Trace Elements in Biliary Calculi

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Abstract

Ten biliary calculi, removed at surgery from 10 patients, were analysed for the presence of trace elements, using a Siemens ETEC Autoescan(s) scanning electron microscope, an ETEC Autospec(TM) spectrometer and a Hilger large quartz spectrograph. Four calculi contained predominantly cholesterol, four bilirubin and two calcium salts (estimated by infrared spectroscopy and X-ray diffraction). Calcium and sodium were detected in all the stones at concentrations of more than 1000 ppm. Concentrations of 100-1000 ppm of magnesium were found in all the calculi; of copper and aluminium in six, of silicon in four, of manganese in two and of bismuth, iron and lead in one. The other stone samples contained these and other trace elements in less than 100 ppm concentration. There was no significant correlation between the type of the stone and the trace elements detected.

Key words: Trace elements, biliary calculi, emission spectroscopy, X-ray microanalysis.

Introduction

In addition to major well-known constituents such as bilirubin, cholesterol and calcium salts, biliary calculi have been found to contain traces of a large number of elements. The present study reports the trace elements detected in biliary calculi from Western India using X-ray microanalysis and emission spectroscopy.

Material and Methods

Representative samples of biliary calculi removed at operation from ten patients were analysed.

A Siemens ETEC Autoescan(s) scanning electron microscope (SEM) and an ETEC Autospec(TM) spectrometer were used for elemental analysis and mapping of these elements on the surface and cross-section of the stones. A Hilger large quartz spectrograph was used for quantitative emission spectroscopic estimation of trace elements in the same calculi. Owing to beam target interactions in the SEM, characteristic X-rays are produced from the different constituents of the sample. Using a wavelength dispersive spectrometer, the elements present in the sample can be detected and mapped by the principle of diffraction of different X-rays over known crystal gratings. The crystals used in this study were:

(a) Lithium fluoride (LiF)—to detect iron, copper, nickel, manganese, chromium, gold, cobalt, arsenic and tin;
(b) Pentazolinyrrol (PET)—for calcium, silver, phosphorus, lead, potassium, chlorine and tin;
(c) Rubidium acid phthalate (RAP)—for aluminium, silicon, magnesium and sodium.

The spectrometer also had a gas flow counter containing PiO gas (a mixture of 90% argon with 10% methane).

The biliary calculi were cut into pieces which were glued onto an aluminium strip using colloidal graphite, dried and coated with a 100-200 A thick layer of copper in an S 150 Edwards mini sputter coater. X-ray analysis of these ions was carried out by crystal. Up to 5-7 counts were considered background counts, and those exceeding background were considered significant. Photographs were taken of the distribution of elements in the samples, the areas of concentration being represented as close dots on the viewing screen.

For emission spectroscopy, a portion of each stone was powdered and 10-15 mg of the powder was dissolved in 2-3 ml of pure nitric acid and water (1:1); 100 mg of graphite was added to it. The solution was evaporated on a sand bath. When the sample was dried, it was thoroughly ground and 20 mg of it was loaded in a 1/4 inch drilled graphite electrode (anode). A 1/8 inch pointed graphite rod was used as a cathode. The emission spectra were excited using a large quartz spectrograph in the region 2400 A-3300 A for 45 seconds on a Kodak 3A-1 emulsion. Single charges of the samples were exposed along with a set of standard elements in the range 1-500 ppm of 45 elements. The intensities of the analytical lines were compared with those of the standards using a projector and an approximate evaluation was made.

Results

A study of the chemical composition of the 10 calculi by infrared spectroscopy and X-ray diffraction revealed predominantly cholesterol in four, bilirubin in four, and calcium salts in two of the gallstones. The results of the X-ray micro-analysis are presented in the Table and those of emission in the Figure.

All the samples were calcium rich (more than 1000 ppm). Over 1000 ppm sodium was present in all the calculi; Copper too was identified in all the calculi; however, only six of them, including all four bilirubin stones, had copper in significant quantities. A very high value of bismuth (1000 ppm) was recorded in one of the samples (a pigment calculus). Another pigment stone showed more than 1000 ppm of aluminium. The other calculi had concentrations of 2-100 ppm of aluminium. Concentrations of 100-1000 ppm of magnesium were detected in all the calculi. Silicon in similar concentrations was recorded in four calculi.

