Abstract

The aim of the study was to evaluate whether significant changes in the prostatic tissue levels of ratios Rb/trace element contents exist in the malignantly transformed prostate. Contents of Rb and other 42 trace elements [Ag, Al, Au, B, Be, Bi, Br, Cd, Ce, Co, Cr, Cs, Dy, Er, Fe, Gd, Hg, Ho, La, Li, Mn, Mo, Nb, Nd, Ni, Pb, Pr, Sb, Sc, Se, Sm, Sn, Tb, Th, Ti, Tl, Tm, U, Y, Yb, Zn, and Zr] in normal [N, n=37], benign hypertrophic [BPH, n=32], and cancerous human prostate [PCa, n=60] were investigated. Measurements of trace element contents were performed using a combination of neutron activation analysis and inductively coupled plasma mass spectrometry. Then the levels of ratios Rb/trace element contents in every sample were calculated. It was observed that the ratio to Rb of Ag, Al, Au, B, Be, Bi, Br, Ce, Cr, Cs, Dy, Er, Fe, Gd, Hg, Ho, La, Li, Mn, Mo, Nb, Nd, Ni, Pb, Pr, Sb, Sc, Se, Sm, Sn, Tb, Th, Ti, Tl, Tm, U, Y, Yb, Zn, and Zr mass fraction were significantly lower in cancerous tissues than in normal and BPH prostate. Finally, we propose to use the Rb/Ag, Rb/Al, Rb/B, Rb/Be, Rb/Bi, Rb/Br, Rb/Ce, Rb/Li, Rb/Mn, Rb/Pr, Rb/Sn, Rb/Sn, Rb/Tb, Rb/Tl, Tb, and Rb/Zr mass fraction ratios in a needle-biopsy core as an accurate tool to diagnose prostate cancer. Further studies on larger number of samples are required to confirm our findings and to investigate the impact of the trace element relationships on prostate cancer etiology.

Keywords: Trace Elements; Trace Element Content Ratios; Prostate; Benign Prostatic Hypertrophy; Prostatic Carcinoma; Neutron Activation Analysis; Inductively Coupled Plasma Mass Spectrometry.

Introduction:

The prostate gland may be a source of many health problems in men past middle age, the most common benign prostatic hyperplasia [BPH], and prostatic carcinoma [PCa]. BPH is a noncancerous enlargement of the prostate gland leading to obstruction of the urethra and can significantly impair quality of life. The prevalence of histological BPH is found in approximately 50-60% of males age 40-50 and greater than 90% of men over 70 years old [1, 2]. In many Western industrialized countries, including North America, PCa is the most frequently diagnosed form of noncutaneous malignancy in males. Except for lung cancer, PCa is the leading cause of death from cancer [3-8]. Although the etiology of BPH and PCa is unknown, some trace elements have been highlighted in the literature in relation to the development of these prostate diseases [9-29].

Trace elements have essential physiological functions such as maintenance and regulation of cell function and signalling, gene regulation, activation or inhibition of enzymatic reactions, neurotransmission, and regulation of membrane function. Essential or toxic [mutagenic, carcinogenic] properties of trace elements depend on tissue-specific need or tolerance, respectively [30]. Excessive accumulation, deficiency or an imbalance of the trace elements may disturb the cell functions and may result in cellular degeneration, death and malignant transformation [30].

