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A proton induced X-ray emission (PIXE) analysis of concentration of trace elements in varicose veins

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Background: Proton induced X-ray emission (PIXE) has been applied as reliable and improved techniques in this study to compare concentration of various trace elements in normal and abnormal varicose veins. ***Materials and Methods:*** Five samples from normal veins and 13 samples from varicose veins bombarded by 2.0 MeV energy proton beams produced by a Van de Graff accelerator in vacuum. Two specimens from each sample, approximately 1cm, were processed for PIXE (proton induced X-ray emission) analysis. Both qualitative and quantitative analyses of potassium, iron, copper and zinc concentration were performed with respect to calcium concentration. ***Results:*** The concentration of potassium and iron in the varicose vein group has been significantly higher than the normal group. Copper and zinc concentration were also higher in the varicose veins, and the elevation of bromine was seen in the normal group. ***Conclusion:*** PIXE analysis showed higher concentrations of trace elements in veins derived from varicos patients compared to normal group. The difference in normal and abnormal vein might be independent of age. **Iran. J. Radiat. Res., 2010; 8 (2): 117-121**

Keywords: *Varicose vein, trace elements, PIXE.*

INTRODUCTION

Varicose veins are the most common vascular disorders affecting human beings. There are four factors that affect the development and progression of varicose vein: heredity, female sex hormones, gravitational hydrostatic force and hydrodynamic muscular compartment forces. A familial tendency toward the development of varicosities may be the most important predisposing factor. Varicose veins are a common occurrence in pregnancy, usually appearing in the first trimester (70 to 80 percent) when the corpus luteum is secreting progesterone. Progesterone is known to inhibit smooth muscle contractibility and venous distensibility increase. Hydrostatic forces produce venous dilatation from the weight of the blood column transmitted through incompetent valves. Homans noted in 1917 that "the overstretching of the vein walls and the destruction of the valves upon which the mechanism principally depends bring about a degree of surface stasis which obviously interferes with the nutrition of the skin and subcutaneous tissues" [(1)]. According to several theories, the physical property of the vein may be affected by biochemical abnormalities such as trace elements which can play a role in the occurrence of varicose [(2)]. Varicose veins may be due to the weakness of the vein wall as a result of structural problems. There are conflicting findings in the literature about these problems, especially concerning collagen, elastin and smooth muscles content. In recent years, endovenous laser therapy (EVLT) as a minimally invasive alternatives to surgical treatment of varicose vein been developed with promising results. The goal is to cause nonthrombotic vein occlusion by heating the vein wall and collagen contraction and denudation of endothelium resulting in vein -wall thickening with eventual fibrosis of the vein [(3)]. No available studies have

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compared the structural problems of varicose veins in the case of the presence of valvular incompetence and normal valves [4-6]. Also, Elsharawy *et al.* histopathologic findings [7] support the theory of primary weakness of the vein wall as a cause of varicosity [8]. Weakness in the walls of the veins and the toxicity of the blood (due to constipation and poor diet or digestion), also, play their part. Unlike the arterial circulation, which has a pump in the form of the heart, the venous circulation has no such pump. Some nutrition guidelines for taking the minerals and vitamins are suggested for preventing the progress of vascular diseases. Recently, it has been reported that varicose veins can develop without valvular incompetence [9, 10].

The method of proton induced X-ray emission (PIXE) has been used to determine the elemental distribution profiles in gall stone, thyroid, breast and other tissues, simply by irradiating the sample with a focused proton beam [11, 12]. The aim of this work was to study the structural wall abnormalities of varicose veins and compare the analytical results with normal vein walls in order to find some nutritional intake differences.

MATERIALS AND METHODS

Sample preparation

The normal and varicose vein tissues were obtained from 18 patients (thirteen males and five females with a wide age range from 23 to 77) by surgical operations. Five samples belonged to normal vein tissues and the other 13 belonged to varicose leg veins of the patients. This study was approved by the Ethical Committee of Tehran University of Medical Sciences. Both normal and varicose veins were washed with serum during the surgery. The selected areas had the maximum damage and were preferred to be bombard without any further processing (homogenization and lyophilization), and put as thick target on a capton foil whose thickness is about few mm. The samples were washed out by normal saline to remove the blood and clot from their surface. Since the chlorine and sodium were not the elements considered for analysis, there was no limitation in washing them out. The samples were suspended on aluminum sample holders with holes at the center. IAEA (International Atomic Energy Agency) MA-B-3/TM Fish tissue was used as the standard for the calibration of the PIXE set up. The standard target was prepared by pressing 250 mg of the powdered standard into a pellet (1.7 cm in diameter) without any additions. All the samples are sprayed by Carbon foil in order to making them conductive.

