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A pixe analysis for measuring the trace elements concentration in breast tissue of Iranian women

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ABSTRACT

Background: A powerful and improved technique, Proton Induced X-ray Emission (PIXE) has been performed-yielding the elemental composition of 17 samples of surgically excised malignant and normal tumors of breast tissue. The samples without any further process as thick targets were put on capton foil backing. There are no homogenizing processes. The PIXE spectra analysis was performed using he non-linear least square fitting code AXIL and GUPIX.
Materials and Methods: The samples are taken from patients in the wide range of age and are bombarded by 2.0 MeV energy proton beams produced by van de graff accelerator in vacuum. The quantitative comparison between two types of tissues was evaluated by assessing the presence of Calcium, Potassium, Iron, Copper and Zinc, as minor and trace elements.
Results: Results in this study indicate that relative values of Cu / Zn, P / K and also Ca and S in benign type were higher than those in malignant type, but the concentration of Fe and Zn in cancerous tissues was significantly higher than those for benign type.
Conclusion: Results suggest significant elevation of Zinc in the pathological tissues. Cu/Zn ratio for both type of tissues are evaluated. The results show that this ratio in patients with breast cancer is significantly lower than the normal group. Selenium and Arsenic was not obtained in any of 17 samples. Most of the tissues of benign kind (Fibrocystic and Fibro adenoma) contain Cadmium. Calcium concentration in normal tissues is significantly higher than tumorous tissues. *Iran. J. Radiat. Res., 2003; 1(1): 23 - 27.*

***Keywords**: Pixe, breast tissue, trace element, calcium, copper, zinc.*

INTRODUCTION

Inadequacy or unbalance of trace element supply consequently affects a number of physiological functions; which is involved in almost every biochemical process in body cells. Elemental analysis by a developed and improved techniques have strongly contributed to an increase understanding of the role of trace elements for health and disease. An adequate nutrient supply is particularly in periods of growth especially for infants, children and pregnant women (Walter *et al.* 1996). Comparing the concentration of elements for tissue characterization is to show if there is such correlation between element concentration and breast cancer.

It is known that many trace elements participate in biological processes in the organism, which include the activation or inhabitation of enzymatic reactions, competition between elements, metalloproteins for binding positions, and modifications in the permibility of

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cellular members. It can therefore be presumed that these elements might influence carcinogenic processes. Today tumor diseases are the second most frequent cause of death on industrially developed countries. In the inception and development of tumor diseases a combination of internal, genetically conditioned and external factors are present.Geraki and Farqurharson (2001).

The human breast develops in the thicked portion of ectodernal tissue. Appreciation of the stages of breast development is necessary to understand many benign and even malignant states that come to clinical attention. During adolescence, the breast is composed primarily of dense fibrous stroma and scattered ducts lined with epithelium. Puberty begins at about 12-15 years of age, where there is hormone-dependent maturation of the genital organs. In the breast, this process entails increased deposition of fat, formation of new ducts by branching and elongation, and the first appearance of lobular units. This process of growth entails cell division and is under control. In diagnostic of breast disease, for patients with benign condition, the history is an exceedingly important part of the overall evaluation. For patients suspected of having cancer, the history directly aids in the approach to the patient, and the ultimate treatment if cancer is confirmed, and help estimate the risk that cancer will be found. It is well known that women with fibrocystic complex and those who have undergone breast biopsy are at increased risk (Sabiston and Duke 1997). Among some multielemental analytical techniques, Pixe technique is chosen for simultaneous determination of ultra-trace quantities of elements in human breast tissues. Such systematic studies, which include a broad spectrum of less abundant, trace metals in cancerous and normal breast tissue. Data reported by different authors are not consistent. According to the authors, the elevation of concentrations of elements are probably due to the increased cellular activity in malignant tissue and active enzymatic systems leading to increased amounts of trace elements. Assessing the full diagnostic value of calcification, or microcalcifications, frequently find within breast tissue is also of interest. Radiologists currently use these as a first line indicator of disease. Among some tentative but intriguing observations associated with calcifications are a correlation between the risk of developing breast cancer and the presence of calcifications and/or trace element concentrations. Calcifications are calcium deposits found within the breast tissue. These deposits can be identified by mamography. They are extremely common and are frequently due to non-cancerous causes. They can, however, be an early sign of breast cancer. There are two main types of calcifications: Macrocalcifications and Microcalcifications (Rogers *et al.* 2002).

