Research Article

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Using prostatic fluid levels of rubidium and zinc concentration multiplication in non-invasive and highly accurate screening for prostate cancer

Abstract

Prostate specific antigen (PSA) does not provide the high reliability and precision that is required for an accurate screening for prostate cancer (PCa). The aim of our study was to search for a simple, rapid, direct, preferably non-invasive, and highly accurate biomarker and procedure for the screening for PCa. For this purpose the levels of rubidium (Rb) and zinc (Zn) were prospectively evaluated in expressed prostatic fluid (EPF). Also RbZn concentration multiplication was calculated for EPF samples, obtained from 38 apparently healthy males and from 33, 51, and 24 patients with chronic prostatitis (CP), benign prostatic hyperplasia (BPH) and PCa, respectively. Measurements were performed using an application of energy dispersive X-ray fluorescent (EDXRF) microanalysis developed by us. It was found that in the EPF of cancerous prostates the levels of Rb and Zn, and also RbZn multiplication were significantly lower in comparison with those in the EPF of normal, inflamed, and hyperplastic prostates. It was shown that "Sensitivity", "Specificity" and "Accuracy" of PCa identification using the Zn and RbZn levels in the EPF samples were all significantly higher than those resulting from of PSA tests in blood serum. It was concluded that the Zn and RbZn levels in EPF, obtained by EDXRF, is a fast, reliable, and non-invasive diagnostic tool that can be successfully used by local, non-urologist physicians at the point-of-care to provide a highly effective PCa screening and as an additional confirmatory test before a prostate gland biopsy.

Keywords: urology screening, prostate cancer, benign prostatic hyperplasia, chronic prostatitis, expressed prostatic fluid, trace element content, trace element concentration multiplication, energy-dispersive X-ray fluorescent analysis

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Abbreviations: PCa, prostate cancer; PSA, prostate-specific antigen; BPH, benign prostate hyperplasia; CP, chronic prostatitis; EPF, expressed prostatic fluid; TE, trace element; EDXRF, energy dispersive X-ray fluorescent microanalysis; [109]Cd EDXRF, EDXRF with using radiation from the radionuclide source, [109]Cd; CRMs, certified reference materials; IAEA, international atomic energy agency

Introduction

The prostate gland is subject to various disorders and of them prostate cancer (PCa) is one of the prostate's most important medical, scientific and public health problems. Worldwide, PCa is the second most commonly diagnosed cancer and the fifth leading cause of cancer deaths in men.[1] PCa is especially prevalent in industrialized countries including North America, Northern and Western Europe and Australia.[2] The American Cancer Society declares PCa, with a lifetime prevalence of one in six men, is the most common cancer in males and the second leading cause of cancer death.[3] Moreover, PCa is the leading cancer in terms of incidence and mortality in men from Africa, Oceania, and the Caribbean.[1,2] PCa in China has also become a major public health concern.[4]

The survival rate is proportional to the stage reached at diagnosis, hence early-stage diagnosis using effective diagnostic tools is a key to reducing mortality due to PCa.[5] It is widely acknowledged that screening and early diagnosis of PCa are of vital importance for improving the likelihood of recovery. Current screening relies on prostate-specific antigen (PSA) testing in blood serum and a PSA level of 4ng/mL is used as the highest level compatible with for non-malignant conditions. However, PSA screening of PCa has some significant disadvantages.

Firstly, PSA is not a cancer-specific biomarker. So there can be an elevated serum concentration (above 4ng/mL) among patients with benign prostate hyperplasia (BPH) and urogenital infections, including chronic prostatitis (CP). Reliance on PSA testing can result in significant over-detection of alleged PCa and hence inappropriate treatment of non-malignant disease.[6] Nearly 70-75% of prostate biopsies fail to detect PCa in men who undergo prostate biopsy procedures due to elevated PSA levels discovered after blood serum-test screening.[5,7] In other words, it has been confirmed that only 25-30% of patients with a PSA value ≥4ng/mL were finally diagnosed with PCa, leading to the over-treatment of low-risk patients, unnecessary biopsies and nonessential radical prostatectomies.[8] Thus, the level of PSA test specificity (selectivity) can be estimated as about 25-30%.

