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§Short Report

Relative measurement of heavy elements in the bile, gallbladder and gallston

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Background: PIXE (Particle Induced X-Ray Emission) is a suitable method for the analysis of biological samples in which heavy trace elements are contained in light matrix elements. It is very important to know which factors or probably elements act as initial seed and lead to growing the sands. The goal of this study was to compare the relative values of Fe/K, Cu/K and Zn/K for gallstones, gallbladder, and bile of a specific patient for studying the origination of forming the gallstones. **Materials and Methods:** Human gallbladder, bile, and gallstone samples were obtained by surgical operation from 15 patients and are bombarded by 2.0 MeV energy proton beams produced by van de Graff accelerator in vacuum. All the gallstones were chosen of pigment type of stones and, all the patients were adults. In contrast with conventional methods, the shell and center of the sands has been analyzed separately. The PIXE spectrum analysis was performed using the nonlinear least square fitting code AXIL and GUPIX. **Results:** The results of detected minor and trace elements shows that the precipitation of calcium salt in the bile lead to reduction of crystals' formation. Elemental comparison of pigment type of gallstone and bile shows that the concentration of calcium in the shell of the stones is four times more than that in the bile. **Conclusion:** Precipitation of the calcium from the saturated bile on the cholesterols as a seed of gallstones led to reduced sands formation. Analysis of the gallbladder of the same patients revealed no relation between elemental concentrations of bile and gallstones. **Iran. J. Radiat. Res., 2006; 3 (4): 195-198**

Keywords: *Bile, gallbladder, gallstone, trace elements, PIXE*

INTRODUCTION

The extra hepatic biliary system begins with the hepatic ducts and ends at the stoma of the common bile duct in the duodenum. The common bile duct is approximately 8-11.5 cm in length and 6-10 mm in diameter. The gallbladder, which is located in the bed of the liver, contains most of the smooth muscle of the organ, in contrast to the body, which is the major strong area and contains most of the elastic tissue. The main constituent of bile is water, electrolytes, bile salt, proteins, lipid, and bile pigments. Sodium, potassium, calcium and chloride have the same concentration in the bile. The major elements involved in the formation of the gallstones are cholesterol, bile pigment, and calcium (1). The suitability of PIXE spectroscopy for trace elements analysis in human tissues lies in the simultaneous analysis of wide range of light and heavy elements. This method is extremely sensitive and multielemental analysis. Absorption of necessary amount of elements for biological organs is from the surroundings or blood. Therefore, a breakdown of the homostatisis is indeed one of the most important indications of diseases (2, 3). The role of trace elements in the biological samples is very important in some diseases. The aim of this study was to compare the relative values of Fe/K, Cu/K and Zn/K for gallstones, gallbladder and bile of specific patient for studying the origin formation of gallstones. In contrast to conventional methods, the shell and center of the sands have been analyzed separately (4).

MATERIALS AND METHODS

Sample preparation

Human gallbladder, bile and gallstone samples were obtained by surgical operation from 15 patients. The sample without any further process were put as thick target on a

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capton foil backing normally dried whose thickness is about few mm. The samples were suspended on aluminum sample holders with holes at the center. International Atomic Energy Agency (IAEA) MA-B-3/TM Fish tissue was used as a standard for calibration of PIXE set up. The standard target was prepared by pressing 250 mg of standard powder into a pellet (1.7 cm in diameter) without any addition. All the gallstones were chosen of pigment type stones, and all the patients were adults. A thin carbon foil was sprayed over the samples to make the target conductive for collecting the charge.

Equipment and measurement

The 3.0 MeV van-de-Graff electrostatic accelerators at nuclear research center of Atomic Energy Organization of Iran were used for the PIXE measurement. In the PIXE technique, measurements were carried out in vacuum (10^{-6} torr) by irradiation with beams of 2.0 MeV energy proton ions whose spot size is 2.8 mm^2. The Samples were placed at an angle of 90° with respect to the incident beam. To analyze many samples without breaking the vacuum, a cassette type of sample installing system was used, where 16 sample holders could be loaded at once. Beam current was around 5nA in order to keep the counting rate below 1000 cps, and the integrated beam current was 10 μC for each measurement.

