.38	1.574	0.600	1.46–13.21	.01 (6.07)
Prednisolone therapy									
Prednisolone therapy duration (≥7.0 months)	19	3	3	35	71,7	4.272	0.948	11,15–461,22	.000001 (33.64)
Current steroid dosage (≥8.66 mg.)	20	2	0	38	274,1	5.613	1.502	14,45–5231,26	.000001 (47.81)
Minimum maintenance dosage of prednisolone (≥10.2 mg)	12	10	7	31	6.425	1.860	0.601	1.98–20.9	.009 (6.82)
Maximum maintenance dosage of prednisolone (≥28.5 mg)	16	6	10	28	5.842	1.765	0.562	1.94–17.61	.0013 (10.41)
Monthly steroid dose (≥10.4 mg)	22	0	5	33	285.0	5.652	1.501	15.06–5428.6	.000001 (39.09)
Cumulative steroid dose (≥498.5 mg)	19	3	0	38	221.6	5.401	1.493	11.9–4155.8	.000001 (49.1)
Pregnancy history									
Pregnancy in past history	3	14	15	12	9.41	2.241	0.635	2.71–32.73	.0296 (4.73)
SLE-related features									
Enanthema of mucosal soft palate	5	17	21	17	−1.06	−1.064	0.548	0.12–1.01	.02 (4.75)
Thrombocytopenia	2	20	15	23	3.41	1.227	0.579	1.09–10.63	.026 (4.93)
Hemolytic anemia	8	14	26	12	0.35	−1.053	0.528	0.12–1.02	.032 (4.60)
Central nervous system lesion	13	9	9	29	4.65	1.477	0.562	1.46–13.21	.013 (6.07)
Lupus nephritis	2	20	15	23	4.61	1.528	0.783	0.99–21.43	.0265 (4.93)
IgG-anticardiolipin antibody (≤11.6)	5	17	23	15	2.08	0.734	0.530	1.78–5.90	.0105 (6.55)
Antiphospholipid syndrome	8	14	6	32	3.93	1.369	0.639	1.124–13.77	.0459 (3.99)
Laboratory results									
TCh (≥169.0 mg/dL)	19	3	19	19	3.86	1.350	0.587	1.22–12.21	.0111 (6.45)
HDL-Ch (≤45.5 mg/dL)	15	8	9	29	5.89	1.773	0.569	1.93–17.99	.0032 (8.69)
ApoB-LDL(≤74.0 mg/dL)	11	11	6	32	0.036	−3.336	1.472	0.002–0.637	.0112 (6.43)
LDL-Ch (≤136.0 mg/dL)	11	11	8	30	2.793	1.027	0.548	0.95–8.19	.0419 (4.14)
ApoA1-HDL (≥116.5 mg/dL)	18	4	18	20	2.899	1.064	0.548	0.99–8.49	.0187 (5.53)
Ratio of TCh and HDL-Ch (>4.5)	12	10	10	28	3.222	1.170	0.548	1.10–9.44	.0563 (3.64)
Ratio of HDL-Ch and HDL-PhL (≥0.504)	12	10	8	30	3.29	1.191	0.560	1.098–9.88	.0361 (4.39)

TCh: Total cholesterol; HDL-Ch: HDL cholesterol; LDL-Ch: LDL cholesterol; HDL-PhL: HDL phospholipids.



(a) (b) (c)

Figure 1: CCL-SEM micrographs of bilirubin (a), cholesterol (b), high molecular weight protein (c) and their corresponding histograms obtained after color separation.

of 32 corticosteroid-treated SLE patients demonstrated the association of the gallstone formation with the dosage of prednisolone and its treatment duration. Significantly higher mean monthly doses of prednisolone ($P < .001$), its higher cumulative dose ($P < .001$), and its longer treatment use ($P < .001$) were observed in patients with gallstone disease. Almost every patient who had taken prednisolone for more than 5 years had gallstones. Parameters, such as age, disease duration, and SLEDAI score, did not differ significantly between the corticosteroid-treated patients with and without gallstones.