MTERIALS AND METHODS

Sample preparation

Human breast tumors were taken by surgurical operation from patients. The age of the patients were between 30 to 60 years. A thin film of carbon spray to make them conductive coated the samples. The samples without any further process as thick targets were put on capton foil backing whose thickness is about 1mm; there are no homogenizing processes. International Atomic Energy agency (IAEA) MA-B-3/TM Fish tissue was used as standard for calibration of PIXE set up. The standards were samples of~250mg powder was pressed into pellets (1.7 cm in diameter) without any additions.

Equipment and measurement

The Van-de-Graff 3.0 MeV electrostatic accelerators at nuclear research center of Atomic Energy Organization of Iran was used for the PIXE measurements. In the PIXE technique, the measurement was carried out in vacuum ($10^{e\ -6}$ torr) with a 2.0 MeV energy proton beam whose spot size is 0.28 cm^2 for irradiation of the samples. The samples were placed at an angle of 90 with respect to the incident beam. A beam current of about 5nA was applied, and the integrated beam current was 10 µc for each target. The counting rate was kept below 1000cps. A thin carbon foil is sprayed over the samples to make the target conductive for collecting the charge.

Characteristic X-rays excited in the target were detected by a Si (Li) detector in order to decrease the intense low energy X-rays originating from the matrix elements. A 175 µm-thick Mylar absorber was positioned in front of the detector, while the light elements were detected without the mentioned absorber. The energy resolution of detector was 180ev at 6.399 Kev. The solid angle was limited to be $3.3e^{-3}$ sr that is corrected by one of the possible calibration techniques which 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 were taken in separate run, then according to sensitivity of detector for calcium and Fe elements for both spectrum sensitivity curve of si (Li) detector in this region were almost flat. Then those two elements were chosen for comparing two pixe spectrum for specific samples. By using this approach and charge correction two spectrums were matched.

RESULTS AND DISCUSSION

The PIXE spectra analysis was performed using the non-linear least square fitting code AXIL and GUPIX (Maxwell and Campbell 1989). Data obtained from the computer program were net peak areas of K & L X-rays; errors were coming from counting statistics and values for the background. Some peaks like K (kβ) and Ca (kα) overlap on each other, but since the using software was able to calculate each peak information separately, the concentration of all detected trace elements were concerned. The Maximum, minimum, mean value, standard deviation (SD) and statistical error (% stat. error) of detected minor and trace elements (for both normal and cancerous breasts tumors) are shown in table S1. The absolute and relative analysis is performed for a thick target. All elements with high and low value of concentration were expressed in ppm.

Among 21 samples, 7 samples are malignant type and 14 refer to benign type (fibroadenome, fibrocystic, hyper plazy and papilom).

The relative analysis, which expressed in relation of some elements like Cu/Zn and P/K are shown in table 2. Since the most important element that is discussed in breast diseases is calcium, then the concentration of Ca are concerned separately.

Table 1. Trace and minor elements in both normal and cancerous human breast tissues.

