Distinguish Between Benign and Malignant Prostate Using the Trace Element Content Ratios in Prostatic Tissue as Tumor Markers

Vladimir Zaichick¹, Sofia Zaichick²

¹Department of Radionuclide Diagnostics, Medical Radiological Research Centre, Russia.
²Department of Medicine, University of Illinois College of Medicine, USA.

Corresponding Author: Vladimir Zaichick, Department of Radionuclide Diagnostics, Medical Radiological Research Centre, Koroleva Str- 4, Obninsk 249036, Kaluga Region, Russia, Tel: +7 (48439) 60289; Email: vezai@obninsk.com

Received Date: 28 Jul 2016
Accepted Date: 18 Aug 2016
Published Date: 24 Aug 2016

INTRODUCTION

The prostate gland may be a source of many health problems in men past middle age, the most common being 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 [1]. The prevalence of histological BPH is found in approximately 50-60% of males age 40-50, in over 70% at 60 years old and in greater than 90% of men over 70 [2, 3]. In many Western industrialized countries, including North America, PCa is the most frequently diagnosed form of noncutaneous malignancy in males and, except for lung cancer, is the leading cause of death from cancer [4-9]. Although the etiology of BPH and PCa is unknown, many trace elements have been highlighted in the literature in relation to the development of these prostate diseases [10-29].

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 [31].

In previous studies significant changes of trace element contents in hyperplastic and cancerous prostate in comparison with those in the normal prostatic tissue were observed [32-53]. Moreover, a significant informative value of Zn content as a tumor marker for PCa diagnostics was shown [54]. Hence it is possible that besides Zn, some other trace elements also can be used as tumor markers for distinguish between benign and malignant prostate.

Current methods applied for measurement of trace element contents in samples of human tissue include a number of methods. Among these methods the instrumental neutron activation analysis with high resolution spectrometry of long-lived radionuclides (INAA-LLR) is a non-destructive and one of the most sensitive techniques. It allows measure the trace element contents in a few milligrams tissue without any treatment of sample. Analytical studies of the Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn contents in normal, BPH and PCa tissue were done by us using INAA-LLR [16, 21, 29, 48, 51, 55]. Non-destructive method of analysis avoids the possibility of changing the content of chemical elements in the studied samples, which allowed for the first time to obtain reliable results [56-59]. In particular, it was shown that the average mass fraction of Zn in PCa tissues is 7 times lower than in healthy or BPH tissue [55]. Obtained results formed the basis for a new method for differential diagnosis of BPH and PCa, the essence of which is to determine the content of Zn in the material of trans-rectal needle biopsy of prostate indurated site. For the first time it was proposed to use INAA-LLR to determine zinc and other trace element contents in needle-biopsy cores [55]. Moreover, it was shown in the study that in a normal prostate tissue mass fraction of some trace elements tend to be correlated with Zn, while in BPH and PCa tissues these relationships are partially broken or changed [55]. These findings open the additional possibilities for developing new methods of PCa diagnostics using Zn content/trace element content ratio.

Thus, this work had three aims. The first was to calculate in intact prostate and malignant prostate.

**MATERIAL AND METHODS**

**Samples**

All patients studied (n = 103) were hospitalized in the Urological Department of the Medical Radiological Research Centre. Trans-rectal 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 BPH (n = 43) and PCa (n = 60) has been confirmed by clinical and morphological results obtained during studies of biopsy and resected materials.

Intact (normal) prostates were removed at necropsy from 37 men who had died suddenly. 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 [16, 21, 29].

**Sample preparation, instrumentation, methods and certified reference materials**

Details of sample preparation, the relevant nuclear reactions, radionuclides, gamma energies, methods of analysis and the results of quality control were presented in our earlier publications concerning the chemical elements of human prostate tissue investigated by INAA-LLR [16, 21, 29, 48, 51, 60].

**Computer programs and statistic**

A dedicated computer program for INAA mode optimization was used [61]. All prostate samples for INAA-LLR were prepared in duplicate and mean values of trace element contents were used in final calculation. Using the Microsoft Office Excel software, the summary of statistics, arithmetic mean, standard deviation, standard error of mean, minimum and maximum values, median, percentiles with 0.025 and 0.975 levels was calculated for trace element mass fraction ratios in normal, benign hyperplastic and cancerous prostate tissue. The difference in the results between BPH and Norm, PCa and Norm, and PCA and BPH was evaluated by Student’s t-test and nonparametric Wilcoxon-Mann-Whitney U-test. We applied a significance level of 0.05. For the construction of “individual data sets for Zn/Ag, Zn/Co, Zn/Cr, Zn/Fe, Zn/Hg, Zn/Rb, Zn/...
Sb, Zn/Sc, and Zn/Se mass fraction ratios in normal, benign hypertrophic and cancerous prostate tissue" diagrams the Microsoft Office Excel software was also used.