In earlier reported studies [31-68] significant changes of trace element contents in hyperplastic and cancerous prostate in comparison with those in the normal prostatic tissue were observed. Moreover, a significant informative value of Zn/Rb content as a tumor marker for PCa diagnostics was shown by us [69,70]. Hence it is possible that besides Zn, the ratio of Rb to
other trace elements also can be used as tumor markers for
distinguish between benign and malignant prostate. Currently
number of methods was applied for the measurement of chemical
elements contents in samples of human tissue. Among these
methods, the instrumental neutron activation analysis [INAA] is a
non-destructive and one of the most sensitive techniques.
It allows measure the chemical element contents in few milligrams
tissue without any treatment of sample. Analytical studies of the
Ag, Br, Ca, Co, Cr, Fe, Hg, K, Mg, Mn, Na, Sb, Sc, Se, and Zn contents
in normal, BPH and PCa tissue were done by us using INAA
[14,15,20,27,28,53,54,60-62,64]. Nondestructive method of
analysis avoids the possibility of changing the content of trace
elements in the studied samples [71-74], which allowed for the
first time to obtain reliable results. In particular, it was shown
that the average mass fraction of Co, Cr, Hg, Sb, and Se in BPH
were higher than normal levels [66]. In adenocarcinoma of prostate
the mean values of Ag, Br, Cr, Fe, Hg, Mn, and Sb were
higher, while those of Ca, Co, Mg, Rh, Sc, and Zn were lower than
in healthy prostatic tissue [60, 61, 67, 68]. Obtained results
formed the basis for a new method for differential diagnosis of
BPH and PCa, the essence of which was to determine the ratios
of chemical element contents changed in opposite directions during
malignant transformation of prostate.

It is obvious that the most effective will be non-destructive
analytical methods, because they involve a minimal treatment of
sample, since the chance of significant loss or contamination would be decreased. However, instrumental
neutron activation analysis with high resolution spectrometry of long-lived radionuclides [INAA-LLR] allows only determine
the mean mass fractions of 10-11 trace elements in the
samples of normal and cancerous prostate glands [15,28].
The inductively coupled plasma mass spectrometry [ICP-MS] is
more power analytical tool than INAA-LLR [18], but sample
digestion is a critical step in elemental analysis by this method.
In the present study two these analytical methods were used
and the results, obtained for some trace elements by ICP-MS,
were under the control of INAA-LLR data.

The present study had three aims. The main objective was
to obtain reliable results about the 43 trace elements: Ag, Al,
Au, B, Be, Bi, Br, Cd, Ce, Co, Cr, Cs, Dy, Er, Fe, Gd, Hg, Ho, La, Li,
Mn, Mo, Nb, Nd, Ni, Pb, Pr, Rb, Sb, Sc, Se, Sm, Sn, Tb, Th, Ti, Tl,
Tm, U, Y, Yb, Zn, and Zr contents in intact prostate of healthy
men aged over 40 years and also in the prostate gland of age-
matched patients, who had either BPH or PCa, combining in
consecutive order non-destructive INAA-LLR with destructive
ICP-MS method. The second aim was to calculate Rb/trace
element content ratios and compare the levels of these ratios
in normal, hyperplastic, and cancerous prostate. The third and
final aim was to evaluate the ratios of Rb/trace element
contents for diagnosis of prostate cancer.

All studies were approved by the Ethical Committees of
the Medical Radiological Research Centre, Obninsk.

Material and Methods:

Samples: The patients studied [n=92] were hospitalized in the
Urological Department of the Medical Radiological Research
Centre [Obninsk, Russia]. All of them were European-Caucasian,
citizens of Moscow and Obninsk [a small city in a non-industrial
region 105 km south-west of Moscow]. Transrectal puncture
biopsy of suspicious indurated regions of the prostate was
performed for every patient, to permit morphological study of
prostatic tissue at these sites and to estimate their chemical
element contents. In all cases the diagnosis has been confirmed
by clinical and morphological results obtained during studies of
biopsy and resected materials. The age of 32 patients with BPH
ranged from 56 to 78 years, the mean being 66±6 [M±SD] years.
The 60 patients aged 40-79 suffered from PCa [stage T1-T4].
Their mean age was 65±10 [M±SD] years. Intact prostates [N]
were removed at necropsy from 37 men aged 41-87 who had
died suddenly. All deceased were European-Caucasian, citizens of
Moscow. Their mean age was 55±11 [M±SD] years. The majority
of deaths were due to trauma. Tissue samples were collected
from the peripheral zone of dorsal and lateral lobes of their
prostates, within 2 days of death and then the samples were
divided into two portions. One was used for morphological study
while the other was intended for chemical element analysis. A
histological examination was used to control the age norm
conformity, as well as to confirm the absence of
microadenomatosis and latent cancer [14,15,20,28].

