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Serum Squalene in Patients with Gallstones*)

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ABSTRACT

Serum squalene concentration, which represents the degree of cholesterol synthesis, and other serum and biliary lipids were determined and characteristic abnormalities of lipid metabolism were discussed in patients with gallstones having high serum squalene.

The serum squalene concentration was significantly high in patients with pure cholesterol and mixed stones. It remained in normal range in patients with combination and bilirubinate stones. The high serum squalene concentration was accompanied by a high lithogenic index and serum triglyceride concentration. Patients with high serum squalene were also accompanied by increased percent cholic and decreased percent chenodeoxycholic acid.

These results may indicate that patients with high serum squalene in our present study have similar lipid abnormalities as type IV hyperlipoproteinemia which has increased cholesterol synthesis, hypertriglyceridemia and increased cholic acid pool and normal chenodeoxycholic acid pool size.

INTRODUCTION

Biliary cholesterol which increases in patients with cholesterol gallstones is estimated to be originated from cholesterol carried from the intestine (exogenous)[2,7] and carried by high density lipoprotein from peripheral tissues (endogenous)[11], or synthesized in the liver. Among these origins of excess biliary cholesterol that develops into gallstone formation, the greatest importance is placed on hepatic cholesterol synthesis.

The hepatic synthesis of cholesterol is regulated by rate-limiting enzyme, hepatic HMG CoA reductase, which activity is reported to increase in gallstone patients[10]. However, it can be easily estimated that the increased synthesis of cholesterol may occur not only in the liver but also in many other organs, e. g., the intestine of patients. Of course, it is impossible to collect biopsy specimens from many human organs to determine individual enzyme activities.

Nestel and Kudchodkar[9] recently reported that plasma squalene, a metabolite to cholesterol, is useful as an index of the degree of cholesterol synthesis in humans. Exogenous squalene disappears from serum within several hours after meal and, therefore, the serum squalene level at fast seems to be originated from synthesized squalene and reflect the amount of cholesterol synthesized in the whole body tissues.

In our present study the serum squalene concentration was determined in patients with gallstones and compared to other serum and biliary lipids to evaluate the roles of synthesized cholesterol in the development of cholesterol gallstones.

MATERIALS AND METHODS

Twenty-eight patients with gallstones, 6 males and 22 females, were admitted to our department for preoperative examinations. Gallstones were diagnosed by oral or dripinfusion cholecystography, endoscopic retrograde choledochography

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and ultrasonography. The types of surgically extracted stones were determined by stereoscopic microscopic observation of the surface and cross-section.

Age-matched normal volunteers were the medical staffs, laboratory assistants and patients in the healing stage of peptic ulcer with normal nutrition and liver function and without symptoms. Cystic bile was collected at fast by duodenal intubation or direct puncture of the gallbladder during surgical removal. Twenty milliliters of blood were drawn before breakfast from the median vein. Serum was separated by centrifugation at 3,000 r. p. m. and stored at −20°C until analysis. Blood from one gallstone patient and three normal subjects was drawn at 8 am, 12 am, 6 pm and 12 pm before meals to observe the diurnal rhythm of serum squalene concentration. Serum squalene was determined by the method described by Liu et al.[8] with minor modification as shown in Fig. 1. Analysis of squalene was made with a Shimadzu-GC-6A gaschromatograph equipped with a hydrogen flame ionization detector and a glass column packed with 2.5% OV-1 on 60-80 mesh Shimalite. Squalane, an internal standard, was purchased from WAKO Pure Chemical Industries, Ltd.

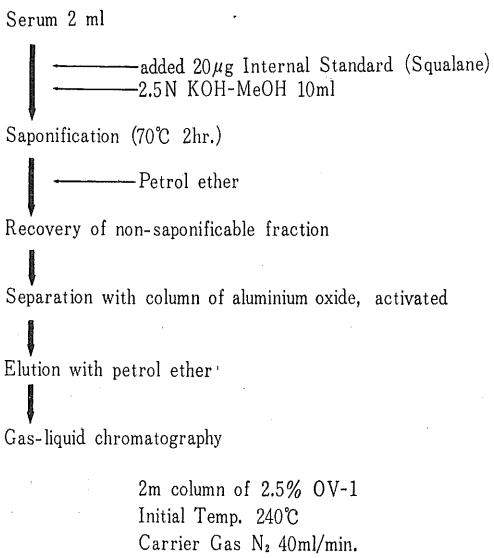
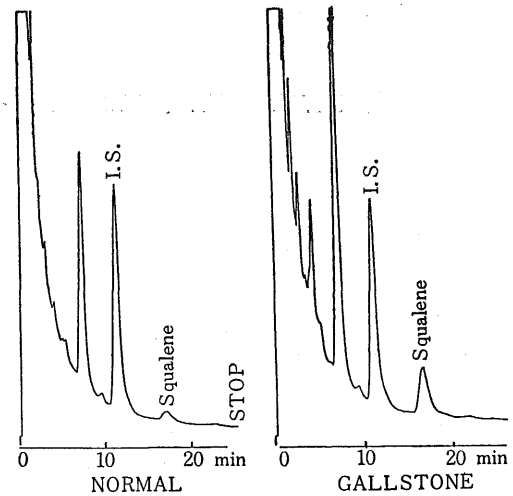