RESULTS

The age of 43 patients with BPH ranged from 38 to 83 years, the mean being 66 ± 8 years (M ± SD). The 60 patients aged 40-79 suffered from PCa. Their mean age was 65 ± 10 years. Mean age of men with intact prostates (Normal group) was 55 ± 11 years (range 41-79).

Table 1 presents certain statistical parameters (arithmetic mean, standard deviation, standard error of mean, minimal and maximal values, median, percentiles with 0.025 and 0.975 levels) of the Zn/Ag, Zn/Co, Zn/Cr, Zn/Fe, Zn/Hg, Zn/Rb, Zn/Sb, Zn/Sc, and Zn/Se mass fraction ratios in normal, benign hypertrophic and cancerous prostate tissue.

Table 1: Some statistical parameters of Zn/Ag, Zn/Co, Zn/Cr, Zn/Fe, Zn/Hg, Zn/Rb, Zn/Sb, Zn/Sc, and Zn/Se mass fraction ratios in normal, benign hypertrophic and cancerous prostate.

<table>
<thead>
<tr>
<th>Tissue Mass fraction ratio</th>
<th>Mean</th>
<th>SD</th>
<th>SEM</th>
<th>Min</th>
<th>Max</th>
<th>Median</th>
<th>Per. 0.025</th>
<th>Per. 0.975</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (n = 37)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zn/Ag</td>
<td>32271</td>
<td>27330</td>
<td>5360</td>
<td>3036</td>
<td>113139</td>
<td>23563</td>
<td>4263</td>
<td>90351</td>
</tr>
<tr>
<td>Zn/Co</td>
<td>27011</td>
<td>20354</td>
<td>3716</td>
<td>4236</td>
<td>82272</td>
<td>21419</td>
<td>4798</td>
<td>69552</td>
</tr>
<tr>
<td>Zn/Cr</td>
<td>2654</td>
<td>1780</td>
<td>356</td>
<td>248</td>
<td>7073</td>
<td>2404</td>
<td>330</td>
<td>6369</td>
</tr>
<tr>
<td>Zn/Fe</td>
<td>10.9</td>
<td>8.0</td>
<td>1.4</td>
<td>1.68</td>
<td>30.5</td>
<td>8.66</td>
<td>2.06</td>
<td>29.9</td>
</tr>
<tr>
<td>Zn/Hg</td>
<td>27011</td>
<td>18957</td>
<td>3717</td>
<td>2533</td>
<td>69231</td>
<td>24331</td>
<td>4110</td>
<td>64394</td>
</tr>
<tr>
<td>Zn/Rb</td>
<td>73.6</td>
<td>37.1</td>
<td>6.6</td>
<td>18.4</td>
<td>181</td>
<td>72.1</td>
<td>19.2</td>
<td>149</td>
</tr>
<tr>
<td>Zn/Sb</td>
<td>34333</td>
<td>33149</td>
<td>6156</td>
<td>2935</td>
<td>122891</td>
<td>18500</td>
<td>4970</td>
<td>118907</td>
</tr>
<tr>
<td>Zn/Sc</td>
<td>46794</td>
<td>34288</td>
<td>7866</td>
<td>14274</td>
<td>121364</td>
<td>35309</td>
<td>15874</td>
<td>116182</td>
</tr>
<tr>
<td>Zn/Se</td>
<td>1548</td>
<td>908</td>
<td>166</td>
<td>421</td>
<td>3652</td>
<td>1335</td>
<td>442</td>
<td>3439</td>
</tr>
<tr>
<td>BPH (n=43)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zn/Ag</td>
<td>39748</td>
<td>19357</td>
<td>4328</td>
<td>13499</td>
<td>83073</td>
<td>39623</td>
<td>14448</td>
<td>76749</td>
</tr>
<tr>
<td>Zn/Co</td>
<td>20798</td>
<td>14641</td>
