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THE INFLUENCE OF AGING UPON GALLSTONES -CHANGES IN SERUM LIPIDS, LITHOGENESITY OF BILE AND CROSS-SECTIONAL VIEWS OF STONES WITH AGE-*)

By

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ABSTRACT

Differences in lithogenesity of bile, and the type of stones of the radiolucent gallstone patients were investigated by both the roentgenogram (solitary and multiple) and cross-sectional views in comparison between the patients of ages below 39 and above 40 either with or without hyperlipidemia and lipoproteinemia.

Hypercholesterolemia was rare in patients of age below 39 in contrast to those of above 40 in cases of solitary ($p<0.05$) and multiple stones (N. S.). The incidence of hypertriglyceridemia was high in these patients of both generations. In the lipoprotein DISC electrophoresis, 14 (37.8%) of 37 patients of age below 39 had hyperlipoproteinemia, whereas 41 (47.1%) of 87 patients of age over 40 had hyperlipoproteinemia that belonged to type IIa, IIb or IV.

Among the types of hyperlipoproteinemia, IIa and IIb were seldom seen in the patients of age below 39 but IIa, IIb and IV were almost equally spreaded over in the patients of age above 40. The solitary stone, on the whole, was accompanied with a high incidence of supersaturated bile irrespectively of the generation and presence or absence of hyperlipoproteinemia. The incidence of supersaturated bile was rare in the normolipidemic patients with multiple stones, particularly the patients of age above 40. This incidence in the patients of age above 40 with multiple stones was significantly lower in the normolipidemic than hyperlipoproteinemic patients ($p<0.01$). The patients with solitary stone had highly concentrated cholesterol stones as compared with those with multiple stones which sometimes contained other stones than the cholesterol stone. The stones of hyperlipoproteinemic patients also contained generally the higher concentration of cholesterol than those of normolipoproteinemic patients.

The patients of age above 40 had more combination stones (11 : 1) and other stones (remainder) (5 : 1) than those of age below 39.

INTRODUCTION

Although the gallstone disease has been well known to be highly prevalent as the age advances, quite a number of younger patients with gallstones are clinically encountered, as has been exemplified by the recent studies on a very high incidence of gallstones in 70% of

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American Pima Indian girls by the age of 27[1]. The recent population study also suggested that excess food intake was influential in inducing the prevalence of gallstones resulting in the high occurrence of this disease in a younger generation[2].

The initiation and development of cholesterol gallstone are not only regulated by the relative amount of cholesterol to bile acids and lecithin in bile but also by the core formation by cell debris, precipitation of pigment calcium or bile congestion caused by biliary inflammation.

It was suggested in the previous studies of the authors that serum and lipoproteins were closely related to the initiation, precipitation and aggregation of cholesterol crystals and development of cholesterol gallstones though lipid metabolism of the whole body[3]. However, the disturbance of lipid metabolism differs among the individual patients. The difference in the lipid metabolic disturbance is considered due directly to and/or affected indirectly by such factors as heredity, excess calorie consumption, kind of foods, pregnancy, sex, age and diseases of various kinds other than gallstones.

In the present study, serum lipids, lipoproteins, lithogenesity of bile and type of stones were analyzed and compared between the younger and older generations in order to inquire wher there are differences in them between the two generations and how these differences are associated with the formation of cholesterol gallstone.

In this investigation, the younger generation was defined as the generation of age below 39 and the older generation above 40, because the forties is the decade (one of the five F's for gallstones) in which the gallstone diseases dramatically increase.

Figure 1 shows the age distribution of gallstone disease studied in this examination. The incidence is clearly distinguished between thirties and forties especially in female patients.

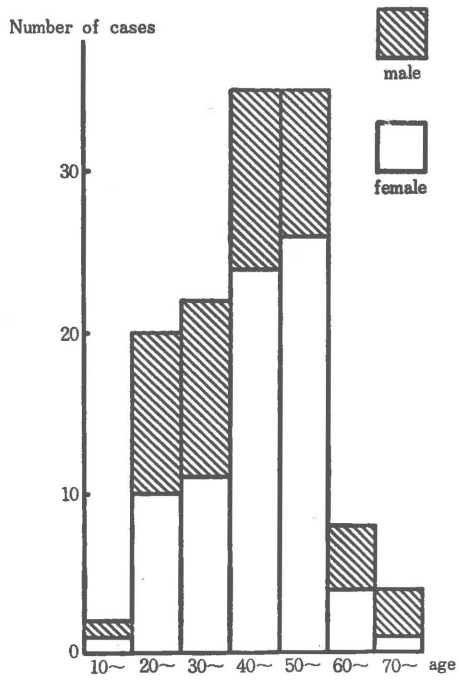

Fig. 1. Age distribution of radiolucent stones in patients examined in this study