A Si (Li) detector at 135° detected characteristic X-rays emitted from the targets. A 175 µm-thick Mylar absorber was positioned in front of the detector in order to decrease the intense low energy X-rays originating from the matrix elements, while the light elements were detected without an absorber. The energy resolution of detector was 175eV at 5.9 keV. The solid angle was limited to be $3.3e^{-3}$ sr that was corrected by one of the available calibration techniques, which has been chosen amongst a wide variety techniques for thick specimens using small number of trace elements in standard and known samples, e.g. calculated calcium concentration in the IAEA standard fish tissue serves as a good reference.

The PIXE spectrum analysis was performed using the nonlinear least square fitting code AXIL and GUPIX [5]. Data obtained from the computer program were net peak areas of K and L shell X-ray (figure 1); errors are coming from counting statistics and values for the background. Since the spectra of low Z elements and high Z elements were obtained separately, they must have been calibrated according to the efficiencies of the detector for calcium and iron. Then, these two elements were chosen for comparing the two PIXE spectra obtained with and without absorbers for the specific samples. In this way, the two spectra were matched after charge correction

RESULTS AND DISCUSSION

The elemental concentrations of bile, gallbladder and gallstone (shell and center) are shown in table 1. The concentrations in the table are mean value of concentrations determined for 10 targets of the same samples. In general, three major components of bile are bile salt (80%), lecithin (15%) and cholesterol (5%). In this work the light elements ($z \leq 19$) were not concerned and for gallstones only the pigment types which were affected by the salts were analyzed. All the measurements were relative, so could not have been compared with similar studies [4, 6]. Since the potassium has no important role in the formation of gallstones, all the elements were measured relative to the potassium. Comparing the calcium concentration in the bile, gallbladder and gallstone (table 1), it shows the precipitation of calcium salt in the bile reduces the formation of crystals, where the macroscopic size during the period of entrapment in the gallbladder, gallstones is formed [1]. The results show a high relative concentration of calcium in the bile and stones but not in the gallbladder. The measured value of the calcium in the shell of gallstones offers that the seed of gallstones are cholesterols as well as heavy trace elements such as Fe, Cu, Zn added to this