To evaluate the impact of various factors on the development of cholelithiasis in SLE patients, all clinical signs and laboratory data were compared in the gallstone and nongallstone groups. Table 2 shows the results of comparing the individual variables in these groups. Both groups did not differ significantly in gender and median age at diagnosis. The SLE patients with gallstones had statistically a longer history of the disease ($P < .000001$) and statistically lower SLEDAI scores ($P < .000145$). There were the most significant differences between the groups in both the duration of therapy with prednisolone ($P < .0001$), its mean daily ($P < .0001$), mean monthly ($P < .0001$), and cumulative doses ($P < .0001$).

The signs showing the significance of group differences of less than 0.05 were regarded as risk factors for gallstone disease in SLE patients. For univariate analysis, the most informative signs were chosen from all the variables included into the study and found to differ significantly. The frequency of this variable (present, absent) in a group, odds ratio (OR), natural logarithm of the odds ratio (ln OR), standard error in the odds ratio (SE ln OR), 95% confidence interval (CI) for the odds ratio, and significance P by the χ^2 test (Table 3) were derived for each variable.

The data given in Table 3 were used to calculate two logistic regressions that allow us to define with an accuracy of 100% the signs by which the patients are divided into two groups (SLE patients with gallstones and SLE patients without gallstones). Such variables were age at the onset of the disease; cumulative steroid dose; monthly steroid dose; current steroid dosage; prednisolone therapy duration.

Table 4: The predisposing factors to gallstone formation in the study SLE patients.

Variable	The Fisher exact test	P-value
Age at the onset of the disease	93.8	<.000001
Current steroid dosage	109.2	<.0000001
Steroid therapy duration	152.0	<.0000001

The values of the study variables at $P < .05$ in SLE patient with and without gallstones underwent discrimination analysis. This yielded for the examined patient cohort following discrimination equation: $G(X) = 0.298$ (age at the onset of the disease) + 0.746 (current steroid dosage) + 0.908 (prednisolone therapy duration). The equation predicts the development of cholelithiasis in SLE patients if the condition $G(X) \geq 19.0$ is met. If $G(X)$ is <19.0, the prediction of development of gallstone disease is negative. The 100% validity of the classification of all the patients by the gallstone group and nongallstone one was confirmed.

Table 4 gives the data obtained using the Fisher exact test for the examined SLE patients, which showing that age, mean daily dose of prednisolone and duration of its therapy are the most likely predictors of cholelithiasis in SLE patients.

3.2. Lipid-Protein Blood Spectrum and Gallstone Disease in SLE Patients. As we expected, the levels of plasma lipids, ApoB-LDL ($P = .005$) and ApoAI-HDL ($P = .05$) differed significantly in SLE patients with and without gallstones. SLE patients with gallstones had significantly higher levels of TCh ($P = .0004$), LDL-Ch ($P = .006$), HDL-Ch ($P = .008$) than those without gallstones.

When the group of SLE patients treated with corticosteroids were additionally compared, significantly higher levels of TCh ($P < .02$) and LDL-Ch ($P < .03$) were observed in patients with gallstones than in those without gallstones.

Significantly reduced levels of HDL-Ch ($P < .02$) and ApoAI-HDL ($P < .04$) were found in SLE patients treated with corticosteroids without gallstone disease.

Interestingly, the levels TG and ApoB-LDL, as well as TCh/HDL-Ch and LDL-Ch/HDL-Ch ratios, did not differ significantly in corticosteroid-treated SLE patients as compared with patients who had never been treated with corticosteroids.

3.3. The Composition and Structure of Gallstones in SLE Patients. Series of CCL-SEM color images of 10 gallstones were taken for analysis. Each image was compared with control images to distinguish the deposition of bilirubin, cholesterol, and protein components. The major components of the gallstones under examination were cholesterol (Figure 2(b)) and protein (Figure 2(c)). They were all detected over the entire surface of the scanned gallstone while rare bilirubin insertions (Figure 2(a)) were seen only at the periphery of the gallstone. All 10 of examined gallstones from 4 SLE patients with cholelithiasis due to long-term prednisolone therapy were structurally identical. The findings have provided evidence for the protein-cholesterol composition of the gallstones in corticosteroid-treated patients with SLE.