Fig. 1. Determination of Squalene (Liu's method)

Fig. 2 shows a representative chart of squalene and squalane (internal standard) in normal subjects and patients with gallstones. Serum triglycerides were determined by the enzyme method using a GSA-2D autoanalyzer (Grainer). Biliary lipids were analyzed and lithogenic index was calculated as described earlier[5,12].



Internal Standard : Squalane
Column 2.5% OV-1 length 2m
Initial Temp. 240℃
Flow rate N_2 40ml/min.

Fig. 2. Gaschromatographic Charts of SQUALENE

RESULTS

1. Diurnal change in serum squalene

The diurnal change in serum squalene showed the highest elevation at 12 pm in both normal subjects and a patient with gallstone (Fig. 3).

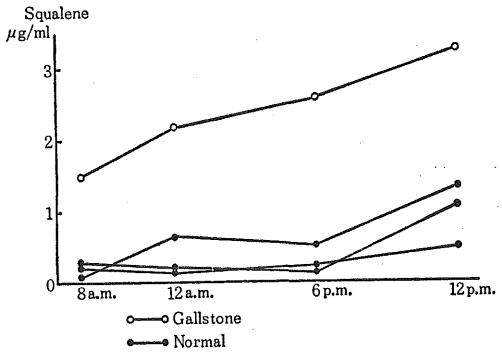


Fig. 3. Diurnal variations of serum squalene (Before meal)

2. Serum squalene concentration in normal subjects and patients with gallstones.

The serum squalene concentration of twelve

normal subjects ranged from 0.42 to 0.96 (0.69±0.27 μg/ml) (mean±standard deviation). Patients with pure cholesterol stone ($p<0.05$) and mixed stone ($p<0.001$) had a significantly high squalene concentration but patients with combined stone and bilirubinate stone had no different concentration from normal subjects (Table 1).

Table 1. Serum squalene in patients with gallstones

	n	M μg/ml	S D	
Normal subjects	12	0.69	0.27	$p<0.05$
Pure cholesterol stone	5	1.76	1.00	$p<0.001$
Mixed stone	15	1.70	0.84	
Combined stone	3	0.83	0.47	
Bilirubinate stone	5	1.04	0.47	

3. Correlation between serum squalene and triglycerides and between serum squalene and lithogenic index.

As shown in Figs. 4 and 5, significant correlations were noted between them.

4. Bile acid composition of bile in patients with cholesterol gallstones.

On comparing the bile acid composition in patients with gallstones between the groups of the squalene concentration below 1.0 and above 1.5 μg/ml, the patients with squalene concentration above 1.5 μg/ml had higher percent cholic acid and lower percent chenodeoxycholic acid, as compared with those below 1.0 μg/ml, although there was no statistic significance between the two groups (Table 2).