	Number of samples		Elements and concentrations (ppm)										
Type			Si	P	S	Cl	K	Ca	Fe	Cu	Zn	Br	Cd
		Min	0	419	421	413	218	128	9	0	11	0	0
		Max	903	7632	7583	9144	6133	864	501	67	498	25	696
Malignant	**7**	Mean	422	220.7	2788	4347	2229	426.7	136.3	17.5	135.5	5.14	94.4
		SD	318	2207	2952	3232	2016	262	172	22.7	121	8.9	243
		%State. Error	7.5	8.8	1.2	3.4	1.9	2.4	1.9	12	17	15.5	22
		Min	0	240	183	791	125	286	13	0	0	0	0
		Max	784	8659	7718	17746	3934	3137	232	109	162	30	342
Benign	**14**	Mean	381.7	3086	6543	5861	1157	977	67.7	19.8	50.5	4.5	46.1
		SD	216	3716	3425	3783	895	879	61.5	22.86	35.8	8.6	91.2
		%State. Error	9.3	5.8	2	7.5	3.6	2.9	8	9.5	11	23	19

Table 2. Relative calculation for Cu/Zn, P/K, and absolute value for calcium concentration for each samples of both benign and cancerous type of breast tissues.

							Benign							
No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Ca	28	386	1222	721	198	119	299	2289	895	3137	388	2015	90	1908
Cu/Zn	0.15	0	1.16	0	0	?	0.45	0.22	0	0	1.29	0	0	0
P/K	2	0.84	1.2	4.5	16.2	1	5.2	3.6	0.49	3.32	1.9	3.8	2.9	2.3
							Malignant							
No.	1	2	3	4	5	6	7	***	***	***	***	***	***	***
Ca	577	124	193	864	128	592	509	***	***	***	***	***	***	***
Cu/Zn	1.55	0.14	0	0.16	0	0.37	0	***	***	***	***	***	***	***
P/K	1.24	1.6	3.3	1.8	1.85	0.25	1.4	***	***	***	***	***	***	***

Benign (non-cancerous) breast tumors

Most often occur during the reproductive period of life or just afterward. These are often difficult to distinguish from malignant tumors and must be watched for a change in size, or lymphatic involvement, where case the growth should be cut and analyzed. In tumors the cells do not spread to other parts of the body. According to the obtained results, it is concluded that table 1 trace and minor elements in both normal and cancerous human breast tissues.

The concentration of Calcium in benign type tissues was large in comparison with those in malignant type. From statistical point of view, the P/K and Cu/Zn ratio for benign kind of tissues are higher than those for malignant type. From 14 samples of this type only 5 samples contained cadmium and 3 samples have bromium. The mean value of sulfur in non-cancerous tissues was higher than the cancerous type.

Malignant (cancerous) tissues

Breast cancer is the most common type of cancer in women and is a major public health problem in some regions .Some women are expected to be diagnosed sometime during their lives. Cancer of the breast is treated by several measures dependent on the patients’ age, clinical status, the type and size of the tumors, the degree of the spread, and the estrogen responsiveness of the tumor. Some 35% of breast cancer in women of child bearing age are estrogen-dependent, meaning that their continued growth is dependent on the presence of estrogen. According to results obtained in table 1 the concentration of Zn in malignant tissues were large comparing with those in benign type. From 7 samples of this kind only one of samples had cadmium and in 2 samples was bromium observed. Iron for this type of samples was significantly greater than benign type as shown in diagram of figure 3. Typical spectrum for cancerous and benign type of human breast tissue is shown in Figure 1 and 2 respectively. In those figures (a) represents for low Z elements and (b) for high Z elements.

