The concentration of heavy trace elements in pigment and cholesterol human gallstones: Comparative studies by PIXE analysis

J. Salimi[1*], K. Moosavi[2], S. Vatankhah[2]

Sina Hospital[1], Tehran University of Medical Sciences, Tehran, Iran
Van de Graaff Laboratory[2], AEOI

ABSTRACT

Background: The method of proton induced X-ray emission (PIXE) has been widely used as a sensitive technique to trace elemental analysis in both biological and medical fields. The sensitivity of this powerful method is in the order of ppm .The aim of this work is to analyze structural composition of 4 heavy trace elements with Z greater than 24 (Mn, Fe, Cu, Zn) in two kinds of gallstones and to compare the results for various ages of patients by PIXE quantitatively.
Materials and methods: The pigment and cholesterole gallstones were obtained from 12 patients during surgical operations and were used for in vitro study. The age of the patients were between 22 and 78 years. Both cholesterol and pigmented types were analyzed for shell and center. The samples were bombarded using a 3.0 MeV van-de-Graff accelerator at nuclear research center of Atomic Energy Organization of Iran for the PIXE measurement.
Results: In pigment gallstones, the mean value of Mn, Fe, and Zn in shell is greater than in the center and Cu in the shell is smaller than center. In cholesterol gallstones, neither in the shell nor in center, Zn was not observed. The composition of Mn and Fe in center is greater than in shell. In the patients older than 40 years, the concentration of heavy trace elements increases, and those in cholesterol stones are nearly two times larger than in pigment stones.
Conclusion: Comparison of two types of gallstones shows that the center of the pigment stones is very similar to cholesterol type .It is concluded that the origination of gallstone in human is common but formation and growth are different. *Iran. J. Radiat. Res.; 2003; 1(2): 93 – 97.*

Keywords: *Gallstone, heavy trace elements, PIXE.*

INTRODUCTION

It is commonly known that gallstones occure in a wide variety of size and color and is comprised of various constituent elements. They are generally classified into one of the following three categories: 1) Cholesterol gallstones (pure cholesterol, combination or mixed); 2) Pigment gallstone (black or calcium bilirubinate) and 3) Rare gallstones (calcium carbonate, fatty acid calcium) (Sabiston *et al.* 1997, Schwarts *et al.* 1999).

Except calcium, which is the third matrix element in various types of gallstones highly concentrated trace elements such as Cu and Fe play a role in the formation of black pigment stones (Verma *et al.* 2002). In order to determine the mechanism for the formation of each of these gallstones, more information is required about their respective trace elements and structures (Sahuquillo *et al.* 2000).

The method of Proton Induced X-ray Emission (PIXE) has been widely used as a technique for trace element analysis in both the

* **Corresponding author:**
Dr. J. Salimi, Sina Hospital, Tehran University of Medical Sciences, Tehran, Iran.
P.O.Box:11365-8486, Tehran, Iran Fax: +98-21-6716545
E-mail: mjsalimi@sina.tums.ac.ir

biological and medical fields (Malmqvist *et al.* 1995, Shizuma *et al.* 1997). Further more, it has also been used to determine the elemental distribution profiles in hairs, breast and other tissue, simply by irradiating the sample with a focused proton beam (Vatankhah *et al.* 2003). Although many authors have used the PIXE method to investigate these properties in kidney stone, but few results of the elemental analysis of gallstones have been published (Shizuma *et al.* 1997). The aim of this work is to quantitatively analyze two kinds of gallstones and to compare the results for various ages of patients. The elemental analysis of 12 gallstone was investigated. Determination of the heavy trace elemental distribution (Mn, Fe, Cu, Zn) in human gallstone is carried out by irradiating of the sample with a focused proton beam.

MATERIALS AND METHODS

Twelve human gallstones were taken by cholecystectomy from 12 patients and were used for *in vitro* study. The age of the patients were between 22 and 78 years. Both cholesterol and pigmented types were analyzed for shell and center.

A thin film of carbon spray to make them conductive coated the samples. These samples were analyzed without any further process as a thick target. Since the center and the shell of stones were concerned separately, stones were cut into two half; there are no homogenizing processes. IAEA MA-B-3fTM Fish tissue was used for calibration of PIXE set up. The standard target was prepared by pressing 250 mg of powdered standard into a pellet (1.7 cm in diameter) without any additive. The samples were bombarded using a 3.0 MeV Van-de-Graff accelerator at nuclear research center of Atomic Energy Organization of Iran for the PIXE measurement. In the PIXE technique, measurements were carried out in vaccum (10-6 torr) with a 2.0 MeV energy proton beam whose spot size is 0.28 cm^2. The samples were placed at an angle of 90 with respect to the incident beam. Beam currents were around 5nA in order to keep the counting rate below 1000 cps, and the integrated beam current (using charge integrator in the setup) was 10 ~C for each measurement.