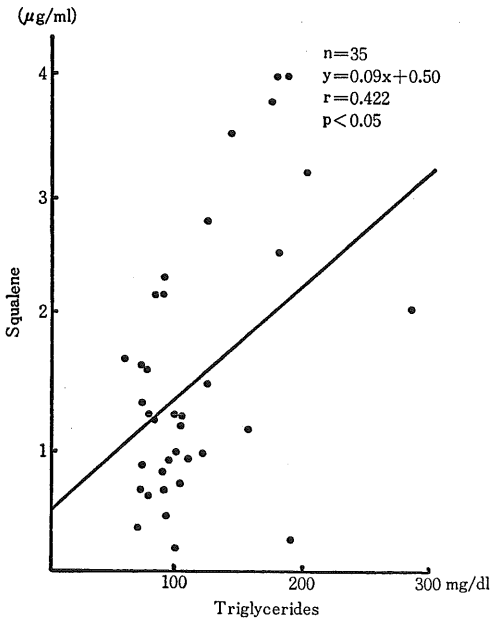


Fig. 4. Correlation between serum squalene and triglycerides

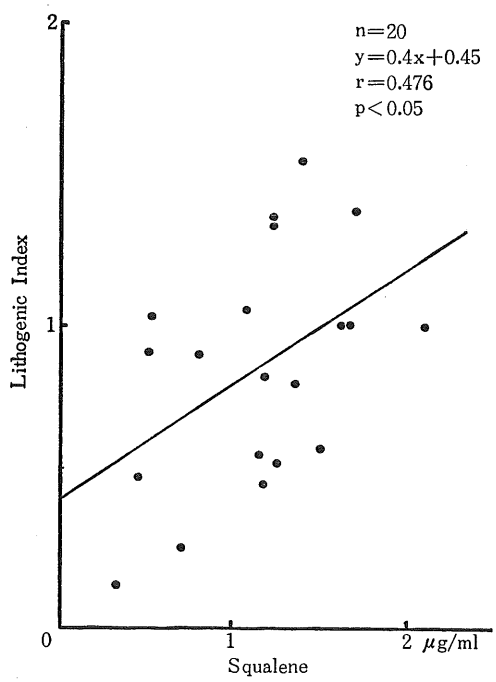


Fig. 5. Correlation between serum squalene and lithogenic index (Admirand-Small)

Table 2. Bile acid composition of bile in patients with cholesterol gallstones

		Squalene (μg/ml) 1≦1.0 (n = 6)	>1.5 (n = 6)
Cholic acid	%	30.5± 8.5	41.0± 8.0
Chenodeoxycholic acid	%	41.4± 9.6	36.9±14.1
Ursodeoxycholic acid	%	5.5± 4.1	6.0± 1.7
Deoxycholic acid	%	19.6±10.4	14.0± 8.2
Lithocholic acid	%	4.2± 2.6	2.0± 1.2
Cholic/Chenodeoxy-cholic acid		0.74± 0.2	1.11± 0.7

±S D

DISCUSSION

It has been well known that cholesterol synthesis is more accentuated at night than day time. This phenomenon has been proven by the diurnal fluctuations of cholesteral synthesis limiting enzyme, HMG CoA reductase, which

activity is highly accentuated at night[4,6]. The nocturnal elevation of serum squalene in our present experiment, therefore, seems to support that the serum squalene concentration represents the degree of cholesterol synthesis in the whole body tissue.

The significant high elevation of serum squalene concentration occurred in pure cholesterol and mixed stones, reflecting accentuated tissue synthesis of cholesterol and its excess excretion into bile and resulting in a formation of lithogenic bile. Two combination stones also contained cholesterol in their inner part, but the patients showed no elevation of serum squalene concentration. Most of the patients with bilirubinate stones which are formed by an entirely different mechanism from cholesterol gallstones also showed no elevation of serum squalene concentration.

From the correlation between serum squalene and serum triglycerides and between serum squalene and lithogenic index, it can easily accepted that patients with cholesterol gallstones having a high serum squalene concentration have frequently hypertriglyceridemia. In earlier studies, it has been reported that type IV hyperlipoproteinemic patients (=hypertriglyceridemia) have significantly elevated HMG CoA reductase as compared with normal subjects[1] and that type IV hyperlipoproteinemic patients have more cholic acid synthesis and, therefore, a larger pool size of cholic acid as compared with normal subjects and other types of hyperlipoproteinemia[3]. From one of our results, patients with higher serum squalene concentration (>1.5 μg/ml) were found to have higher percent cholic acid than those with lower squalene concentration, although there was no statistically significant difference.

Therefore, we can speculate that cholesterol gallstones with increased serum squalene (mostly pure cholesterol and mixed stones) are often accompanied by an increased cholic acid pool as well as increased cholesterol synthesis and high serum triglyceridemia. In the earlier study of Vlachevic[13] it was emphasized that patients with cholesterol gallstones have not only elevated hepatic cholesterol synthesis but also a decreased bile acid pool size (cholic acid) with decreased bile acid synthesis limiting enzyme 7α-hydroxylase activity.

The patients in our present study may have different mechanisms of gallstone formation from those described by Vlahcevic et al.[13] as mentioned above.

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