<td>3359</td>
<td>6285</td>
<td>58902</td>
<td>15637</td>
<td>7496</td>
<td>54869</td>
</tr>
<tr>
<td>Zn/Cr</td>
<td>1161</td>
<td>678</td>
<td>156</td>
<td>282</td>
<td>2720</td>
<td>902</td>
<td>359</td>
<td>2619</td>
</tr>
<tr>
<td>Zn/Fe</td>
<td>10.4</td>
<td>6.82</td>
<td>1.15</td>
<td>1.88</td>
<td>30.2</td>
<td>9.30</td>
<td>2.22</td>
<td>28.8</td>
</tr>
<tr>
<td>Zn/Hg</td>
<td>6490</td>
<td>3302</td>
<td>688</td>
<td>1575</td>
<td>14404</td>
<td>6588</td>
<td>1829</td>
<td>13492</td>
</tr>
<tr>
<td>Zn/Rb</td>
<td>79.4</td>
<td>40.2</td>
<td>7.0</td>
<td>24.8</td>
<td>182</td>
<td>73.6</td>
<td>26.1</td>
<td>175</td>
</tr>
<tr>
<td>Zn/Sb</td>
<td>10115</td>
<td>9947</td>
<td>2344</td>
<td>1765</td>
<td>39016</td>
<td>7255</td>
<td>1903</td>
<td>36005</td>
</tr>
<tr>
<td>Zn/Sc</td>
<td>39678</td>
<td>12156</td>
<td>3372</td>
<td>18545</td>
<td>60576</td>
<td>40462</td>
<td>21415</td>
<td>58160</td>
</tr>
<tr>
<td>Zn/Se</td>
<td>886</td>
<td>413</td>
<td>90</td>
<td>236</td>
<td>1562</td>
<td>825</td>
<td>283</td>
<td>1540</td>
</tr>
<tr>
<td>PCa (n=60)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zn/Ag</td>
<td>723</td>
<td>680</td>
<td>133</td>
<td>64.5</td>
<td>2968</td>
<td>492</td>
<td>72.3</td>
<td>2474</td>
</tr>
<tr>
<td>Zn/Co</td>
<td>4293</td>
<td>2933</td>
<td>554</td>
<td>926</td>
<td>12136</td>
<td>3186</td>
<td>1021</td>
<td>11264</td>
</tr>
<tr>
<td>Zn/Cr</td>
<td>78.1</td>
<td>68.2</td>
<td>13.4</td>
<td>11.4</td>
<td>256</td>
<td>58.2</td>
<td>12.4</td>
<td>247</td>
</tr>
<tr>
<td>Zn/Fe</td>
<td>1.08</td>
<td>0.79</td>
<td>0.12</td>
<td>0.054</td>
<td>2.97</td>
<td>0.84</td>
<td>0.114</td>
<td>2.83</td>
</tr>
<tr>
<td>Zn/Hg</td>
<td>1216</td>
<td>618</td>
<td>115</td>
<td>1.84</td>
<td>2301</td>
<td>1106</td>
<td>207</td>
<td>2172</td>
</tr>
<tr>
<td>Zn/Rb</td>
<td>15.7</td>
<td>8.0</td>
<td>1.2</td>
<td>1.30</td>
<td>32.4</td>
<td>15.4</td>
<td>1.69</td>
<td>31.1</td>
</tr>
<tr>
<td>Zn/Sb</td>
<td>334</td>
<td>253</td>
<td>44</td>
<td>37.1</td>
<td>857</td>
<td>256</td>
<td>40.8</td>
<td>843</td>
</tr>
<tr>
<td>Zn/Sc</td>
<td>13157</td>
<td>8593</td>
<td>1624</td>
<td>2222</td>
<td>33750</td>
<td>10693</td>
<td>2703</td>
<td>30178</td>
</tr>
<tr>
<td>Zn/Se</td>
<td>270</td>
<td>155</td>
<td>28</td>
<td>69.2</td>
<td>706</td>
<td>234</td>
<td>90.0</td>
<td>644</td>
</tr>
</tbody>
</table>

M - arithmetic mean; SD – standard deviation; SEM – standard error of mean; Min – minimum value; Max – maximum value; Per. 0.025 – percentile with 0.025 level; Per. 0.975 – percentile with 0.975 level, n - number of samples.