4. Discussion

Gallstone disease is one the most common disorders of the digestive system. In several past decades, there has been a tendency toward the increased incidence of gallstone disease. It can be explained by better diagnosis and by the new noninvasive ultrasound techniques being introduced into clinical practice.

Any publications on the incidence and mechanisms of cholelithiasis in SLE have not been found in the literature. This study has examined the relationship between gallstone disease and corticosteroid treatment in SLE. As far as we know, this is the first study to show the high frequency of gallstone disease in SLE patients treated with prednisolone. Regression and discrimination analyses reveal a strong association of gallstone formation in SLE patients with age at the onset of the disease, current steroid dosage, and duration of prednisolone therapy. Cholesterol metabolic disturbance is one of the possible causes of gallstone formation in SLE patients on prednisolone therapy.

Since there is a strong correlation between cholelithiasis and blood lipid-protein spectral changes in patients on corticosteroid therapy, we hypothesize that cholesterol metabolism occurring on P_{450} cytochrome of the agranular endoplasmic reticulum is altered and may be involved in gallstone formation.

Our findings suggest that higher total cholesterol levels in SLE patients with gallstones are most likely to be caused by cholesterol catabolic disorders. Up to 80% of the total cholesterol pool is oxidized to bile acids [13]. Bile acids are synthesized from cholesterol, by involving P_{450} cytochrome of the hepatic agranular endoplasmic reticulum [13]. They are the final products of cholesterol metabolism and one of the most important ways of its excretion from the body [13].

The effects of corticosteroids on the lipid-protein blood spectrum have been studied before. The plasma lipid abnormalities reported in SLE corticosteroid treatment patients are similar to those in other dyslipoproteinemias [14–16].

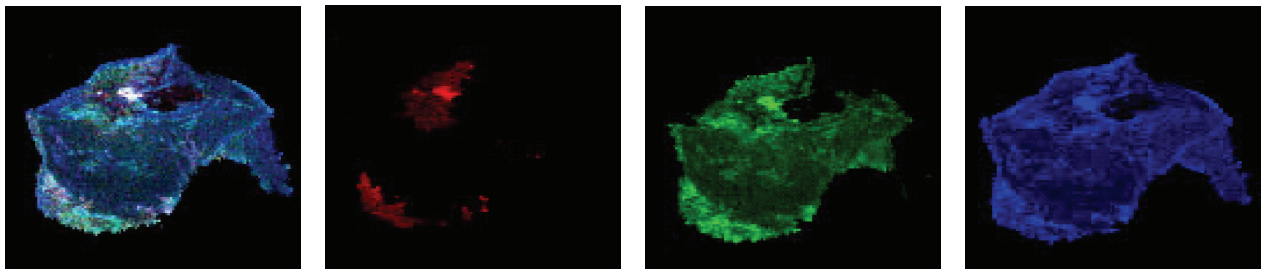
Our SLE patients with gallstones had significantly elevated LDL-Ch and HDL-Ch levels, as compared to those without gallstones (Table 2). The increased level of HDL responsible for cholesterol transport from tissues and organs to the liver may be accounted for by diminished cholesterol biotransformation to bile acids, which should stimulate cholesterol delivery to hepatocytes through the feedback system. Since HDL-cholesterol, both from the intestine and peripheral sources, is the preferred type of cholesterol for biliary secretion, increased HDL transport to the liver can also cause cholesterol hypersecretion in bile [17]. Enhanced cholesterol outflow from the peripheral tissues and cells by HDL causes an increase in LDL levels via the feedback system to maintain the normal levels of cholesterol in the tissues and cells in SLE patients long taking prednisolone. Interestingly, the ApoB-LDL levels and the LDL-C/HDL-C ratio are in the normal range, and do not differ significantly in the patients untreated or treated with prednisolone. ApoB-LDL plays a major regulatory role in the response of biliary cholesterol secretion to high-dietary cholesterol [18]. These findings suggest that cholesterol transport by means of lipoproteins is unchanged in SLE patients receiving corticosteroids long.

