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The Composition of Gallstones from Geriatric Patients Methods for the Determination of Cholesterol and of Black Substances in Gallstones

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Summary: 78 female and 31 male Danish gallstone cases found by autopsy were studied. The mass of the gallstone(s) from each patient was recorded and the stones were ground. The content of cholesterol in the mixed material from each patient was determined, using the *Liebermann-Burchard* reaction. The ratio Gallstone(s) ash – mass/Gallstone(s) – mass was determined, and where this ratio exceeded 0.10, calcium and phosphate determinations were carried out. Subjective description of the stones as 'mixed stones' or 'pigment stones' was substituted by visual comparison of the ground gallstones with a 'grey scale' (black was given the highest value). The values of this quantity and the ash values generally increased with a decreasing content of cholesterol. The content of cholesterol in the gallstones increased with increasing stone mass. The chemical composition of gallstones found by surgery seems to differ from that of gallstones found by autopsy.

Die Zusammensetzung von Gallensteinen geriatrischer Patienten. Methoden zur Bestimmung von Cholesterin und schwarzen Substanzen in Gallensteinen

Zusammenfassung: Die bei der Autopsie gefundenen Gallensteine von 78 weiblichen und 31 männlichen dänischen Patienten wurden untersucht. Das Gewicht der Gallensteine jedes Patienten wurde festgestellt. Die Steine wurden gemahlen. Der Cholesteringehalt wurde in dem gemischten Material eines jeden Patienten mit der *Liebermann-Burchard*-Reaktion bestimmt. Das Verhältnis Aschegewicht der Gallensteine/Rohgewicht der Gallensteine wurde bestimmt; Calcium- und Phosphatbestimmung wurden durchgeführt, wenn das Verhältnis 0,10 überschritt. Die subjektive Beschreibung der Steine als „gemischte Steine“ oder „Pigmentsteine“ wurde ergänzt durch visuellen Vergleich der gemahlenen Gallensteine mit einer Grauskala (schwarz ergibt den höchsten Wert). Diese Werte und der Ascheghalt stiegen generell mit fallendem Cholesteringehalt der Steine an. Der Cholesteringehalt der Gallensteine stieg mit steigendem Steingewicht. Die chemische Zusammensetzung von Gallensteinen, die bei der Operation gefunden wurden, scheint sich von der bei Autopsie gefundener Gallensteine zu unterscheiden.

Introduction

Few papers describing the chemical composition of human gallstones have been published. The main component in gallstones is usually cholesterol. In Germany the substance content of cholesterol in gallstones from most patients was above 2.33 mol/kg (1) (the substance content of cholesterol in chemically pure cholesterol is 2.59 mol/kg); in one out of 100 cases the substance content was as low as 0.26 mol/kg. The average substance content of cholesterol in 331 gallstones from 8 countries was 1.81 mol/kg (2); most of the residual part of the gallstones consisted of calcium salts; sex differences and differences between countries with respect to the composition of gallstones was found. The present paper presents the composition of gall-

stones from Danish geriatric patients and the methodologies used. The results are compared with similar material of German origin.

Materials and Methods

The study was carried out with gallstones found by autopsy of urban patients (78 female and 31 male cases) from the Geriatric Unit, De Gamles By. The age by the time of death varied between 67 and 99 with 84 years as the arithmetic mean value.

The mass of the gallstone(s) from each patient was recorded, the stone(s) was ground and passed through a stainless steel sieve (holes 0.6 × 0.6 mm).

The fraction of black components in the mixed material from each patient was evaluated by visual comparison under 'day-light' from fluorescent tubes with an arbitrary 'grey scale'

(1–6 arbitrary units), corrected for colour influence (3). Gallstone(s) material too dark to correspond to 6 arbitrary units in the scale was given the value 7 arbitrary units.

The ratio $Ash - mass/Gallstone(s) - mass$ was determined according to standard procedure (4) as were the determination of the substance content of calcium(II)(Ca) and of phosphate(P) (5, 6). The two latter determinations were only carried out on stones when the above ratio exceeded 0.1.

The relative loss of mass after drying at 105 °C (until stable mass) was recorded as the *water content*.

Cholesterol was extracted from about 125 mg mass of the dried gallstone powder using 25.0 ml chloroform saturated with a solution of sodium hydroxide (substance concentration = 5 mol/l) in deionized water. The extraction time was only 0.1 ks (= approximately 2 minutes) in order to minimize the extraction of coloured substance, e. g. bilirubin, which increases the apparent cholesterol values. When the colour of the extract was more dense than that of a chloroform solution of bilirubin, substance concentration = 30 µmol/l, the bilirubin was removed from the extract by washing with the above mentioned sodium hydroxide solution saturated with chloroform. The concentration of cholesterol in the chloroform extract was determined by a *Liebermann-Burchard* reaction according to *Carr & Drechter* (7), using the procedure described by these authors under 'Standard'; the volume of the cholesterol solution (cholesterol dissolved in glacial acetic acid) was replaced by the chloroform extract. The calibration of the method was based on 'Standard Reference Material 911, Cholesterol' from the United States' Bureau of Standards. The other reagents were pro analysi grade from Merck, Darmstadt. A pool of gallstones (cholesterol, substance content = 2.10 mol/kg) was run as a control through the 27 main series of the analyses and the relative standard-deviation for this group was 0.012.

All analytical results refer to the undried gallstone(s) material.

Quantities and units

A quantity is described with the format System–Component, kind of quantity = numerical value × unit; if the system is

identical with the Component the latter is omitted in order to avoid repetitions. In the table the heading is given the form $\frac{\text{quantity}}{\text{unit}}$ so that only numerical values are given in the field.