Secondly, the PSA test misses some aggressive tumors. For example, as was found by Thompson et al. (2004) that 20–25% men diagnosed with PCa including those with a poorly differentiated form (Gleason Score ≥8) have PSA levels below 4ng/mL.[6,9] Data from other research shows that only 40% of patients with PCa have an abnormal PSA level.[10] Thus, the PSA test's sensitivity can be estimated as somewhere between 40-75%.

The limitations and potential harm associated with PSA screening

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stimulate investigation of novel biomarkers with superior ability to detect PCa, compared with traditional PSA tests, so decreasing unnecessary biopsies. Much attention is now turning to fluid-based biomarkers, because obtaining fluid samples is in effect is a minimally invasive liquid biopsy. Other relevant factors of great significance for any novel method of PCa detection include cost-effectiveness, capacity to generate real-time results, "simplicity-of-use", robustness, and functionality without excessive prior-processing of samples.[11]

In our previous studies the significant involvement of Zn, Ca, Mg, Rb and some other trace elements (TEs) in the function of the prostate was studied.[12–22] One of the main functions of the prostate gland is the production of prostatic fluid.[23] It contains a high concentration of Zn and elevated levels of Ca, Mg, Rb, and some other TEs, in comparison with levels in serum and other human body fluids.

The first finding of remarkably high levels of Zn in human expressed prostatic fluid (EPF) was reported in the early 1960s.[24] After analyzing EPF expressed from the prostates of 8 apparently healthy men, aged 25-55 years, it was found that Zn concentrations varied from 300 to 730mg/L. After this finding several investigators suggested that the measurement of Zn levels in EPF may be useful as a marker of abnormal prostate secretory function.[25,26] This suggestion promoted more detailed studies of the Zn concentrations in the EPF of healthy subjects and in those with different prostatic diseases, including PCa.[26,27] A detailed review of these studies, reflecting the contradictions within accumulated data, was given in our earlier publication.[27] Moreover, the method and apparatus for micro analysis of Zn and some other TEs in the EPF samples using energy dispersive X-ray fluorescence (EDXRF) activated by radiation from the radionuclide source ^{109}Cd (^{109}Cd EDXRF) was developed by us.[28] We reasoned that apart from total amounts of TEs the ratios of Zn to some other TE content in EPF are likely to reflect a disturbance of prostate function. It was found that data on changes of TE content and Zn/TE concentration ratios in EPF of patients with PCa are very important, because these significant changes increase our knowledge and recognition of PCa pathogenesis and may prove useful as PCa diagnostic markers.[29–39]

The present study had three aims. The main objective was to obtain reliable results about the Rb and Zn concentrations in the EPF of healthy men as well as in the EPF of patients with CP, BPH and PCa using the ^{109}Cd EDXRF method. The second aim was to calculate RbZn concentration multiplication and to compare the levels of all EPF parameters investigated for normal, inflamed, hyperplastic, and cancerous prostates. The final aim was to select the optimal PCa biomarker among the TEs' concentrations and RbZn concentration multiplication by evaluating appropriate characteristics of each potential diagnostic test, with regard to its sensitivity, specificity, and accuracy.

All studies were approved by the Ethical Committees of the Medical Radiological Research Centre, Obninsk. All the procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments, or with comparable ethical standards.

