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THE EFFECT OF DIABETES MELLITUS AND ITS TREATMENT ON THE LITHOGENESITY OF BILE IN MAN*)

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ABSTRACT

Bile lipid and bile acid compositions of twenty-five diabetic patients (eight males and seventeen females) were compared with those of twenty normal subjects and twelve non-diabetic patients with cholesterol gallstone. Diabetic patients were grouped into the untreated, insulin-treated and untreated patients with gallstone.

The results are as follows. The average molar percentage of cholesterol and incidence of lithogenic bile were higher in all the groups of diabetic patients as well as non-diabetic cholesterol gallstone patients than in normal subjects. However, the incidence of lithogenic bile was distinctly higher in diabetic patients treated with insulin and patients with gallstone as compared with untreated patients. The average molar percentage of phospholipids was significantly higher in diabetic patients untreated and treated with insulin than normal controls but was almost normal in diabetic patients with gallstone and non-diabetic cholesterol gallstone patients.

Diabetic patients treated with insulin and those with gallstone tended to have a low molar percentage of chenodeoxycholic acid, which was significant in non-diabetic gallstone patients. These results may indicate that the diabetic patients who fall into gallstone have the bile lipid and bile acid compositions that differ from those of diabetic patients without gallstone and that the insulin administration deteriorates lithogenesity of bile in diabetic patients.

INTRODUCTION

A gallstone is frequently associated with a lot of complications such as hyperlipidemia[1], diabetes mellitus[2,3], ileal diseases (ileostomy)[4,5] and hepatic injuries[6].

It has been reported by Lieber that the incidence of gallstone was 30.2% in Caucasian patients with diabetes mellitus and increased with age, exceeding those in a control population (11.6%)[2].

Many investigators have clarified the disturbed lipid metabolism as well as glucose metabolism resulting in a formation of abnormality of bile lipid and bile acid compositions in diabetic patients[7,8]. An inhibited turnover rate[9] and increased secretion rate of cholesterol[10] accompanied by the increased bile acid secretion into

*) 梶山梧朗，小山田健，中尾精治，三好秋馬：ヒトにおける糖尿病およびその治療が胆汁の胆石形成度に及ぼす影響

bile[10] were seen in diabetic rats, in which, furthermore, the trihydroxy bile acid pool sizes and their synthesis were enlarged and the cholesterol absorption rate was accerelated through intestines[10].

Many patients with diabetes mellitus, particularly the maturity-onset diabetes, have lithogenic bile with an increased proportion of cholesterol[8] which results in a production and development of gallstone. However, the above findings are mostly attributed to Caucasians and are observed in the Japanese only inadequately at present. The recent increased incidence of gallstone is said to be due to the increased incidence of cholesterol gallstone rather than pigment-calcium stone that has been prevalent among the Japanese patients.

In this study bile lipid and bile acid compositions in maturity-onset diabetic patients and gallstone patients were analyzed in order to clarify the influence of diabetes mellitus and its therapy on the biliary lipid composition and eventually on lithogenesity.

MATERIALS AND METHODS

Patients and specimens: Twenty-five patients with maturity-onset diabetes mellitus (eight males and seventeen females) were compared with twenty normal subjects (ten males and females each) and twelve gallstone patients (six males and females each) whose surgically extracted gallstones had been proven by infrared spectrophotometric analysis and stereoscopic microscopic observation of cross-sectional features to be the pure cholesterol gallstones. Twenty-five diabetic patients consisted of twelve who had no medical treatment until the present examination, five who were undergoing insulin therapy (15–25 μ lente insulin) and eight who had a gallstone disease but had been untreated for diabetes.

The diagnosis of diabetes mellitus was based essentially on the glucose tolerance test which produced an abnormal result in all the patients: the two hour-blood glucose levels were above 160 mg/dl, clinical diabetes had begun between the fourth and fifth decade of life in all the patients. None of the patients had been treated with oral hypoglycemic drugs. Of eight diabetic patients with gallstone, one had the stone that had proven to be the cholesterol gallstone by the examination of surgically extracted stone. The remaining seven patients revealed radiolucent solitary or multiple stones by roentgenography. All the untreated patients were admitted for regular diet of an average of 2450 calorie per day at least for one week (four patients maintained a low calorie diet ranging between 1200 and 1500 cal./day).