Characteristic X-rays were emitted from the target and detected by a Si (Li) detector at 135°. A 175~-thick Mylar absorber was positioned in front of the detector in order to decrease the intense low energy X-rays originating from the low Z elements, while the light elements were detected without absorber. The energy resolution of detector is 175 e V at 5.9 keV. The solid angle was limited to be 3.3 e^{-3} sr that is corrected by one of the possible calibration techniques. It has been chosen among wide variety techniques for thick specimens using small number trace elements in standard and known samples, e.g. calculated calcium concentration from the I.A.E.O standard fish tissue is a good reference element. Since the spectrums of low Z elements and high Z elements without filter and with Mylar filter are taken in separate run, then according to sensitivity of detector for calcium and Fe elements for both spectrum (sensitivity cmve of si (Li) detector in this region are almost flat) then those two elements are chosen for comparing two pixe spectrum for specific samples. By using this approach and charge correction two spectrums are matched.

RESULTS

The PIXE spectra analysis was performed using the non-linear least square fitting code AXIL and GUPIX (Maxwell *et al.* 1989). Data obtained from the computer program were net peak areas of K&L X-rays; errors are coming from counting statistics and values for the background. In table 1, the type of the gallstones (cholesterol, pigment) age and sex of the patients are shown. Four major elements detected in the shell and the center of the gallstones are listed in Table 2. In patients older than 40, the concentration of heavy elements such as Mn, Fe,

Table 1. Kind of stones, Sex and Age for each sample.

no:	1	2	3	4	5	6	7	8	9	10	11_1	11_2	12
kind of stone	cho	cho	cho	cho	cho	cho	cho	cho	pi	pi	pi	pi	pi
female	***	***	***	***	***	***	***	***	***	***			
male											***	***	***
age	23	35	29	78	22	63	34	41	68	43	32	32	38

Table 2. Type of gallstones and concentration of heavy trace elements in shell and center.

				Heavy trace elements& concentration (ppm)			
	Type	No.Samples		Mn	Fe	Cu	Zn
Center	Chol.	7	min	0	0	0	0
			max	2194	4973	9624	0
			mean	1104	2271	5182	
			SD	1019.6	1321	2735	
			%Stat.Error	±17	±320	±270	0
	Pig.	4	min	212	0	0	0
			max	831	955	7701	642
			mean	606	636	5333	160
			SD	252	335	2654	185
			%Stat.Error	±8	±80	±276	±85
Shell	Chol.	8	min	0	0	0	0
			max	1126	1486	9582	0
			mean	715	1367	5661	0
			SD	131	437.7	2135	0
			%Stat.Error	±9	±170	±283	0
	Pig.	5	min	0	677	1236	0
			max	2257	5126	4386	1445
			mean	895	1985	2306	1195
			SD	521.8	1099	916	523
			%Stat.Error	±10	±260	±140	±130

(*; Units in %)

Cu and Zn increases, and those in cholesterol stones were nearly two times larger than in pigment stones.

Typical PIXE spectra for a human gallstone of both pigment and cholesterol types (shell and center) for heavy elements are shown in figures 1-4.

The minimum, maximum and mean value for 5-6 samples of each type of gallstones are listed where errors are indicated by the standard deviation. The absolute analysis is performed for a thick target.

In pigment gallstones, the mean value of heavy metal elements Mn, Fe, and Zn in shell is grater than in the center and Cu in the shell was smaller than in the center.

In cholesterol gallstones, neither in the shell nor in the center, Zn was not observed. The composition of heavy metal elements like Mn and Fe in the center was greater than in the shell.

In the shell of stones, the value of Mn and Fe in pigment stones was greater than cholesterol stones and the value of Cu was equal in both of them.

In the center of stones, the value of Mn and Fe in cholesterol stone was greater than pigment stones and the value of Cu in pigment stone was two times of cholesterol stones.