Sample Preparation: All tissue samples were divided into
two portions. One was used for morphological study while the
other was intended for trace element analysis. After the
samples intended for trace element analysis were weighed,
they were freeze-dried and homogenized. The sample
weighing about 10 mg [for biopsy materials] and 50-100 mg
[for resected materials] was used for trace element
measurement by INAA-LLR. The samples for INAA-LLR were
wrapped separately in a high-purity aluminum foil washed
double rectified alcohol beforehand and placed in a nitric
acid-washed quartz ampoule.

After INAA-LLR investigation, the prostate samples were
taken out and used for ICP-MS method. The samples were
decomposed in autoclaves; 1.5 mL of concentrated HNO3,
[nitric acid at 65 %, maximum [max] of 0.0000005 % Hg; GR,
ISO, Merck] and 0.3 mL of H2O2 [pure for analysis] were added
to prostate tissue samples, placed in one-chamber autoclaves
[Ancon-AT2, Ltd., Russia] and then heated for 3 h at 160-200
°C. After autoclaving, they were cooled to room temperature
and solutions from the decomposed samples were diluted with
deionized water [up to 20 mL] and transferred to the plastic
measuring bottles. Simultaneously, the same procedure was
performed in autoclaves without tissue samples [only
HNO3+H2O2+ deionized water], and the resultant solutions
were used as control samples.

Instrumentation and Methods: A vertical channel of a nuclear
reactor was applied to determine the trace element mass
fractions by INAA-LLR. The quartz ampoule with prostate
samples and certified reference materials was soldered,
positioned in a transport aluminum container and exposed to a
24-hour neutron irradiation in a vertical channel with a neutron
flux of 1.3·1013 n·cm⁻²·s⁻¹. Ten days after irradiation samples
were reweighed and repacked. The samples were measured for period
from 10 to 30 days after irradiation. The duration of
measurements was from 20 min to 10 hours subject to pulse
counting rate. The gamma spectrometer used for INAA-LLR
included the 100 cm² Ge[Li] detector and on-line computer-based
multichannel analyzer. The spectrometer provided a resolution of
1.9 keV on the 60Co 1332 keV line. Other details of the INAA-LLR analysis were presented in our previous publication [15]. An ICP-MS Thermo-Fisher “X-7” Spectrometer [Thermo Electron, USA] was used to determine the content of trace elements by ICP-MS. The element concentrations in aqueous solutions were determined by the quantitative method using multi elemental calibration solutions ICP-MS-MA78A and ICP-AM-6-A produced by High-Purity Standards [Charleston, SC 29423, USA]. Indium was used as an internal standard in all measurements. Information detailing the results of this quality assurance study was presented in our previous publication [18].

**Certified Reference Materials:** For quality control, ten subsamples of the certified reference materials [CRM] IAEA H-4 Animal muscle and IAEA HH-1 Human hair from the International Atomic Energy Agency [IAEA], and also five subsamples INCT-SBF-4 Soya Bean Flour, INCT-TL-1 Tea Leaves and INCT-MPH-2 Mixed Polish Herbs from the Institute of Nuclear Chemistry and Technology [INCT, Warsaw, Poland] were analyzed simultaneously with the investigated prostate tissue samples. All samples of CRMs were treated in the same way as the prostate samples. Detailed results of this quality assurance program were presented in earlier publications [15, 18].

**Computer Programs and Statistics:** A dedicated computer program for INAA mode optimization was used [76]. All prostate samples for INAA-LLR were prepared in duplicate and mean values of trace element contents were used in final calculation. For elements investigated by both INAA-LLR and ICP-MS methods the mean of all results was used. Using the Microsoft Office Excel software Rb/trace element contents for prostate samples for INAA-LLR were prepared in duplicate and mean values of trace element contents were used in final calculation. The arithmetic mean ±standard errors of mean were calculated for trace element mass fraction and for ratios of Rb/trace element mass fraction in normal, benign hyperplastic and cancerous prostate. The difference in the results between BPH and N, PCa and N, as well as PCa and BPH was evaluated by parametric Student’s t-test and non-parametric Wilcoxon-Mann-Whitney U-test. Values of p≤0.05 were considered to be statistically significant. For the construction of “individual data sets for Rb/trace element mass fraction ratios in normal, benign hypertrophic and cancerous prostate” diagrams the Microsoft Office Excel software was also used.