The ratios of means and the difference between mean values of Zn/Ag, Zn/Co, Zn/Cr, Zn/Fe, Zn/Hg, Zn/Rb, Zn/Sb, Zn/Sc, and Zn/Se mass fraction ratios in BPH and Norm, PCa and Norm, and PCA and BPH evaluated by Student’s t-test and nonparametric Wilcoxon-Mann-Whitney U-test are presented in Table 2.
Table 2: Ratio of means and the difference between mean values of Zn/Ag, Zn/Co, Zn/Cr, Zn/Fe, Zn/Hg, Zn/Rb, Zn/Sb, Zn/Sc, and Zn/Se mass fraction ratios in normal, benign hypertrophic and cancerous prostate.

<table>
<thead>
<tr>
<th>Mass fraction ratio</th>
<th>BPH and Normal (N)</th>
<th>PCa and Normal (N)</th>
<th>PCa and BPH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ratio p</td>
<td>t-test</td>
<td>U-test</td>
</tr>
<tr>
<td>Zn/Ag</td>
<td>1.23</td>
<td>=0.28.</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Zn/Co</td>
<td>0.77</td>
<td>=0.22.</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Zn/Cr</td>
<td>0.44</td>
<td>&lt;0.001</td>
<td>≤0.01</td>
</tr>
<tr>
<td>Zn/Fe</td>
<td>0.95</td>
<td>=0.78.</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Zn/Hg</td>
<td>0.24</td>
<td>&lt;0.001</td>
<td>≤0.01</td>
</tr>
<tr>
<td>Zn/Rb</td>
<td>1.08</td>
<td>=0.55.</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Zn/Sb</td>
<td>0.29</td>
<td>&lt;0.001</td>
<td>≤0.01</td>
</tr>
<tr>
<td>Zn/Sc</td>
<td>0.85</td>
<td>=0.41.</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Zn/Se</td>
<td>0.57</td>
<td>&lt;0.001</td>
<td>≤0.01</td>
</tr>
</tbody>
</table>

Table 3: Parameters of the importance (sensitivity, specificity and accuracy) of some trace element mass fraction ratios for the diagnosis of PCa calculated in this work.

<table>
<thead>
<tr>
<th>Mass fraction ratio</th>
<th>Upper limit for PCa (M+2.5SD)</th>
<th>Sensitivity %</th>
<th>Specificity %</th>
<th>Accuracy %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zn/Ag</td>
<td>2420</td>
<td>96±4</td>
<td>100±2</td>
<td>99±1</td>
</tr>
<tr>
<td>Zn/Cr</td>
<td>250</td>
<td>96±4</td>
<td>98±2</td>
<td>97±2</td>
</tr>
<tr>
<td>Zn/Fe</td>
<td>3.0</td>
<td>100±2</td>
<td>89±4</td>
<td>94±2</td>
</tr>
<tr>
<td>Zn/Hg</td>
<td>2760</td>
<td>100±3</td>
<td>94±3</td>
<td>96±2</td>
</tr>
<tr>
<td>Zn/Sb</td>
<td>970</td>
<td>100±3</td>
<td>100±2</td>
<td>100±1</td>
</tr>
</tbody>
</table>

M - arithmetic mean, SD – standard deviation.

DISCUSSION

As was shown by us the use of CRM IAEA H-4 as a CRM for the analysis of samples of prostate tissue can be seen as quite acceptable [16, 21, 29]. Good agreement of the trace element mass fractions analyzed by INAA-LLR with the certified data of CRM IAEA H-4 indicates an acceptable accuracy of the results obtained in the study of trace elements of the prostate presented in Tables 1-3 and Figure 1.

The mean values and all selected statistical parameters were calculated for 9 mass fraction ratios (Zn/Ag, Zn/Co, Zn/Cr, Zn/Fe, Zn/Hg, Zn/Rb, Zn/Sb, Zn/Sc, and Zn/Se) (Table 1). The mass fraction ratio of these elements were calculated for all, or a major portion of normal, BPH and PCa samples. No published data referring to mass fraction ratios of trace elements in the human prostate was found.

From Tables 1 and 2, it is observed that in benign hypertrophic tissues the mass fraction ratios of Zn/Cr (p < 0.001), Zn/Hg (p < 0.001), Zn/Sb (p < 0.001), and Zn/Se (p < 0.01) are lower than in normal tissues. In cancerous tissue all investigated mass fraction ratios are lower (p < 0.001) than in normal and benign hypertrophic tissues of the prostate.