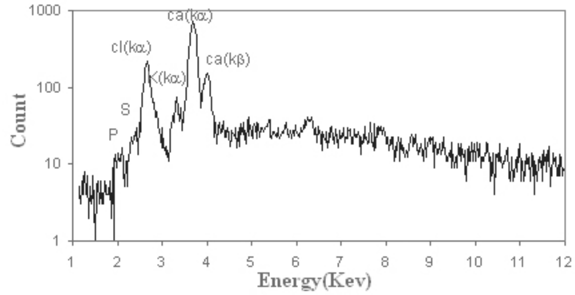

Figure 1. Typical spectra for shell of pigment stones (With a 175 µm Mylar filter).

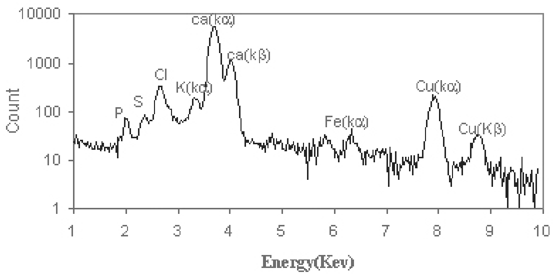

Figure 2. Typical spectra for center of pigment stones (With a 175 µm Mylar filter).

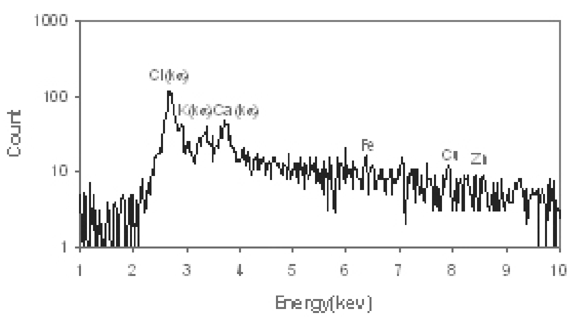

Figure 3. Typical spectra for shell of cholesterol stones (With a 175 µm Mylar filter).

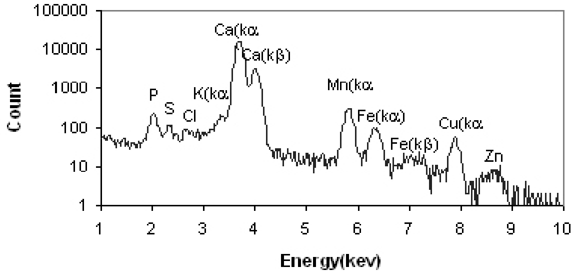

Figure 4. Typical spectra for center of cholesterol stones (With a 175 µm Mylar filter).

CONCLUSION

Four heavy trace elements (Fe, Zn, Mn, Cu) in human gallstones were determined by PIXE method. The samples were cut in two half for analyzing the shell and center of stones separately. Elemental comparison of two essential types of stones (cholesterol and pigment) shows that the center of the pigment stones was very similar to the cholesterol type. According to the figure.5 and figure.6 this small difference of major elements, whose concentrations are expressed in percent, is not large.

It is concluded that the origination of gallstones in man are common but their formation and growth are different.

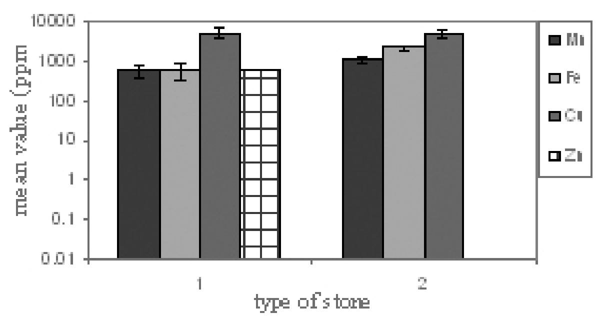

Figure 5. Heavy trace element concentration of gallstones shell (Vertical axis 1: Cholesterol stones; Vertical axis 2: Pigment stones).

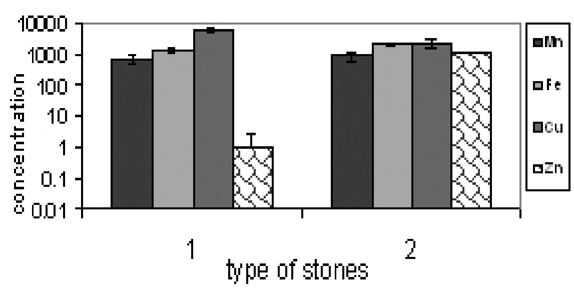

Figure 6. Heavy trace element concentration of gallstones center (Vertical axis 1: Cholesterol stones; Vertical axis 2: Pigment stones).

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