The normal control group was formed with staff members of our department and patient-volunteers. However, those having liver, gastrointestinal and other metabolic diseases had been excluded.

Collection of bile: Subjects were intubated with duodenal tube between 7 : 00 and 9 : 00 am after an overnight fast. Gallbladder bile was obtained by a subcutaneous injection of 2γ/kg of Ceosnin (cerulein derivatives, Kyowa Hakko, Japan).

Bile lipid analysis: Cholesterol and bile acids were analyzed from 0.5 ml of gallbladder bile by the method described by Kawamoto[11]. Zero point five milliliters of bile were added with 10 ml of chloroform methanol (2 : 1, v/v) and the extract was hydrolyzed in 1N NaOH at 15 p. s. i. for 3 hours at 120°C. After extraction of cholesterol in diethyl ether, the solution was acidified with HCl and bile acids were extracted in diethyl ether.

Bile acids were methylated in diazomethane ether solution and propionated in pyridine at 110–120°C for 2 hours. The gas-chromatographic analysis of cholesterol and bile acid derivatives was performed using a 2.5% OV-1 column with F. I. D. detector connected to a Shimadzu chromatopac A-1-C peak analyzer.

Determination of bile phospholipids: Bile phospholipids (mainly phosphatidyl choline) were determined by the Takayama's enzyme method[12]. To avoid the influence of bile pigment, the non-reactive bile solution distilled with saline was adopted for the back ground.

The lithogenic index was calculated by the formula described by Thomas and Hofmann[13] based on the triangular coordinate graph of Admirand and Small[14]. The lithogenic index below 1.0 indicates the unsaturated bile and that equal to or above 1.0 indicates the saturated or supersaturated bile with cholesterol.

Table 1. Bile lipid composition (molar percent)

	Cholesterol (%)	Phospholipds (%)	Total bile acids (%)
Normal Controls	6.98± 1.80	20.29± 5.90	72.71± 6.47
Diabetes Mellitus (Untreated)	11.22± 6.71*	29.91± 6.60***	58.87± 9.29***
Diabetes Mellitus (Insulin)	17.51± 9.79*	30.62± 4.74**	51.87± 7.62***
Diabetes Mellitus with gallstones	18.41± 5.49***	21.74±10.97	59.88±13.54*.
Non-diabetic cholesterol gallstones	13.22± 5.14**	21.92± 4.93	64.85± 5.61**

*p<0.05 **p<0.01 ***p<0.001
statistically significant aginst control

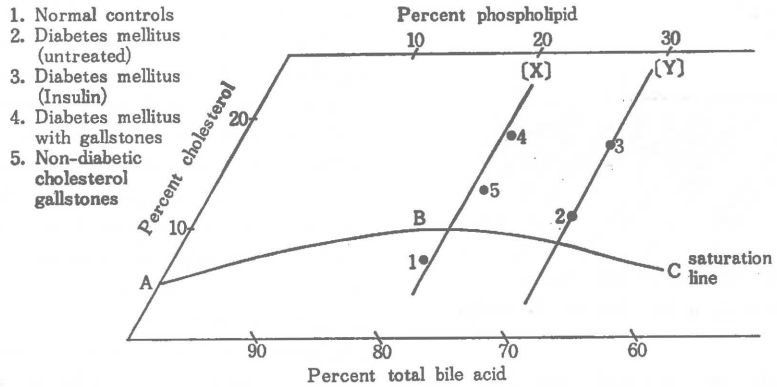

Fig. 1. Bile lipid composition expressed on the triangular coordinate of Admirand-Small

RESULTS

Table 1 lists the molar percentages of cholesterol, phospholipids and total bile acids in normal controls, diabetic and gallstone patients. The molar percentage of cholesterol and total bile acids rose in all the groups of diabetic and gallstone patients. The diabetic patients both untreated and treated with insulin therapy presented significantly higher molar percentage of phospholipids than the normal controls but the diabetic patients with gallstone and cholesterol gallstone patients had almost the normal percentage of phospholipids.

Figure 1 shows the bile lipid composition on the triangular coordinate graph of Admirand-Small. All the groups of diabetic and gallstone patients were positioned above the saturation line A-B-C. However, two groups were formed fitting in either line X or line Y that were drawn approximately parallel with the percent cholesterol axis line of the triangular coordinate. The normal controls and gallstone patients, whether complicated with diabetes mellitus or not, were situated on the line X, and the diabetic patients without gallstone were situated on the line Y distinctly according to the molar percentage of phospholipids.