Analysis of trace element mass fraction ratios 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 [62]. 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 all cases we analyzed a part of the material obtained from a puncture transrectal biopsy of the prostate.
indurated site in the prostate. Therefore, our data allow us
to evaluate adequately the importance of trace element ra-
tios information for the diagnosis of PCa. As is evident from
individual data sets (Figure 1), the Zn/Ag, Zn/Cr, Zn/Fe, Zn/Hg,
and Zn/Sb mass fraction ratios are the most informative for a
differential diagnosis. For example, if 2420 (M ± 2.5SD) is the
value of Zn/Ag mass fraction ratio assumed to be the upper
limit for PCa (Figure 1) and an estimation is made for “PCa or
intact and BPH tissue”, the following values are obtained:

Sensitivity = \( \frac{\text{True Positives (TP)}}{\text{TP} + \text{False Negatives (FN)}} \) •100% = 96 ± 4%;

Specificity = \( \frac{\text{True Negatives (TN)}}{\text{TN} + \text{False Positives (FP)}} \) •100% = 100-2%;

Accuracy = \( \frac{\text{TP+TN}}{\text{TP+FP+TN+FN}} \) •100% = 99 ± 1%.

The number of people (samples) examined was taken into
account for calculation of confidence intervals [63]. In other
words, if Zn/Ag mass fraction ratio in a prostate biopsy sample
do not exceed 2420, one could diagnose a malignant tumor
with an accuracy of 99 ± 1%. Thus, using the (Zn/Ag)-test
makes it possible to diagnose cancer in 96 ± 4% cases (sen-
sitivity). The same way parameters of the importance (sensitiv-
ity, specificity and accuracy) of Zn/Cr, Zn/Fe, Zn/Hg, and Zn/Sb
mass fraction ratios for the diagnosis of PCa were calculated
(Table 3).

Mass fraction ratios of Zn/Ag, Zn/Co, Zn/Cr, Zn/Fe, Zn/Hg, and Zn/Sb
in the needle-biopsy cores could be used as a tool to diagnose
PCa and are comparable with characteristics of the Zn mass
fraction-test [55]. However, it is our opinion that application
of the trace element mass fraction ratios is more suitable for
PCa diagnosis. Trace element mass fraction depends on the
sample mass, which decreases with loss of its moisture. The
needle-biopsy core is a small piece of tissue with a relatively
high “surface/volume” ratio. After sampling, it begins to lose
mass very fast. Weight loss of samples depends on the humid-
ity of operating and store rooms [56]. Thus, it is very difficult
to determine the fresh mass of needle-biopsy cores and to
calculate the precise mass fraction of trace elements. Sample
freeze-dry, storage in air-tight vials until weighing, and then
calculating mass fraction on dry weight basis is the only pos-
sible method that eliminates the variation in sample weight.

Conversely, accuracy of trace element mass fraction ratios
does not depend on sample mass and changes in moisture
content. Therefore, this method does not require dry samples.
Moreover, the use of the relations between mass fractions of
chemical elements is particularly promising for the develop-
ment of in vivo diagnostic methods, including the diagnosis
of PCa.

**CONCLUSION**

In this work, mean values of Zn/Ag, Zn/Co, Zn/Cr, Zn/Fe, Zn/
Hg, Zn/Rb, Zn/Sb, Zn/Sc, and Zn/Se mass fraction ratios in normal, benign hypertrophic and cancerous prostate tissue were calculated using data of INAA-LLR. It was observed that in benign hypertrophic tissues the Zn/Cr, Zn/Hg, Zn/Sb, and Zn/Se mass fraction ratios are lower than in normal tissues. In cancerous tissue all investigated mass fraction ratios are lower than in normal and benign hypertrophic tissues of the prostate. It was shown that the Zn/Ag, Zn/Cr, Zn/Fe, Zn/Hg, and Zn/Sb mass fraction ratios are the most informative for a differential diagnosis among all investigated mass fraction ratios. Finally, we propose to use the estimation of Zn/Ag, Zn/Cr, Zn/Fe, Zn/Hg, and Zn/Sb mass fraction ratios in a needle-biopsy core as an accurate tool to diagnose prostate cancer.

REFERENCES
22. Zaichick V and Zaichick S. (2014). Determination of trace elements in adults and geriatric prostate combining neutron activation with inductively coupled plasma atomic emission...


47. Zaichick V and Zaichick S. (2016). The Bromine, Calcium,
Potassium, Magnesium, Manganese, and Sodium Contents in Adenocarcinoma of Human Prostate Gland. J Hematology and Oncology Research. 2(2), 1-12.