Recent advances in bile molecular biology and biochemistry have demonstrated that gallstone disease is a complex disorder: cholesterol supersaturation, decreased bile acid/cholesterol ratio, hydrophobic bile salts, pronucleating proteins, inflammation changes, and impaired gallbladder motility [19]. The SLE patients are prone to biliary sludge and stone formation because of biliary dyskinesia caused by ischemia of the gallbladder. Increased incidence of cholelithiasis in SLE patients may also be related to vasculitis and thrombosis. Gallbladder bile from gallstone patients contains factors that stimulate formation of macroscopic cholesterol crystals and cholesterol stones [17, 20]. Supersaturation of bile with cholesterol is generally considered to be a major trigger of cholesterol precipitation [20, 21].

Steroid hormone catabolism also takes place in the hepatocytes through the P_{450} cytochrome-dependent hydroxylation. It is one of the principal pathways of their hepatic inactivation in mammals [13, 22].

We assume that prednisolone competitively inhibits hepatocytic alpha-hydroxylases and thus decelerates cholesterol transformation into bile acids. Subsequently, it raises the serum levels of total cholesterol, LDL-Ch, and HDL-Ch, and also increases cholesterol excretion into bile. Simultaneously, enhanced cholesterol excretion is accompanied with the reduced hepatocytic secretion of bile acids into the bile capillaries, resulting in a decrease in the bile acid/cholesterol ratio. In SLE patients receiving long steroid hormones, gallbladder inflammatory changes and immunological changes in the presence of abnormal cholesterol and protein metabolisms may initiate gallstone formation (Figure 3).

Cholesterol gallstones are more common in women than in men, and exposure to oral contraceptive steroids and conjugated estrogens increases the risk for gallstones



(A) (a) (c) (b)

Figure 2: CCL-SEM micrographs demonstrating the structural organization and chemical composition of a gallstone obtained from SLE patient: (A) general gallstone structure before color separation; (a) bilirubin inclusions; (b) cholesterol component; (c) protein.

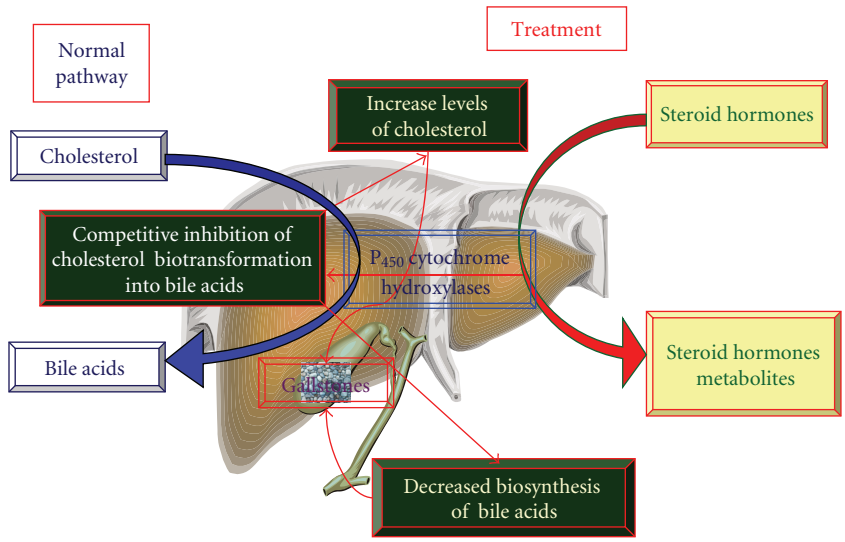


Figure 3: Diagram of prednisolone (steroid hormones)-induced inhibition of cholesterol catabolism (see the text for comments).