Equipment and measurement

The 3.0 MeV van-de-Graff Electrostatic Accelerator (High Voltage Company, USA) of Nuclear Science Research School (Nuclear Science and Technology Research Institute, Iran) was used for PIXE analysis. A 2.0 MeV proton beam with the spite size of 2.8 mm^2 was irradiated on the samples placed in the vacuum chamber at 10^{-6} torr. The samples were placed at the angle of 90° with respect to the incident beam. The samples' wheel of the system could accommodate up to 16 sample holders at the same time without breaking the vacuum. The beam current was adjusted at about 3 nA in order to keep the counting rate below 1000 cps (count per second). The integrated charge of the beam was monitored to be 10 mC for each sample. A tungsten filament inside the vacuum chamber and close to the samples was turned on during data collection for reducing the background of the spectra.

The characteristic X-rays emitted from the samples were detected by an Si (Li) detector positioned at 135° with respect to the incident beam. A 175 mm thick Mylar absorber foil was placed in front of the detector in order to decrease the intensity of low energy X-rays originating from the matrix elements. However, the light elements were detected and measured with-

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out using the maylar foil. The energy resolution of the detector was 175 eV at 5.9 keV. The solid angle of the detector was limited to 3.3×10^{-3} sr (Stradian). This value was set using one of the calibration techniques chosen form among a wide variety of techniques available for thick specimens using a 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 codes AXIL (Analytical X-ray analysis by iterative least-squares) and GUPIX (Guelf pixe group) [13]. The data obtained from the computer program were net peak areas of K&L X-ray. Errors normally come from counting statistics and background values. Since the spectra of low Z elements and high Z elements were collected individually, they should be calibrated based on the efficiencies of the detector for calcium and Iron, separately. Each pair of PIXE spectra obtained with and without Mylar absorbers for the specific samples (figure 1).

RESULTS AND DISCUSSION

The results are summarized in table 1 using descriptive statistics and shown in figure 2. The mean and standard deviation are compared with certified values [14]. All the concentrations are expressed in ppm with an acceptable value of LOD (limit of detection), except for copper and bromine. Some of the patients had the clinical evidence of venous insufficiency; the others had arterial injuries. Some of the varicose group had saphenofemoral valve (SFV) and some had saphenofemoral valve. The study of the structural abnormality or weakness of the vein wall, especially concerning collagen, elastin and smooth muscle cell content, were not the concern. Such structural abnormalities might be due to mineral intake or deficiency. While nutrition can do little to correct a genetic weakness of the vein tissue, diet can help patients to prevent the formation of varicose veins by improving blood circulation in legs and ensuring regular bowel movements. Studies have been done in relation to the trace elements with varicose vein walls by

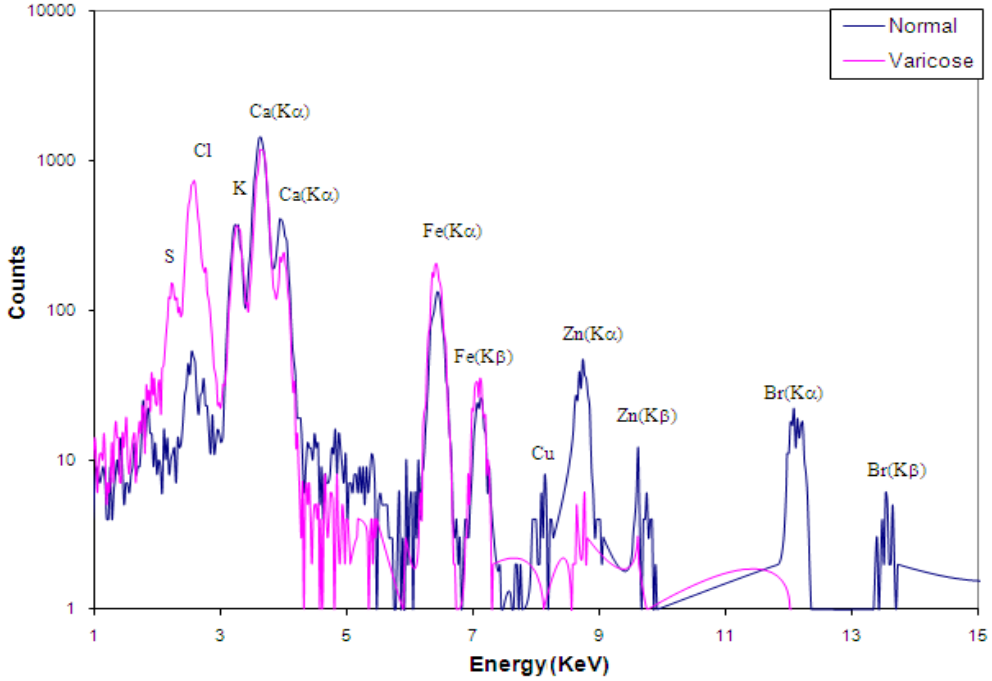

Figure 1. Typical PIXE spectrum of normal and varicose veins with 175mm Maylar absorber in front of Si(Li) detector.

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Ercan *et al.* [2]. Iron and copper have an important role in varicose vein abnormalities and can lead to serious illnesses [15].

As shown in table 1, the concentration of potassium and iron in the varicose vein group is significantly higher than the normal group ($p<0.05$). Copper and zinc concentrations are also higher in the varicose veins, but the elevation of bromine was seen only in normal group. All concentration values were derived while concentration of calcium kept constant according the certified value.

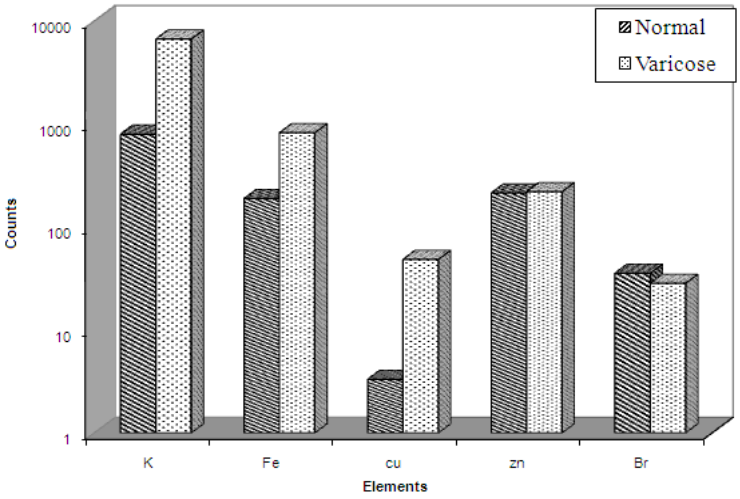

Figure 2. Diagram of mean values of potassium, iron, copper, zinc and bromine for normal and varicose human veins.

Table1. Analytical result of potasium, calcium, iron, cupper, zinc and bromium in the normal and varicose veins wall.

			K	Ca	Fe	Cu	Zn	Br
	Certified value		2100	1700	77	2.2	48	
		1	1369	1701	55	2.7	38	75
		2	541	1701	280	4.6	72	8
		3	1337	1704	242	6.8	571	61
Normal vein wall	This study	4	283	1705	254	0	285	16
		5	461	1705	115	2.5	106	18
	Mean		798.2	1703.2	189.2	3.32	214.4	35.6
	SD		257	0.4	49.2	1.27	110	15
		1	2799	1701	959	3.4	127	15
		2	1555	1702	613	15	73	26
		3	6429	1702	819	8.8	122	54
		4	9495	1705	422	0	629	170
varicose vein wall	This study	5	4664	1701	1272	0	225	0
		6	4871	1702	536	33	304	0
		7	8168	1703	473	0	303	0
		8	759	1701	414	3.6	15.6	38
		9	31625	1702	2829	521	594	0
		10	9885	1702	1202	43	232	0
		11	537	1701	200	3.3	132	0
		12	2413	1701	261	0	35	67
		13	3817	1700	706	0	75	0
	Mean		6693.6	1701.8	823.5	46	220.5	28.4
	SD		2343	0.35	198.6	41	56.8	13.97

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According to the obtained results, the elevation of zinc, iron, copper and the decrease of bromine in this study might have led to varicosity. Also, because the samples were derived from a wide age range of patients, this abnormality might be independent of age of the patients.

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