The average lithogenic index (Admirand-Small) was significantly higher in diabetic and gallstone patients than the normal subjects as shown in Figure 2. The index of the diabetic patients with gallstone was significantly higher than that of the untreated diabetic patients and also the cholesterol gallstone patients.

The incidence of lithogenic bile (supersaturated bile) was also predominant in all the groups of diabetic and gallstone patients beyond

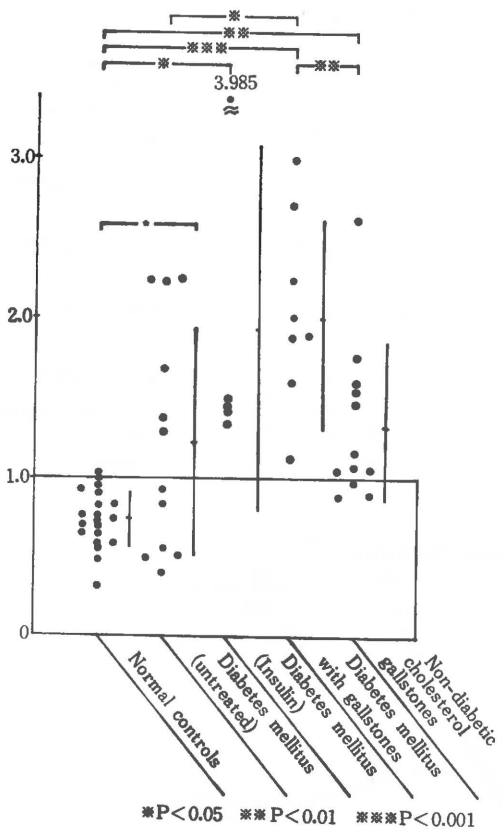

Fig. 2. Lithogenic index (Admirand-Small)

comparison with the normal controls. The incidence in the untreated diabetics was 50% in contrast to the incidence in the diabetic patients treated with insulin and diabetic patients with gallstone in whom it was 100%. The incidence in the cholesterol gallstone patients was 75%.

Table 2 shows the bile acid composition of bile by the group. There was no statistic significance between the normal controls and diabetic or gallstone patients except for chenodeoxycholic and deoxycholic acid in the cholesterol gallstone patients. Chenodeoxycholic acid, however, tended to decrease in the diabetic patients with insulin therapy and with gallstone.

DISCUSSION

A high cholesterol and low bile acid levels were found in diabetic patients, whether or not treated or complicated with gallstone. However, the molar percentage of phospholipids was significantly higher in untreated diabetic patients and patients treated with insulin as compared with normal controls, diabetic patients with gallstone and non-diabetic cholesterol gallstone patients. The increased phospholipid concentration may be beneficial to solubilization of cholesterol in bile, contributing to the prevention of gallstone production and development. As a matter of fact, the molar percentage of phospholipids remained within the normal range in diabetic patients with gallstone. Therefore, on the triangular coordinate graph of Admirand-Small, the lithogenic index points formed two

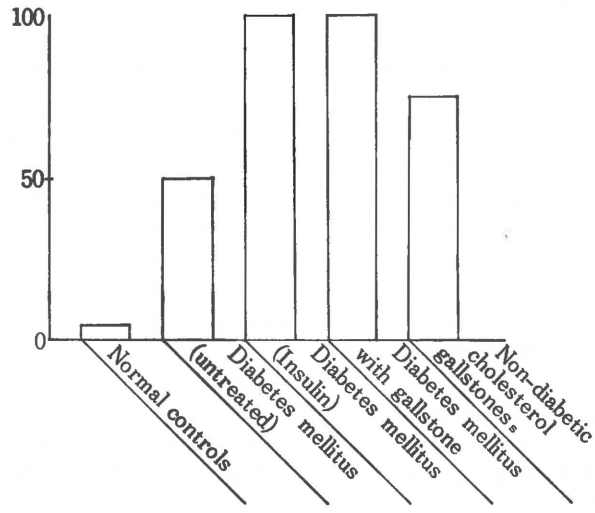

Fig. 3. Incidences of lithogenic bile (supersaturated bile)