### Results:

Tables 1 and 2 depict our data for trace element mass fractions in CRMs measured using INAA-LLR and ICP-MS, respectively, as well as the certified values of these materials.

**Table 3:** represents mean values ± standard error of mean [M±SEM] of the Element Soya Bean Flour Tea Leaves Mixed Polish Herbs

<table>
<thead>
<tr>
<th>Element</th>
<th>Soya Bean Flour</th>
<th>Tea Leaves</th>
<th>Mixed Polish Herbs</th>
</tr>
</thead>
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<tr>
<td>Ag</td>
<td>0.034±0.008</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Al</td>
<td>0.0208±0.0045</td>
<td>0.0360±0.0024</td>
<td>0.0230±0.0044</td>
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<tr>
<td>Be</td>
<td>0.0071±0.0001</td>
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<td>0.0071±0.0001</td>
</tr>
<tr>
<td>Bi</td>
<td>0.0209±0.0004</td>
<td>-</td>
<td>0.0209±0.0004</td>
</tr>
<tr>
<td>Br</td>
<td>0.0980±0.0800</td>
<td>-</td>
<td>0.0980±0.0800</td>
</tr>
<tr>
<td>Cs</td>
<td>0.057±0.023</td>
<td>-</td>
<td>0.057±0.023</td>
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<tr>
<td>Cr</td>
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<td>0.079±0.08</td>
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<td>Cs</td>
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<td>0.0119±0.0065</td>
<td>-</td>
</tr>
<tr>
<td>Dy</td>
<td>0.0014±0.0002</td>
<td>-</td>
<td>0.0014±0.0002</td>
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<tr>
<td>Er</td>
<td>0.0049±0.0005</td>
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<td>0.0049±0.0005</td>
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<td>Gd</td>
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<td>Hg</td>
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<td>Nb</td>
<td>0.0357±0.0023</td>
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<td>0.0357±0.0023</td>
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<tr>
<td>Nd</td>
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<td>0.0519±0.0002</td>
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<td>Zn</td>
<td>53.5±2.1</td>
<td>53.5±2.1</td>
<td>53.5±2.1</td>
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<tr>
<td>Element</td>
<td>Symbol</td>
<td>Prostatic tissue</td>
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<td>Al</td>
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<td>34±23.5</td>
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<td>Ba</td>
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<td>0.080±0.020</td>
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<tr>
<td>Lithium</td>
<td>Li</td>
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<td>0.0419±0.0055</td>
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<td>Manganese</td>
<td>Mn</td>
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<td>Molybdenum</td>
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<td>0.282±0.038</td>
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<td>Niobium</td>
<td>Nb</td>
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<td>0.0035±0.00053</td>
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<td>Ra</td>
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<td>Titanium</td>
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<td>0.0002±0.0003</td>
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<td>Uranium</td>
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<td>0.0070±0.0021</td>
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<td>Yttrium</td>
<td>Y</td>
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<td>Ytterbium</td>
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<td>Zinc</td>
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<tr>
<td>Zirconium</td>
<td>Zr</td>
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<td>0.035±0.006</td>
</tr>
</tbody>
</table>

Table 4: depicts mean values ± standard error of mean (SEM) of the ratio to Rb of Ag, Al, Au, B, Be, Bi, Br, Cd, Ce, Co, Cr, Cs, Dy, Er, Fe, Gd, Hg, Ho, La, Li, Mn, Mo, Nb, Nd, Ni, Pb, Pr, Sc, Sc, Se, Sm, Sn, Tb, Th, Ti, Ti, Tm, U, Y, Yb, Zn, and Zr mass fraction in normal, benign hypertextic and cancerous prostate.

The ratios of means and the difference between mean values of the Rb/trace element mass fraction ratios in normal, benign hypertropic and cancerous prostate are presented in Table 5.
are shown in Figure 1.