METHODS

The radiolucent gallstones of 125 patients were examined, who comprised 48 males and 77 females, of whom 46 were seen having solitary and 79 multiple stones in the roentgenogram. The item and the number of cases examined in this study are listed in Table 1. Serum cholesterol and triglycerides at fast were determined by the enzyme method reported by Allain et al.[4] and Fletcher[5] respectively.

The ranges of 150–250 mg/dl and 50–130 mg/dl were defined as normal for serum cholesterol and triglycerides respectively.

Lipoproteins were analyzed by lipoprotein-DISC electrophoresis using polyacrylamide gel as described by Wada and Mise[6]. The type of hyperlipoproteinemia was determined by the height of β-and pre-β-lipoproteins after densitometry of gel columns according to the WHO report[7].

Bile was collected early in the morning at fast by duodenal intubation and gallbladder bile was served for bile lipid analysis. After bile, added with 1N NaOH, was hydrolyzed under 1 kg/cm^2 at 120°C for 3 hours, cholesterol was extracted in an alkline solution and bile acids were extracted after acidification of the solvent. Bile acids were methylated and propionated, and analyzed by gas-liquid chromatography as described by Kawamoto et al.[8] Cholesterol was also determined by gas-liquid chromatography. Biliary lecithin was determined as described previously[8].

The lithogenic index was calculated by the

Table 1. The item and the number of patients studied

Items studied	Roentgenogram	Serum cholesterol & triglycerides	Lipoprotein electrophoresis	Lithogenic Index	Cross-sectional examination of stones
Number of patients	125	125	124	72	38

formula described by Thomas and Hofmann[9] based on the triangular coordinate graph of Holzbach[10].

The lithogenic index of less than 1.0 indicates the unsaturated bile and that equal to or of more than 1.0 indicates the saturated or supersaturated bile with choleterol.

Thirty-eight patients were subjected to surgical operation immediately or during the period of 6 months after the serum and biliary lipid examination. The stones excised from them were divided by the cross-sectional views using a stereoscopic microscope (×20) into four types; pure cholesterol, combination, mixed and other stones (remainder) without cholesterol content (pigment-calcium and rare forms).

The combination stones were subdivided into three types; (1) pure cholesterol in the center surrounded by pigment-calcium, (2) mixed stone in the center surrounded by pigment-calcium and (3) pigment-calcium in the center surrounded by cholesterol. Mixed stones were also subdivided into two types; (a) subradiated white stone containing highly concentrated cholesterol with a small core in the center and (b) radial concentric brown stone with a core in the center[11].

RESULTS

1. Serum cholesterol and triglycerides:

Figures 2–5 show the serum cholesterol and triglyceride levels in gallstone patients of ages below 39 and above 40. Table 2 and Figures 6–7 indicate the incidences of hyperlipidemia in both the generations.

As shown in Figures 2, 3 and 6, hypercholesterolemia was rare in patients of age below 39 as compared with those of above 40. It was statistically significant in the patients having solitary stone ($p<0.05$) and the same tendency, although without statistical significance, was observed in the patients having multiple stones.

As shown in Figures 4, 5 and 7, the incidence of hypertriglyceridemia was high, on the con-

Table 2. The incidence of hypercholesterolemia and triglyceridemia

Type of stone †	Age	Cholesterol normo-	Cholesterol hyper-	Cholesterol total	Triglycerides normo-	Triglycerides hyper-	Triglycerides total
Solitary	≦39	16	2	18	8	10	18
〃	≧40	16	12	26	16	12	28
〃	Total	32	14	46	24	22	46
Multiple	≦39	24	2	26	19	7	26
〃	≧40	41	12	53	37	16	53
〃	Total	65	14	79	56	23	79
Solitary + Multiple	≦39	40	4	44	27	17	44
	≧40	57	24	81	53	28	81