[23]. Sex hormones are most likely to be responsible for the increased risk. Estrogen increases biliary cholesterol secretion causing cholesterol supersaturation of bile. The hepatic estrogen receptor alpha, but not estrogen receptor beta, plays a critical role in 17β-estradiol-induced formation of cholesterol gallstones [23]. Thus, hormone replacement therapy in postmenopausal women and the use of oral contraceptives have also been described to be associated with an increased risk for gallstone disease [24]. Steroid (sex hormones, glucocorticoids, and mineralocorticoids) hormones can competitively inhibit alpha-hydroxylases of hepatocytes and thus decelerates cholesterol transformation into bile acids (Figure 3). Concomitant inflammatory diseases of the gallbladder and its motor dysfunction may lead to the development of gallstone disease. If there is evidence for this assumption, it tells about drug-induced gallstone disease.

There are also reports on that high biliary protein concentration in lithogenic bile was associated with cholesterol gallstone formation [25–27]. Mucin is supposed to accelerate the crystallization of cholesterol in model bile [28]. Wilhelmi et al. demonstrated a dose-dependent effect of human but not of bovine gallbladder mucin on the formation of cholesterol monohydrate crystals in gallbladder bile of patients with cholesterol stones [28]. An inflammation-dependent epidermal growth factor receptor cascade causes overproduction of the gel-forming mucin MUC5AC, which accumulates in cholesterol gallstone disease [29].

Despite the importance of high protein and cholesterol concentrations on the sequence of events in gallstone formation, very little is known about the relationship between cholesterol and protein to the microarchitectonics of gallstones [27]. The standard technique for visualization of cholesterol stones is scanning electron microscopy (SEM) [30, 31]. However, this technique does not permit identification and localization of chemical organic deposits within the gallstone.

In present study, we used the novel electron microscopy technique, color cathodoluminescence scanning electron microscopy (CCL-SEM), to investigate the chemical composition and microstructure of gallstones obtained from patients with SLE.

The main advantage of CCL-SEM over SEM is its capability to visualize different organic and inorganic components and their distribution over the gallstone section surface [12]. This information can potentially contribute to the elucidation of the mechanisms of gallstone formation.

The CCL-SEM technology allowed us to generate CCL-mapping of color images of 3 constituents of studied gallstones, unesterified cholesterol, unconjugated bilirubin, and protein (Figure 2). Comparative analysis with control images (see Figure 1) has identified cholesterol (Figure 2(b)) and protein (Figure 2(c)) as major components with homogeneous distribution from the center to the periphery of the gallstone. In contrast, rare bilirubin insertions (Figure 2(a)) were observed only at the periphery. CCL-SEM of all study gallstones from SLE patients with gallstone disease demonstrated their similar structure and chemical composition. The protein-cholesterol nature of gallstones in SLE patients may serve as circumstantial evidence for the above assumption that gallstone disease develops due to impaired cholesterol and protein metabolisms during cholepoiesis under the action of corticosteroids.

In conclusion, gallstone disease was detected in 36.7% of the examinees; 68.8% of SLE patients on corticosteroid therapy had cholelithiasis. Age at the onset of the disease, current steroid dosage, and steroid therapy duration are factors that predispose to gallstone formation. The CCL-SEM study identified predominantly the protein-cholesterol structure of gallstones. Multicenter-randomized studies are required to support the aforesaid assumption that long-term steroid hormones therapy is a model of cholesterol lithogenesis. Moreover, if there is evidence that cholelitiasis is induced by steroids, this will provide impetus to the search for and design of new drugs and therapeutic approaches to treating autoimmune diseases.

Acknowledgments

The authors would like to thank Professor Nataliya V. Perova, Dr. Irina N. Ozerova (State Research Center for Preventive Medicine, Moscow, Russia) for obtaining the data on the lipid profile, and Dr. Tatiana B. Prokaeva (Boston University School of Medicine, Boston, Mass, USA) for their assistance and support of this work. They also would like to thank Aleksandr K. Tarasov, Candidate of Engineering Sciences, and Mikhail I. Barsov for assistance in the statistical processing of the findings.

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