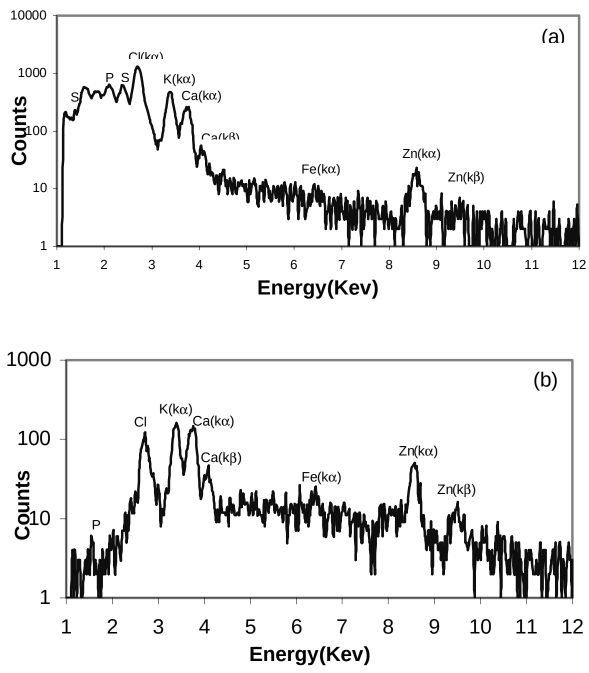

Figure 1. Typical spectrum for malignant type of human breast tissue, (a) for low Z elements (without absorber filter) and (b) for high Z elements with 175μ mylar absorber.

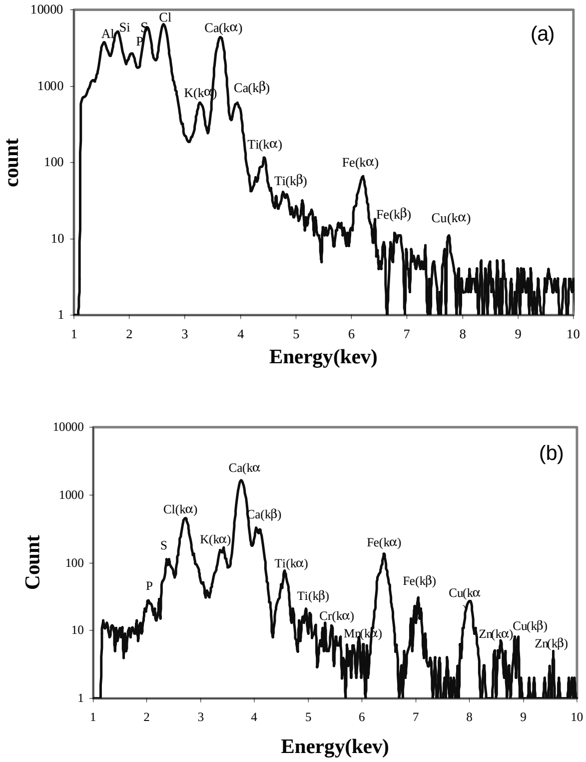

Figure 2. Typical spectrum for benign type of human breast tissue, (a) for low Z elements (without absorber filter) and (b) for high Z elements with 175µ maylar absorber.

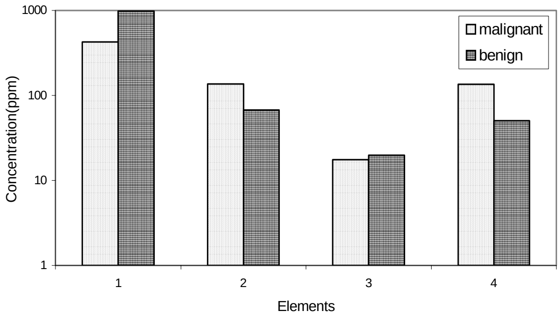

Figure 3. B Diagram of comparative concentration for four elements: 1 (Ca), 2 (fe), 3 (Cu), 4 (Zn) of two types of samples.

CONCLUSIONS

In study of breast diseases, 21 samples of breast tissues of Iranian women, which were supplied by surgerical equipments, were discussed. Among 21 samples 7 samples ,were malignant (cancerous) type , and 14 samples were benign (non cancerous) kind. The samples belonged to wide age range of patients. The tissues were analyzed by PIXE (Proton Induced X-ray Emission) in vacuum by bombarding the targets by 2.0 Mev proton beams. The results are shown in Tables (1) and (2)- absolute and relative analysis respectively. Ca, Fe, Cu and Zn were compared for both types of tissues in diagram of Fig.3. Relative values of Cu/Zn, P/K and also Calcium and S in benign type were higher than those in malignant type,but the concentration of Fe and Zn in cancerous tissues was significantly higher than those for benign type.

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