* $p<0.05$ ∞ NS
** $p<0.025$
† Number of stone in the roentgenogram

Fig. 2. Serum cholesterol levels of patients with solitary stone

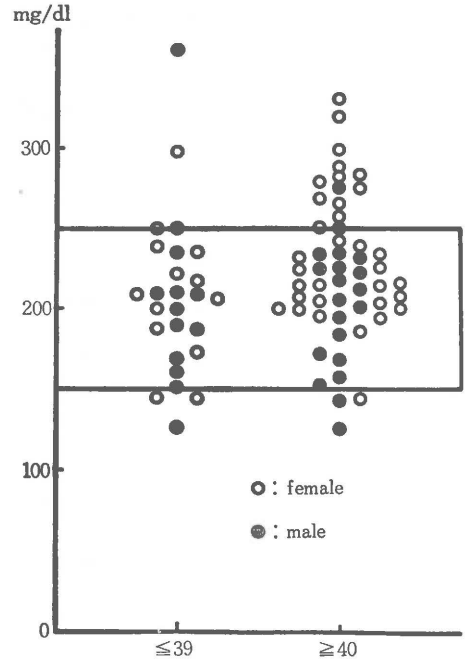

Fig. 3. Serum cholesterol levels of patients with multiple stones

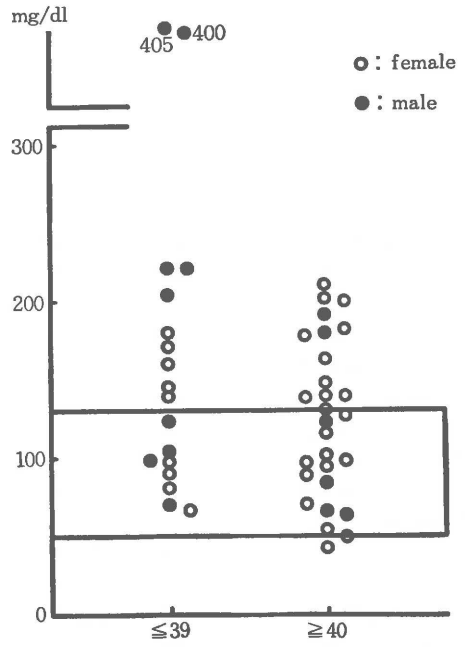

Fig. 4. Serum triglyceride levels of patients with solitary stone

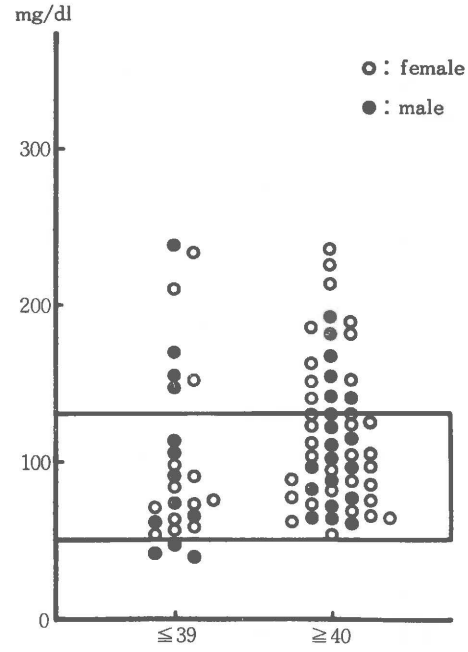

Fig. 5. Serum triglyceride levels of patients with multiple stones

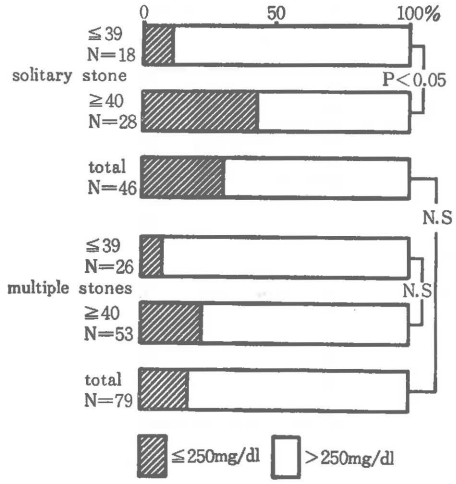

Fig. 6. The incidence of hypercholesterolemia in patients with solitary and multiple stones

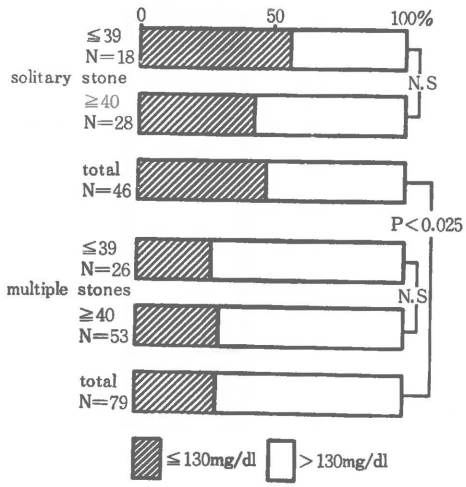

Fig. 7. The incidence of hypertriglceridemia in patients with solitary and multiple stones

trary, in these patients regardless of age, without any statistical significance between the two generations.