<table>
<thead>
<tr>
<th>Element</th>
<th>Rb/Ag</th>
<th>Rb/Al</th>
<th>Rb/Au</th>
<th>Rb/B</th>
<th>Rb/Be</th>
<th>Rb/Bi</th>
<th>Rb/Br</th>
<th>Rb/Cd</th>
<th>Rb/Co</th>
<th>Rb/Cr</th>
<th>Rb/Cs</th>
<th>Rb/Dy</th>
<th>Rb/Er</th>
<th>Rb/Fe</th>
<th>Rb/Gd</th>
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Individual data sets for Rb/Ag, Rb/Al, Rb/B, Rb/Be, Rb/Bi, Rb/Br, Rb/Ce, Rb/Li, Rb/Mn, Rb/Pr, Rb/Sm, Rb/Sn, Rb/Th, Rb/Tl, and Rb/Zr mass fraction ratios in all investigated samples of normal, benign hypertrophic and cancerous prostate, respectively, are shown in Figure 1.
Figure 1: Individual data sets for Rb/Ag, Rb/Al, Rb/B, Rb/Be, Rb/Bi, Rb/Br, Rb/Ce, Rb/Li, Rb/Mn, Rb/Pr, Rb/Sm, Rb/Sn, Rb/Th, Rb/Tl, and Rb/Zr mass fraction ratios in samples of normal (1), benign hypertrophic (2) and cancerous (3) prostate.
Discussion:

As was shown by us [14,15,17,18], the use of CRM IAEA H-4 Animal muscle, IAEA HH-1 Human hair, INCT-SBF-4 Soya Bean Flour, INCT-TL-1 Tea Leaves, and INCT-MPH-2 Mixed Polish Herbs as certified reference materials for the analysis of samples of prostate tissue can be seen as quite acceptable. Good agreement of the trace element contents in these CRMs, measured by us using INAA-LLR and ICP-MS methods, with the certified data [Table 1 and 2] indicates an acceptable accuracy of the results obtained in the present study.

The mean values and standard error of mean [±SEM] were calculated for 43 trace element contents including Rb [Table 3], as well as for 42 ratios of Rb/trace element mass fractions [Table 4]. The mass fraction of Rb and other 42 trace elements were measured in all, or a major portion of normal prostate samples. The masses of BPH and PCa samples varied very strong from a few milligrams [sample from needle biopsy material] to 100 mg [sample from resected material]. Therefore, in BPH and PCa prostates mass fraction ratios of Rb/trace element content were determined in 21 samples [11 BPH and 10 PCa samples, respectively].

From Table 5, it is observed that in benign hypertrophic tissues the Rb/Ag, Rb/Al, Rb/Au, Rb/Be, Rb/Br, Rb/Cd, Rb/Co, Rb/Fe, Rb/Li, Rb/Mn, Rb/Nb, Rb/Ni, Rb/Sc, Rb/Th, Rb/Ti, Rb/Tl, Rb/Yb, and Rb/Zn mass fraction ratios do not differ from normal levels, but the mass fraction ratios of Rb/Ce, Rb/Cs, Rb/Dy, Rb/Er, Rb/Gd, Rb/Ho, Rb/La, Rb/Mo, Rb/Nd, Rb/Pb, Rb/Pr, Rb/Sm, Rb/Sn, Rb/Th, Rb/Tm, Rb/U, and 3/Rb/Y are higher, while the mass fraction ratios of Rb/B, Rb/Bl, Rb/Cr, Rb/Hg, Rb/Sb, Rb/Se, and Rb/Zr are significantly lower. In cancerous tissue the all Rb/trace element mass fraction ratios investigated in the study are significantly lower, than in BPH and normal prostate, with the exception of Rb/Cd, Rb/Co, Rb/Mo, Rb/Nb, Rb/Sc, Rb/Se and Rb/Zn ratios. Analysis of the mass fraction ratios for trace element in prostate tissue could become a powerful diagnostic tool. To a large extent, the resumption of the search for new methods for early diagnosis of PCa was due to experience gained in a critical assessment of the limited capacity of the prostate specific antigen [PSA] serum test [77,78]. In addition to the PSA serum test and morphological study of needle-biopsy cores of the prostate, the development of other highly precise testing methods seems to be very useful. Experimental conditions of the present study were approximated to the hospital conditions as closely as possible. In BPH and PCa cases we analyzed a part of the material obtained from a puncture transrectal biopsy of the indurated site in the prostate. Therefore, our data allow us to evaluate adequately the importance of Rb/trace element mass fraction ratios for the diagnosis of PCa. As is evident from Table 5 and, particularly, from individual data sets [Fig. 1], the for Rb/Ag, Rb/Al, Rb/Bl, Rb/Br, Rb/Be, Rb/Br, Rb/Ce, Rb/Li, Rb/Mn, Rb/Pr, Rb/Sm, Rb/Sn, Rb/Th, Rb/Tl, and Rb/Zr mass fraction ratios are potentially the most informative test for a differential diagnosis. For example, if 90 is the value of Rb/Ag mass fraction ratio assumed to be the upper limit for PCa [Fig. 1] and an estimation is made for “PCa or intact and BPH tissue”, the following values are obtained:

Sensitivity = (True Positives [TP]/[TP + False Negatives [FN]]) ·100% = 90±6%;
Specificity = (True Negatives [TN]/[TN + False Positives [FP]]) ·100% = 96±2%.
Accuracy = ([TP+TN]/[TP+FP+TN+FN]) ·100% = 93±3%.

The number of people [samples] examined was taken into account for calculation of confidence intervals [79]. In other words, if Rb/Ag mass fraction ratio in a prostate biopsy sample is lower 90, one could diagnose a malignant tumor with an accuracy 93±3%. Thus, using the Rb/Ag mass fraction ratio-test makes it possible to diagnose cancer in 90±6% cases [sensitivity]. The same way parameters of the importance [sensitivity, specificity and accuracy] of for Rb/Ag, Rb/Al, Rb/Bl, Rb/Br, Rb/Be, Rb/Br, Rb/Ce, Rb/Li, Rb/Mn, Rb/Pr, Rb/Sm, Rb/Sn, Rb/Th, Rb/Tl, and Rb/Zr mass fraction ratios for the diagnosis of PCa were calculated [Table 6].

Conclusion:

The combination of nondestructive INAA-LLR and destructive ICP-MS methods is satisfactory analytical tool for the precise determination of 43 trace element mass fractions in the tissue samples of normal, BPH and carcinomatous prostate glands. The sequential application of these methods allowed precise quantitative determinations of mean mass fraction of Ag, Al, Au, B, Be, Bi, Br, Cd, Ce, Co, Cr, Cs, Dy, Er, Fe, Gd, Hg, Ho, La, Li, Mn, Mo, Nb, Nd, Ni, Pb, Pr, Rb, Sb, Sc, Se, Sm, Sn, Tb, Th, Ti, Tl, Tm, U, Y, Yb, Zn and Zr. It was observed that the ratio of Rb to Ag, Al, Au, B, Be, Bi, Br, Ce, Cr, Cs, Dy, Er, Fe, Gd, Hg, Ho, La, Li, Mn, Nd, Ni, Pb, Pr, Sb, Sc, Se, Sm, Sn, Tb, Th, Ti, Tl, Tm, U, Y, Yb, Zn, and Zr. It was observed that the ratio of Rb to Ag, Al, Au, B, Be, Bi, Br, Ce, Cr, Cs, Dy, Er, Fe, Gd, Hg, Ho, La, Li, Mn, Nd, Ni, Pb, Pr, Sb, Sc, Se, Sm, Sn, Tb, Th, Ti, Tl, Tm, U, Y, Yb, and Zr mass fraction were significantly lower in cancerous tissues than in normal and BPH prostate. Finally, we propose to use the Rb/Ag, Rb/Al, Rb/Bl, Rb/Br, Rb/Be, Rb/Br, Rb/Ce, Rb/Li, Rb/Mn, Rb/Pr, Rb/Sm, Rb/Sn, Rb/Th, Rb/Tl, and Rb/Zr mass fraction ratios in a needle-biopsy core as an accurate tool to diagnose prostate cancer. Further studies on larger number of samples are required to confirm our findings and to investigate the impact of the trace element relationships on prostate cancer etiology.
References:


