TRACE ELEMENT ANALYSIS OF PROSTATE TISSUES USING SYNCHROTRON RADIATION

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Abstract. *Prostate cancer (PCa) is one of the main causes of illness and death all over the world. In Brazil, prostate cancer currently represents the second most prevalent malignant neoplasia in men, representing 21% of all cancer cases. Benign Prostate Hyperplasia (BPH) is an illness prevailing in men above the age of 50, close to 90% after the age of 80. Metallic elements and their organic compounds have dynamic regulatory functions in cells. The prostate produces and stores concentrations of many important biological substances.*
In this work the samples of human prostate tissues with cancer, HPB and normal tissue were analyzed utilizing the Total Reflection X-Ray Fluorescence spectroscopy by synchrotron radiation (SRTXRF). The SRTXRF was used to investigate the differences in the elemental concentrations among prostate tissues (PCa, BPH and Normal). The X-Ray Fluorescence measurements were performed at the X-Ray Fluorescence Beamline at Brazilian National Synchrotron Light Laboratory (LNLS), in Campinas, São Paulo. It was possible to determine the concentrations of the following elements: P, S, K, Ca, Fe, Cu, Zn, Br and Rb. By using Mann-Whitney U test it was observed that almost all elements presented concentrations with significant differences ($\alpha = 0.05$) among the groups studied. The elements that presented significant differences among groups, were: S, K, Ca, Fe, Zn, Br and Rb (PCa X Normal); S, Fe, Zn and Br (PCa X BPH); K, Ca, Fe, Zn, Br and Rb (BPH X Normal).

***Keywords**: X-Ray Fluorescence; Synchrotron radiation; Trace elements; Prostate Tissues.*

1. INTRODUCTION

In Brazil, prostate cancer is the second kind of cancer more frequent in man - an estimation of 49.000 cases for 2008 - corresponding to a risk of 52 new cases per 100.000 habitants. In the world, it is the sixth more common and predominant cancer in men accounting for approximately 10% of all cancer cases. These incidence rates are about 6-fold higher in developed countries, as compared to the underdeveloped (Estimative 2008, 2007).

Prostatic cancer is typically a neoplasm of the aged, and its incidence increases after 50 years old. Eating habits, ethnic or environmental factors and life style are directly connected to its incidence (Zaichick *et al.*, 2004b). Benign Prostatic Hyperplasia (BPH) is the most predominant illness in prostate, occurring in approximately 50% in men over 50, reaching a 90% incidence after the ninth decade of life. BPH is defined as a continuous enlargement of the prostate causing, among other symptoms, weak urinary stream, nocturia, recurrent urinary tract infection, urinary retention, eventually leading to limitation in social activities (Lee *et al.,* 1995).

The prostate presents a high zinc concentration, about 10 times higher than any other body tissue. Zinc distribution in the prostate is not uniform. Its concentration increases as the bladder distance enlarges and higher concentrations are found in peripheral zone (Zaichick, 1997a; Vartsky, 2003). It has been reported that zinc concentration may vary according to age (Zaichick *et al*., 2004b). Studies show that zinc concentration decreases considerably in prostate cancer and increases in BPH tissues (Zaichick, 1997a; Vartsky *et al*., 2003), except for "Yaman *et al*. (2007)" who found an increase in zinc concentration analyzing cancer tissues.

The total reflection X-ray fluorescence using synchrotron radiation (SRTXRF) is a multielemental technique widely used in the analysis of low concentration ($\mu g.g^{-1}$) in environmental, medical and biological samples (kubala-kukuś *et al*., 2007c). TXRF analysis is a well established analytical technique for the detection of major, minor and trace elements (Streli et al., 2008), especially suited for samples, whenever only small specimen mass is available. On the other hand, synchrotron radiation is an excellent source for exciting X-ray fluorescence (Jones *et al*., 1992; Kwiatek *et al*., 2005).

The aim of this work was to analyze prostate samples through Total Reflection X-ray Fluorescence using synchrotron radiation technique (SRTXRF) to compare the elemental concentrations found in PCa, BPH and normal prostate tissues, and also to verify if zinc can be a PCa bioindicator.

2. MATERIALS AND METHODS

2.1 Population Characteristics

This study was conducted following approval by the Internal Review Board of the Clementino Fraga Filho Teaching Hospital of the Federal University of Rio de Janeiro, Brazil. The prostate samples were collected from 55 patients submitted to surgery at the Andarai Hospital in Rio de Janeiro city - Brazil. The age-range of the patients was 40 – 85 years old. The experimental groups were constituted of 11 patients with PCa and 44 with BPH. The control group was formed by 4 samples of individuals with age-range from 18 - 30 years old who died from unexpected death (accident, murder, cardiac arrest).

2.2 Samples Preparation

The mass of the tissues samples varied from 10 mg to 50 mg. The tissues were washed in MILLI-Q water, lyophilized in a freeze-dryer at -60°C, crushed and afterwards they were digested in HNO_3 at 60°C. The solutions digested were diluted in MILLI-Q water up to 2 mL and a 50 μL aliquot of Ga solution (Standard ICP Ga - 100 μgg^{-1}) was added as internal standard. Next, the solution was homogenized by shaking and a small aliquot of 5 μL was pipetted on Perspex sample. After deposition, the samples were oven dried at 40°C. The methodology was validated through analysis of samples of standard reference material (Bovine liver 1577b), prepared under the same conditions of the prostate samples.

2.3. Experimental Organization

The measurements were carried out using SRTXRF technique in the XRF beam line at the Synchrotron Light National Laboratory, in Campinas, São Paulo, using a polychromatic beam with maximum energy of 20 keV for excitation and an HpGe detector with resolution of 140 eV at 5.9 keV. The acquisition time was set to 100 s for each measurement. The obtained X-ray fluorescence spectra were evaluated by software QXAS distributed by International Atomic Energy Agency (Bernasconi and Tajani, 1996).

3. RESULTS AND DISCUSSIONS

The methodology was validated by analysis of Standard Reference Material from NIST (bovine liver - 1577b). The results with relative error varied from 0.01 for Potassium and 0.21 for Rubidium. Table 1 shows the certified values and the results obtained in this work. The obtained results agree well with the certified values.

By using the SRTXRF technique it was possible to detect the following elements: P, S, Cl, K, Ca, Fe, Cu, Zn, Br and Rb. Figure 1 shows a typical X Ray fluorescence spectrum of a sample of a prostate tissue with BPH. Table 2 shows the mean elemental concentration with confidence intervals ($\alpha = 0.05$) and median values for normal prostate, BPH and PCa tissues. The elements that showed higher concentration levels were P, S and K. The results showed that there was a reduction in the concentration of S, Cl, K, Ca, Fe, Zn and Rb on the two pathologies (PCa and BPH), as comparing with the concentrations obtained in normal tissues. Comparing the results with the ones of literature, the values of elemental concentration determined are close, except for Chlorine, whose difference is being investigated.

Table 1. Mean values and standard deviations (μg.g^{-1})
of concentrations certified reference and analytical results.

Element	**Concentration (μg.g^{-1})**		
	Certified	This work	Relative error
P (%)	1.10 ± 0.03	1.08 ± 0.16	0.02
S (%)	0.785 ± 0.006	0.81 ± 0.07	0.03
K (%)	0.994 ± 0.02	1.01 ± 0.12	0.01
Ca	116 ± 4	127 ± 58	0.09
Mn	11 ± 2	9 ± 1	0.18
Fe	184 ± 15	171 ± 7	0.07
Cu	160 ± 8	166 ± 6	0.04
Zn	127 ± 16	139 ± 8	0.09
Rb	14 ± 1	11 ± 2	0.21

In the literature it has been related that the elemental distribution in biological samples is not symmetric (kubala-kukuś *et al*., 2004b). As a result of these properties, it was observed that an estimation of the median is less sensitive on the tails of distribution. The median of concentration distribution can be systematically more precisely estimated than the mean value and consequently median is a better parameter to describe the distribution of trace element concentration in medical samples (kubala-kukuś *et al*., 2007c). For a non-parametric comparison of two groups Mann-Whitney U test was applied in order to verify if there were statistical differences between BPH and normal population and between PCa and normal population. In this case it was used a level of significance of 5 % in all tests.

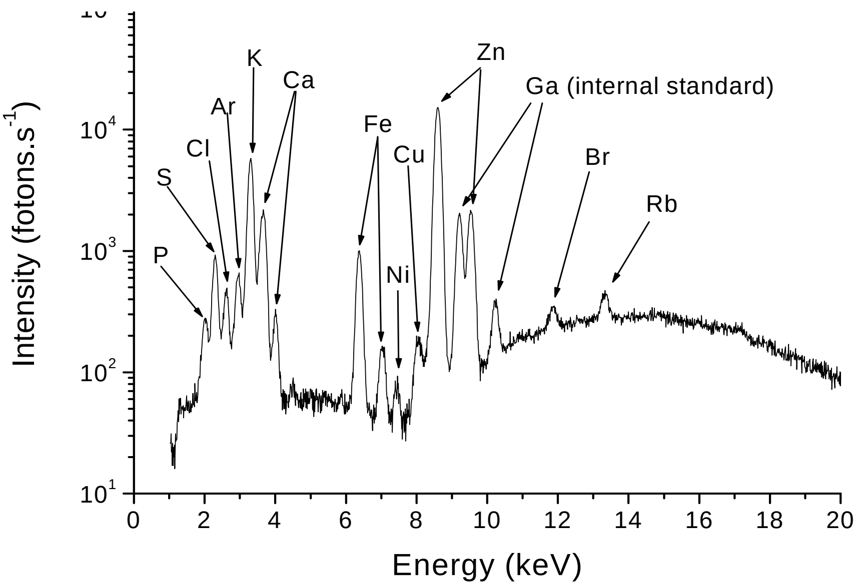

Figure. 1: The X-ray fluorescence spectra of a prostatic tissue sample with BPH

Mann-Whitney U for BPH and normal population presented significant differences between the median concentrations of the elements Cl, K, Ca, Fe, Zn, Br and Rb. It can be observed a decrease of concentration of Cl (70 %), K (62%), Ca (51%), Fe (73%), Zn (25%), Br (14%) and Rb (50%) in samples with BPH as compared to samples of normal prostatic tissue. Besides, the test presented statistical differences between the normal samples and PCa population for the elements Cl, K, Fe, Zn and Rb, particularly, a decrease of concentrations of S (37%), Cl (78%), K (69%), Ca (54%), Fe (82%), Zn (61%), Br (57%) and Rb (38%) in PCa was observed, as compared to the median concentrations obtained in normal tissue. In addition, statistically significant differences between samples with PCa and BPH were found for concentrations of S, Cl, Fe, Zn and Br, a decrease of concentrations of S (24%), Cl (28%), Fe (33%), Zn (48%) and Br (50%) in tissues samples of prostate with cancer was observed as compared to tissues samples with BPH.

Table 2 – Mean values with confidence intervals and medians of concentrations of trace elements in the prostate tissue.

Element	Mean with confidence intervals ($\mu g.g^{-1}$) Median ($\mu g.g^{-1}$)		
	BPH	Cancer	Normal
P	3422 ± 299 3243	3621 ± 684 3751	2852 ± 564 2743
S	8662 ± 546 8548	6871 ± 1140 6516	10756 ± 1587 10331
Cl	817 ± 111 640	515 ± 151 463	2429 ± 386 2127
K	1744 ± 211 1488	1456 ± 327 1236	3839 ± 415 3934
Ca	861 ± 94 707	658 ± 109 665	2715 ± 1207 1435
Fe	63 ± 11 45	35 ± 9 30	173 ± 55 164
Cu	3.5 ± 0.5 3.0	4.5 ± 1.0 4.0	ND(1)
Zn	420 ± 43 372	210 ± 44 192	699 ± 221 493
Br	7 ± 1 6	4 ± 1 3	14 ± 4 7
Rb	7 ± 1 6	8 ± 1 8	12 ± 2 12

(1) ND = Not detected

The changes in mean concentrations of these elements in cancerous tissue are real. Since most of these elements are very important for various biological and enzymatic processes (kubala-kukuś *et al*.,1999a). In this study it was observed that the concentration of zinc decreases in prostate tissue with cancer as compared to normal prostatic tissue. This result is close to another work in literature (Zaichick *et al*., 1997a). Zinc is concentrated in the glandular epithelial cell and it is bound to proteins such as metalloenzymes. Studies show that one of the main functions of the human prostatic gland is the accumulation and secretion of high levels of citrate by the peripheral prostate zone. Furthermore, citrate metabolism noticeably alters in BPH and PCa. The higher levels of zinc are found in the mitochondria and prevent citrate oxidation by Krebs cycle. The decrease in citrate oxidation represents 65 % of the ATP efficiency. The reduction of zinc carry-on to non-inhibition m-acomitase enzyme activity, so citrate becomes fully oxidized. As consequence, an increase in the ATP production occurs (Costello *et al*., 1998a, 1999b, 2000c, 2006d).

4. CONCLUSIONS

SRTXR proved to be a highly efficient technique in the analysis of biological samples, being capable of determining low elemental concentrations ($\mu g.g^{-1}$). The methodology used to prepare the samples proved to be efficient. It was possible to determine the concentrations of the elements P, S, Cl, K, Ca, Fe, Cu, Zn, Br and Rb. Analysis using Mann-Whitney U test showed, with 5 % of significance, that there was a decrease in Cl, K, Ca, Fe, Zn, Br and Rb concentration in BPH as compared to normal prostatic tissues. On the other hand in PCa, the Mann-Whitney U showed that there was a reduction of S, Cl, K, Ca, Fe, Zn, Br and Rb, therefore suggesting an association of these elements with carcinogen processes. The PCa and BPH samples present significant differences only for S, Cl, Fe, Zn and Br. The Pca samples presented a 61% reduction of Zn concentration, as compared to normal prostate tissues, suggesting that this element can be a bioindicator for prostate illness.

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7. RESPONSIBILITY NOTICE

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