Table 2. Bile acid composition (molar percent)

	Primary bile acids		Secondary bile acids	
	Chenodeoxycholic acid (%)	Cholic acid (%)	Lithocholic acid (%)	Deoxycholic acid (%)
Normal controls	45.59± 6.63	41.38± 6.61	1.23± 1.41	11.77± 9.25
Diabetes Mellitus (Untreated)	44.48± 7.42	38.54±10.62	2.34± 1.90	14.62±10.82
Diabetes Mellitus (Insulin)	40.71±10.85	46.93± 8.47	2.44± 1.79	9.90±10.68
Diabetes Mellitus with gallstones	41.58± 6.23	44.31±10.97	1.93± 1.69	12.12± 8.04
Non-diabetic cholesterol gallstones	38.77± 8.66*	38.70± 9.26	1.66± 1.19	20.57±13.03*

*$p<0.05$

groups by taking positions on the two separate lines parallel to the percent cholesterol axis line of the triangular coordinate. The gallstone patiente (diabetic patients with gallstone and nondiabetic cholesterol gallstone patients) shared the line of lithogenic index point with normal controls, and diabetic patients without gallstone (untreated and treated with insulin) shared the line which was drawn separately at a distance from the other line as shown in Figure 1.

The individual and average lithogenic index based on Admirand-Small's saturation line was shown in Figure 2. The diabetic patients with gallstone had significantly higher average index than the untreated diabetic patients, although the former had not been having treatment at the time of bile collection for bile lipid analysis. This in combination with the fact that the diabetic patients with gallstone did not have the higher molar percentage of phospholipids may indicate that the additional factor (s) should be involved in gallstone production in diabetic patients. The incidence of lithogenic bile was 50% in untreated diabetic patients but amounted to 100% in diabetic patients with gallstone who exceeded non-diabetic cholesterol gallstone patients in the average lithogenic index and incidence of lithogenic bile as illustrated in Figures 2 and 3.

The incidence of lithogenic bile amounted to 100% also in insulin-treated patients with diabetes mellitus. The insulin administration may, therefore, contribute to accerelation cholesterol secretion, production of lithogenic bile and eventually formation of gallstone, although the patients had maintained the higher molar percentage of phospholipids, which seems to be the most striking characteristic of the bile lipid composition in diabetes mellitus and also seems to be lost in patients who developed gallstones as seen in the present results (Table 1 and Figure 1).

In the animal experiments[15-17] the cholesterol synthesis and turnover rate are diminished in diabetic rats by induction with pancreatectomy, alloxan or streptozotosin. In the bile acid composition of diabetic rats also cholic acid is low and chenodeoxycholic acid is high. The fact that the insulin administration achieved the cholesterol synthesis in diabetic[18] and normal rats[19] by activating HMG CoA reductase in the liver may reflect the increased lithogenesity due to accerelation of cholesterol excretion into bile and may support the above results in some measure. Results reported on individual bile acid composition in gallstone in earlier studies are diversined. Cahlin et al.[20] and Van Der Linden et al.[21] reported that the gallstone patients, especially with hyper-pre β-lipoproteinemia frequently had a decreased proportion of chenodeoxycholic acid. Other researchers[22] reported, on the other hand, that the gallstone patients had a decreased pool size of cholic acid.

The present study showed significantly lowered molar percentage of chenodeoxycholic acid in non-diabetic cholesterol gallstone patients. Among the diabetic groups, the untreated pa-

tients did not have a decreased molar percentage of chenodeoxycholic acid but the insulintreated patients or patients with gallstone tended to have a lowered proportion of chenodeoxycholic acid in bile, though not statistically significant against the normal controls.

It is possible to conclude from the foregoing results that although lithogenic bile is present in many diabetic patients, their bile lipid composition is altered by the increase in phospholipids which is helpful in keeping the cholesterol solubility of bile by decreasing the relative cholesterol content and that the bile lipid composition of diabetic patients is again altered to contain the normal proportion of phospholipids and increased cholesterol proportion which significantly exceeds that seen in non-diabetic cholesterol gallstone patients when the diabetic patients developed gallstone formation. The insulin treatment increases the incidence of lithogenic bile, producing a tendency to modify the individual bile acid composition to the similitude of what is seen in non-diabetic cholesterol gallstone patients, although phospholipids still maintain higher concentration.

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