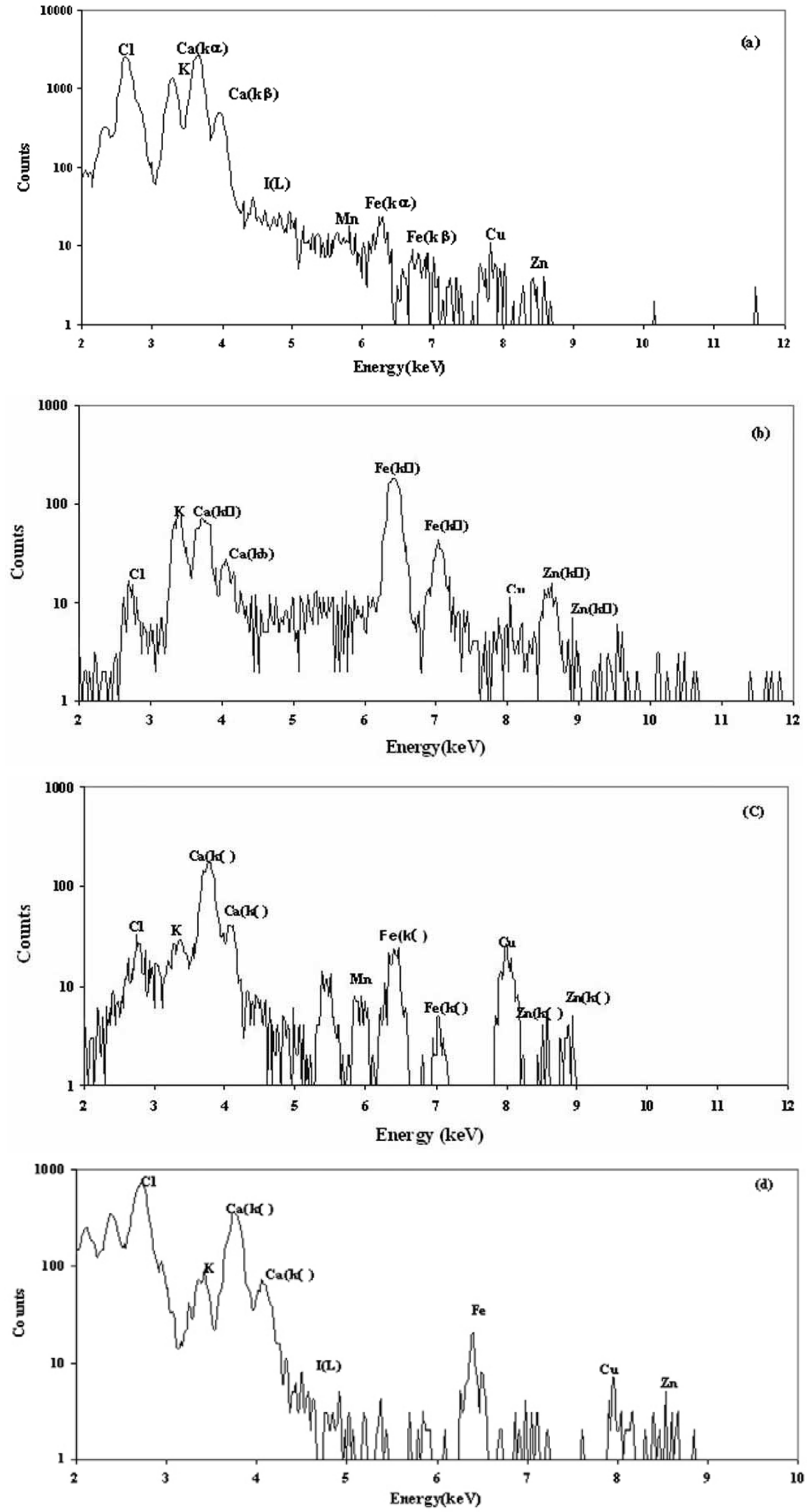

Figure 1. Typical spectra for PIXE analysis of: (a) bile, (b) gallbladder, (c) gallstones (center), and (d) gallstones (shell). A 175 m Maylar filter are used in front of detector.

Table 1. Relative elemental concentrations of bile, gallstones (shell and center) and the gallbladder. All the elements are compared with K

Samples	Number of samples		Elements and Concentrations (ppm) Ca/K	Mn/K	Fe/k	Cu/k	Zn/K	I/K
Bile	10	min	0.448	0	0	0	0	0
		max	8.039	0.002	0.403	0.016	0.011	0
		mean	2.538	0.0006	0.097	0.006	0.003	0
		SD	0.806	0.0003	0.048	0.0018	0.0011	0
Gallbladder	10	min	0.077	0	0.022	0	0.007	0
		max	0.68	0.004	2.191	0.021	0.36	0.222
		mean	0.35	0.0009	0.33	0.006	0.063	0.044
		SD	0.07	0.0005	0.22	0.003	0.036	0.026
Center	10	min	0.762	0	0.012	0	0	0
		max	15.734	0.559	0.839	1.512	0.254	0.46
		mean	6.58	0.088	0.26	0.2	0.09	0.115
		SD	1.712	0.058	0.096	0.154	0.326	0.0591
Shell	10	min	0.584	0	0.056	0	0	0
		max	43.87	0.595	16.512	5.933	1.158	0
		mean	10.94	0.072	1.96	0.7	0.28	0
		SD	5.014	0.062	1.71	0.615	0.125	0

seed. Only the concentration of iodine in the center of the stones was higher than that of the shells and bile. Trace and major elements in human gallstones were determined by PIXE method. Elemental comparison of only pigment type of gallstone and bile showed that the concentration of calcium in the shell of the stones was four times more than that in the bile. Nevertheless, in the center of the gallstones this relation has not been valid. According to the previously published work [(7)] the origin of the formation of gallstones is cholesterols, and then the precipitation of calcium happens by the saturated bile. Heavy element concentration (Fe, Cu and Zn) in the bile was very lower than those in the gallstones were. The amount of Fe in the center of the sands was higher than that in the shell, on the contrary, the value of Cu and Zn in the shell is greater than the center. These results may show the origin of formation of the stones. The analysis of gallbladder of the same patients shows no relationship between elemental concentration of bile and gallstones.

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