Results

Table 1 shows the results grouped according to the substance content of cholesterol in the gallstones. With an increasing substance content of cholesterol the arbitrary substance content of black component and the mass fraction of water decrease, as well as the ratio between the ash mass and the mass of the gallstones; simultaneously the mass of the gallstones increases.

The calcium(II) and phosphate investigation shows 10 patients with no or small amounts of phosphates in their calcium(II) containing stones. 5 additional patients had mixtures of phosphate free calcium salts and some calcium phosphates e. g., 'apatite' ($Ca_{10}(PO_4)_6(OH)_2$). Three further patients showed results which correspond to nearly pure 'apatite'. One patient had much more phosphate than could be bound by the amount of calcium(II) found; this abnormal stone contained nearly no cholesterol and was quite white.

The number of gallstones from each patient varied between 1 and more than 50; the figures, however, showed no correlation to other results of this investigation.

Tab. 1. 109 gallstone patients grouped according to the substance content of cholesterol in their stones^{a), b), c)}.

Gallstone(s) – Cholesterol, substance content		Number of patients		Number of patients/l having values of Gall- stone(s) – Black component, arbitrary substance content							Gall- stone(s) – Water, mass	Gallstone(s) – mass fraction	Gallstone(s) ash – mass Gallstone(s) – mass
mol/kg		1		arbitrary units							g	l	l
interval	recorded mean	male	fe- male	1	2	3	4	5	6	7	mean	mean	mean
0.00–0.25	0.10	3	6	1	–	–	–	1	5	2	2.26	0.0748	0.2331
0.26–1.02	0.56	2	4	–	–	–	–	2	4	–	3.23	0.0210	0.1065
1.03–1.54	1.27	3	5	–	–	–	1	4	3	–	2.99	0.0173	0.1579
1.55–2.06	1.92	2	10	–	–	–	3	9	–	–	4.65	0.0111	0.1081
2.07–2.32	2.21	3	12	–	–	1	8	6	–	–	7.40	0.0070	0.0783
2.33–2.58	2.49	10	25	1	6	7	20	1	–	–	8.74	0.0078	0.0216
2.59–2.75	2.65	8	16	6	4	10	4	–	–	–	8.22	0.0063	0.0069

a) Table with results from the individual patients will be supplied on request.

b) Determination of the substance contents of calcium(II)(Ca) and phosphate(P) in the gallstones was carried out in 19 cases where the ash values exceeded 0.10 (this limit was exceeded in 25 cases; however, in only 19 cases a sufficient amount of gallstone material was available for the determinations). The calcium values were found to be between 0.96 and 4.12 mol/kg. In 10 cases the phosphate values were less than 0.1 mol/kg, in 8 cases the phosphate values were between 0.51 and 1.04 mol/kg and the substance ratios, phosphate(P)/calcium(II)(Ca) were between 0.15 and 0.58. In one case the phosphate value was 4.05 mol/kg and the substance ratio, phosphate(P)/calcium(II)(Ca) was 1.37.

c) A group of gallstone patients having almost only cholesterol in their gallstones is recognised, using Gallstone(s) – Cholesterol, substance content = 2.325 mol/kg as the threshold value. In fourfold tables this group is compared to the residual gallstone patients with respect to Gallstone(s) – Black component, arbitrary substance content and Gallstone(s) – mass and Gallstone(s) – Water, mass fraction and Gallstone(s) ash – mass/Gallstone(s) – mass using 3.5 arbitrary units, 10 g, 0.015 and 0.05 respectively as borderlines in order to divide the results for each type of quantity into two groups. Significantly unequal distribution of the results was found in all fourfold tables ($P \leq 0.0005$ to $P \leq 0.005$).

Discussion

In approximately one fourth of the gallstone cases the cholesterol values exceeded 2.59 mol/kg which is the substance content of cholesterol in the pure compound. A lack of specificity of the *Liebermann-Burchard* reaction when used on biological material is found by other authors (8, 9). The substance content of 7-dehydro-cholesterol in selected gallstones was estimated and the presence of this component cannot fully explain the hyperestimation of cholesterol by the *Liebermann-Burchard* reaction. A similar lack of specificity was found when the $\text{H}_2\text{SO}_4 + \text{FeCl}_3$ -reaction was used on the gallstones.

In classical anatomic pathology the gallstones are described according to their appearance by terms such as 'mixed stones' and 'pigment stones'. This subjective description gives only little chemical information. The present paper shows a simple but more refined technique, the visual comparison of ground gallstones with a *grey scale*; increasing content of black compounds in the gallstones is found to correspond well with a decreasing content of the white component, cholesterol. Only 1 out of 109 gallstone patients showed a disagreement between the grey scale and the cholesterol content, and this stone material was dominated by calcium(II) and phosphate. Thus when the grey scale evaluation is to be used the desirability of an ash determination should also be considered.

The increasing mean values of the *Gallstone(s) - mass* with an increasing proportion of cholesterol is as yet unexplained, but it should be taken into account in future theories on the formation of gallstones.

The present autopsy material is compared to a similar German material (1), to a German surgery material (2) and to surgery material from 8 countries in 5 continents (2). For women only minor differences in the *cholesterol* results between countries and between gallstones obtained by autopsy and by surgery are found; for men the values in the surgical material are lower than in the autopsy material and significantly lower than in women. Less *calcium(II)* is found in the two autopsy materials than in the surgery material; this observation seems also valid for the *phosphate* results for women, however, the very low values in the German autopsy material are remarkable. It should be noted that different methodologies were used for the two groups of autopsy patients and for the surgery patients; however, it seems reasonable to expect a somewhat different chemical composition of the gallstones found by surgery and those found by autopsy, as the latter were accompanied only by minor or no clinical manifestations (silent stones). A table comparing the above autopsy and surgery gallstones will be supplied upon request.

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