However, comparison between the solitary and multiple stones revealed significantly higher occurrence of hypertriglyceridemia in the solitary than the multiple stones ($p<0.025$).

2. Serum lipoprotein electrophoresis:

Of 37 patients of age below 39, 14 (37.8%) presented hyperlipoproteinemia and of 87 patients of age above 40, 41 (47.1%) presented hyperlipoproteinemia of type IIa, IIb or IV. There was no statistically significant difference in the incidence of hyperlipoproteinemia between two generations. But among the three types of hyperlipoproteinemia, type IIa was seldom seen in patients of age below 39 in contrast to those of above 40 both with solitary and multiple stones ($p<0.05$). There was the same tendency with type IIb but a higher rate of type IV was seen in the younger generation.

Unlike the above, however, the incidences of types IIa, IIb and IV were almost even in the patients of age above 40.

3. Lithogenic index:

Table 4 lists the lithogenic index of bile of 72 gallstone patients.

Supersaturated bile in the cases of normoli-

Table 3. Types of hyperlipoproteinemia of gallstone patients

Type of stone †	Age	Normolipo-proteinemic	Hyperlipoproteinemic				Total
			IIa	IIb	IV	Sub-total	
Solitary	≦39	7(50.0)	1(7.1)	1(7.1)	5(35.7)	7(50.0)	14
〃	≧40	12(44.4)	6(22.2)	5(18.5)	4(14.8)	15(55.5)	27
Multiple	≦39	16(69.5)	1(4.3)	2(8.7)	4(17.3)	7(30.4)	23
〃	≧40	34(56.6)	8(13.3)	7(11.7)	11(18.3)	26(43.3)	60
Solitary + Multiple	≦39	23(62.2)	2(5.4) — *	3(9.1)	9(24.3)	14(37.8)	37 — ∞
	≧40	46(52.9)	14(16.1) — *	12(13.8)	15(14.9)	41(47.1)	87 — ∞

( ) = %
* $p<0.05$ ∞ NS
† The number of stone in the roentgenogram

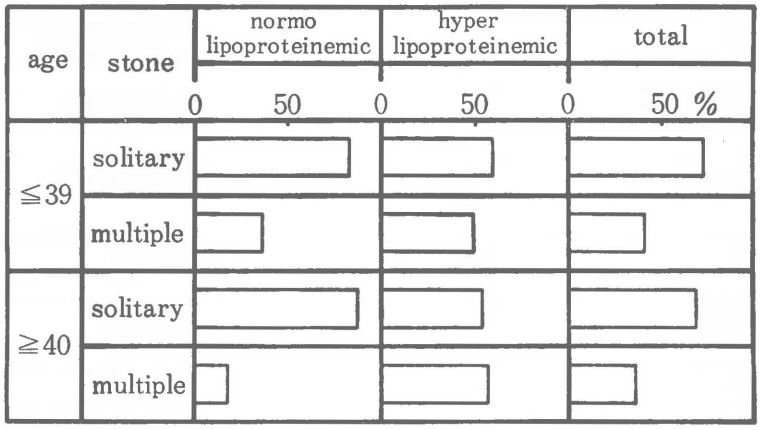

Fig. 8 The incidence of supersaturated bile in normo- and hyper-lipoproteinemic patients of ages below 39 and above 40

Table 4. The incidence of lithogenic bile (supersaturated) in normo- and hyper-lipidemic patients with gallstones <Holzbach>

Serum lipoprotein	Normolipo-proteinemic		Hyperlipoproteinemic									
			IIa		IIb		IV		Sub-total		Total	
†	≧1.0	<1.0	≧1.0	<1.0	≧1.0	<1.0	≧1.0	<1.0	≧1.0	<1.0	≧1.0	<1.0
Type of stone												
Age ≦39												
Solitary	5	1	0	0	1	1	2	1	3	2	8	3
Multiple	3	5	1	0	0	1	1	1	2	2	5	7
Age ≧40												
Solitary	7	1	4	1	1	2	1	2	6	5	13	6
Multiple	3	13	5	1	2	1	1	4	8	6	11	19
		***										

*** $p<0.01$

† Lithogenic index of bile

poproteinemia was rarely incident in the patients with multiple stones (especially in the patients of age above 40) in contrast to those with solitary stone.