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Thanks are due to Dr Mathur, Reader in Surgery.
The elements which showed a considerable variation were lead (from absent to 100 ppm) and manganese (5–100 ppm). Minute amounts (around 1–5 ppm) of unusual elements such as silver, tin, cobalt and antimony were detected in all the samples. Five ppm nickel was noted in four calculi. Indium, cadmium, gold and arsenic were not recorded in any of the samples.

Discussion

Trace elements have been detected in biliary calculi by many workers, using a variety of techniques. However, the role of these elements in lithogenesis remains largely unknown.

Sodium was detected in fairly high amounts in all the samples of the current study. The most likely explanation is co-precipitation when the gallstone forms rapidly. Bogren noted that sodium chloride always occurred mixed with other compounds and he could not observe it in regions containing large, well-shaped cholesterol crystals, which must have grown slowly.

Emission spectrography has been used to detect a wide range of elements in black pigment stones. Copper and iron were supposed to be responsible for the black appearance of these calculi. It was suggested that the black pigment was a bilirubin-copper complex. The present study too detected large concentrations of copper in the bilirubin calculi. Iron, however, was evenly distributed.

An earlier study using an SEM and an X-ray detector also identified trace elements including calcium, phosphorus, sulphur, copper and iron. The same group suggested that calcium salt precipitation was a factor of major importance in stone nucleation. Copper may occur in gallstones as a consequence of precipitation of its protein ligands from bile. Trauma to the gallbladder wall may result in haemorrhage with release of both complexed iron and a fibrous protein. The hard central aggregates of calcium salts within the gallstones are potential agents for causing such a trauma.

Elemental analysis with the aid of SEM is not as accurate as emission studies. The element used for coating the sample cannot be detected very precisely. Mapping of elements can however be done and

Table: Results of X-ray Microanalysis

<table>
<thead>
<tr>
<th>Sample</th>
<th>Chemical Composition</th>
<th>Elements detected</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Calcium palmitate</td>
<td>Calcium</td>
</tr>
<tr>
<td>2</td>
<td>Calcium carbonate and phosphate</td>
<td>Calcium, potassium, silicon, cobalt, iron, nickel, copper</td>
</tr>
<tr>
<td>3</td>
<td>Cholesterol</td>
<td>Calcium, potassium, silicon, chromium</td>
</tr>
<tr>
<td>4</td>
<td>Bilirubin</td>
<td>Calcium, potassium, copper</td>
</tr>
<tr>
<td>5</td>
<td>Bilirubin</td>
<td>Calcium, copper</td>
</tr>
<tr>
<td>6</td>
<td>Cholesterol</td>
<td>Calcium, potassium</td>
</tr>
<tr>
<td>7</td>
<td>Bilirubin</td>
<td>Calcium, copper, iron, sulphur, silicon, potassium, chlorine</td>
</tr>
<tr>
<td>8</td>
<td>Bilirubin</td>
<td>Calcium, potassium, copper</td>
</tr>
<tr>
<td>9</td>
<td>Cholesterol</td>
<td>Calcium, potassium</td>
</tr>
<tr>
<td>10</td>
<td>Cholesterol</td>
<td>Calcium</td>
</tr>
</tbody>
</table>

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photographs taken. The advantage of emission spectroscopy is that minute amounts of samples are used and a photographic record of the spectra is obtained. However, emission spectroscopy can only be used to identify heavy and metallic ions.

Trace elements in biliary calculi have a variable role. Burnett et al. suggested that the role of transition type metals in biliary calculi was minimal and that the ions were generated as a result of the cationic exchange properties of the black pigment, polybilirubinate, which is found in most types of human gallstones.

Some elements like calcium, copper, sodium and iron are present due to important steps in stone nucleation and formation. Others seem to be just incidentally present. These elements are derived from bile, from blood or the gallbladder wall. Defining the specific role of each trace element in gallstones can provide clues to modifying them and hence producing a major effect by a relatively minor manipulation.

References