On the contrary, in the cases of hyperlipoproteinemia, there was no difference in the occurrence of supersaturated bile between the solitary and multiple stones. The incidence of supersaturated bile was low in multiple stones, particularly in the normolipoproteinemic patients when compared with the hyperlipoproteinemic patients ($p<0.01$).

4. Cross-sectional views of gallstones:

The normolipoproteinemic patients of age below 39 with solitary stone had either pure cholesterol stone or mixed stone (a) that contained highly concentrated cholesterol. These patients with multiple stones, however, had mixed stone (a), mixed stone (b) or other stones (remainder) than cholesterol stones. The hyperlipoproteinemic patients of the same age generation with solitary stone had either pure cholesterol or combination stone (1) with pure cholesterol in the center.

The normolipoproteinemic patients of age above 40 with solitary stone had combination

Table 5. Cross-sectional views of solitary and multiple stones

age	serum lipoprotein	roent genogram	cross sectional view of stones									
			pure cholesterol	combination (1)		(2)	(3)	mixed (a)		(b)	others
≦39	normo-	solitary	••					•••			
		multiple						•		••	•
	hyper-	solitary	•	•							
		multiple									
≧40	normo-	solitary		•						••	
		multiple				•	••			••	•••
	hyper-	solitary	••	•			•	••			
		multiple	•	•		••	••	•		•	••

Table 6. Cross-sectional views of gallstones

Age	Pure cholesterol	Combination (1)	(2)	(3)	Mixed (a)	(b)	Others
≦39	3	1			4	2	1
≧40	3	3	3	5	3	5	5

stone (1) or mixed stone (b), and those with multiple stones had combination (2), combination (3), mixed (b) or other stones (remainder) than cholesterol.

The hyperlipoproteinemic patients with solitary stone had stones of high cholesterol content except for one case which had combination stone (3), and those with multiple stones showed all types of stones from pure cholesterol to other stones (remainder).

The solitary stone, on the whole, contained a higher amount of cholesterol (i. e., pure cholesterol, combination (1) or mixed (a)) than the multiple stones. The multiple stones, in contrast, not only contained a low amount of cholesterol (combination (2), combination (3) and mixed (b)) but also were sometimes composed of other stones than cholesterol stones.

The hyperlipoproteinemic patients, irrespectively of the generation, had stones that contained a higher amount of cholesterol than the normolipoproteinemic patients did.

The patients of age above 40 had more combination stones (11 : 1) and other stones (remainder) (5 : 1) than those of below 39 did.

DISCUSSION

The incidences of hypercholesterolemia and triglyceridemia increase with age. Furthermore, such hyperlipidemia is frequently seen in the atherosclerotic patients who also increase with age.

Hyperlipidemia in gallstone patients can be estimated as incidental to aging or atherosclerosis. However, the higher incidence of hypercholesterolemic and triglyceridemia in the patients with radiolucent than radiopaque stone, rareness of hypercholesterolemia in males and affluence in females as previously reported[3] suggest that hyperlipidemia seen in gallstone disease is not simply accompanied by aging or atherosclerotic change of patients but is concomitant with cholesterol gallstone itself, be-

cause the majority of radiolucent stones is the cholesterol stones, most of the radiopaque stones are the pigment-calcium stones which are unassociated with cholesterol metabolism and serum cholesterol increases with age and by atherosclerotic effect not only in females but also in males.

It is, therefore, possible to conclude decisively that the cholesterol gallstone patients are subject to hyperlipidemia (either cholesterolemia or triglyceridemia, or both of them) at a higher rate of occurrence.

The present investigation revealed a lower incidence of hypercholesterolemia in the patients with radiolucent stones of age below 39 as compared with those of above 40 but there was no significant difference in the incidence of hypertriglyceridemia between the two generations. The above results (on the incidence of hypercholesterolemia and triglyceridemia in two generations) also suggest the presence of differences in the incidence of hyperlipoproteinemia by the type between two generations.

62.2% of the gallstone patients of age below 39 proved to be normolipoproteinemic and remaining 37.8% had hyperlipoproteinemia of which types IIa and IIb were very rare in contrast to type IV. 52.9% of the patients of age above 40 proved to be normolipoproteinemic and remaining 47.1% had hyperlipoproteinemic which, however, displayed no difference in the occurrence among the three types of hyperlipoproteinemia.

The lipid metabolism in the liver in three types of hyperlipoproteinemia somewhat differs among one another, which was at times observed as having hereditary origin but was also caused by excess food intake. Intake of excess cholesterol or saturated fatty acids is commonly known to cause types IIa and IIb in human beings as well as in animals. Type IV is usually brought forth by excess intake of refined carbohydrate, frequently with an increase in body weight.

The difference in the incidence of hyperlipoproteinemia and its type seems likely to influence the incidence of stone formation and the number of stones, whether solitary or multiple, because the solitary and multiple stones pass through the different metabolic course in their production and development[12,13]. It is also acceptable that there are different lipid metabolism among the gallstone patients with or without hyperlipoproteinemia and among the types of hyperlipoproteinemia.

Cases of gallstone disease in a young generation have frequently been empirically encountered in a family in which this disease was prevalent. As shown in Figure 1, on the other hand, the occurrence of this disease increases dramatically in human beings after the age of 40.

These facts permit estimation that the young gallstone patients of age below 39 have lipid methbolism that differs from those of age above 40 and that such different lipid metabolism in the liver is involved in the serum lipid levels, cholesterol concentration in bile and eventually the stone type.

The normolipoproteinemic patients with solitary stone had in most cases supersaturated bile (lithogenic bile), regardless of the generation. On the contrary, unsaturated bile was seen in many normolipoproteinemic patients with multiple stones and particularly in most of the patients of age above 40 there was seen unsaturated bile.

This indicates that some of the multiple stones in normolipoproteinemic patients, especially of age above 40 are scarcely or not related to the cholesterol saturation in bile and, therefore, that the initiation and development of gallstone are achieved with the aid of some other factors such as inflammation or biliary congestion, with the consequence of stones being formed of materials other than cholesterol. The cross-sectional views of the stones endorse this fact.

Of the stones obtained from 8 normolipoproteinemic patients of age above 40 with multiple stones, three proved to be the combination stone ((2) or (3)), two the mixed stone (b) and the rest, three, were the other stones (remainder) (pigment-calcium or rare forms).

On the contrary, the patients with hyperlipoproteinemia presented all types of stones covering pure cholesterol, combination (1) and mixed stones (a) that contained rather highly concentrated cholesterol, some of which, however, were formed with a pigment core or outer shell.

The incidence of supersaturated bile in the patients of age above 40 with multiple stones was significantly higher as shown in Table 4 and Figure 8 in hyperlipoproteinemia than normolipoproteinemia. ($p<0.01$)

The difference in the gallstone patients of generation between above 40 and below 39 as shown in Table 6 was distinguishably characterized by the higher production and presence of combination stones (1), (2), (3) and other stones (remainders) in the former than the latter.

A great many questions on the mode of cholesterol gallstone development still remain unsolved, one of the questions, for instance, being what concerns the pathogenesis of gallstone in which two theories have been advocated, one in support of development from the core[14] and the other, the development from outside into the center[15]. However, the general approval seems to have been given to the following: the stones with pure or high cholesterol concentration may be associated with long-lasting excretion of highly concentrated cholesterol which is caused by abnormal lipid metabolism; the stones with a core in the center is developed by the aid of other factors (e. g., inflammation) at the initial stage; and the formation of combination stone is achieved by at least two different causes, e. g., the precipitation of cholesterol from supersaturated bile and pigment aggregation due to inflammation.

As explicated in the foregoing, a variety of factors combined together is considered involved in the formation and development of gallstone under certain regulations, amongst which age and serum lipids appear to exercise large ininfluence upon the composition of gallstone through lipid metabolism in the liver.

The medical treatment of gallstone disease has in recent years attained a remarkable progress. Bile acid preparations (urso- and chenodeoxycholic acid) produce dramatic success in dissolution of gallstone on suitably selected patients. Many problems are still left unsolved. Relapse of stone, for example, is one of the most important subjects to be most urgently solved. The utmost attention has to be paid to establishing the effective treatment after achievement of gallstone dissolution for preventing relapse of this disease. The intermittent or continuous low dose administration of bile acid preparations is recommended for this purpose. At the same time, great attention is being drawn to the importance of the dietary fiber, brawn[16], plant sterols[17] and food restriction[1] for the treatment of stones and the prevention of relapse.

The above results would provide helpful guide for selecting the appropriate procedures not only of medical treatment but also prevention of relapse of gallstone diseases.

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