Materials and methods

Specimens of EPF were obtained from 38 men with apparently normal prostates (N, mean age ± Standard Deviation - 59±11 years, range 41-82 years), from 33 males with CP (mean age 50±9 years,

range 37-65 years), from 51 patients with BPH (mean age 63±6 years, range 52-75 years), and from 24 patients with PCa (mean age 65±10 years, range 47-77 years) in the Urological Department of the Medical Radiological Research Centre (MRRC) using a standard rectal massage procedure. The diagnosis of each prostate condition was made by qualified urologists. In all cases the N classification and CP diagnosis were confirmed by clinical examination and by cytological and bacteriological investigations of the EPF samples. The diagnosis of BPH or PCa had been confirmed by clinical examination and morphological results obtained during studies of biopsy and resected materials. Subjects were asked to abstain from sexual intercourse for three days preceding the procedure. Specimens of EPF were obtained in sterile containers, which were appropriately labeled. Twenty μL (microliters) of fluid were taken in duplicate by micropipette from every specimen for TE analysis, while the rest of the fluid was used for cytological and bacteriological investigations. One 20μL sample of the EPF was dropped on a 11.3mm diameter disk made of thin, ash-free filter paper fixed on pieces of adhesive tape and dried in a desiccator at room temperature. Then the dried sample was covered with a 4μm gage Dacron film and centrally pulled onto a Plexiglas cylindrical frame.[28]

To determine concentration of the TEs by comparison with known standards, aliquots of solutions of commercial, chemically pure compounds were used for calibration.[40] The standard samples for calibration were prepared in the same way as the samples of prostate fluid. Because there were no available liquid Certified Reference Materials (CRMs), ten sub-samples of the powdered CRM IAEA H-4 (animal muscle) were analyzed to estimate the precision and accuracy of results. Every CRM sub-sample weighing about 3 mg was applied to the piece of adhesive tape serving as an adhesive fixing backing. An acrylic stencil made in the form of a thin-walled cylinder with 11.3mm inner diameter was used to apply the sub-sample to the adhesive tape. The polished-end acrylic pestle, which is a constituent of the stencil set, was used for uniform distribution of the sub-sample upon the adhesive tape surface restricted by the stencil's inner cylindrical surface. After the sub-sample was lightly pressed onto the adhesive tape carrier, the stencil was removed. Then the sub-sample was covered with 4μm gage Dacron film. Before the sample was applied, pieces of adhesive tape and Dacron film were weighed using an analytical balance. They were reweighed after the sample had been placed inside to determine precisely the sub-sample mass.

The facility for the radionuclide-induced EDXRF included an annular ^{109}Cd source with an activity of 2.56 GBq, A Si(Li) detector with an electric cooling system and a portable multi-channel analyzer based on a personal computer, comprised the detection system. Its resolution was 270 eV at the 6.4 keV line. The facility functioned as follows. Photons with energy 22.1 keV from the ^{109}Cd source arrive at the surface of the specimen inducing fluorescent K_α X-rays from the TE. The fluorescence reaches the detector after passing through a 10 mm diameter collimator. Then the X-ray's arrival is recorded. The duration of the measurements of Br and Zn concentrations was 60 min for each sample. The intensity of the K_α-line of Rb and Zn for EPF samples and standards was estimated from a calculation of the total area under the corresponding photopeaks in the spectra.

All EPF samples for EDXRF were prepared in duplicate and mean values of Rb and Zn contents were used in the final calculation. Using the Microsoft Office Excel programs, some statistical characteristics, such as arithmetic mean (M), standard deviation (SD), standard error of the mean (SEM), minimum and maximum values (Range),

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and median were calculated for TE concentrations and for the RbZn concentration multiplication in the EPF of normal (N), CP, BPH and PCa prostates. The difference in the results between the five pairs of samples (N and CP, N and BPH, N and PCa, PCa and CP, PCa and BPH) was evaluated by the 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 diagrams illustrating individual data sets for Rb and Zn concentrations and also for RbZn concentration multiplication in the EPF of normal, inflamed, benign hyperplastic and cancerous prostates, the Microsoft Office Excel software was also used.

Results

Table 1 & 2 depict some statistical characteristics of the parameters investigated and relevant to a normal distribution (M, SD, and SEM) (Table 1) and appropriate for a distribution that may not necessarily be normal (Median, Range) (Table 2).

The ratios of means/medians and the difference between mean/median values of all investigated parameters (Rb, Zn, RbZn) in EPF samples of normal, inflamed, benign hyperplastic and cancerous prostates determined by the parametric Student's t-test and non-parametric Wilcoxon-Mann-Whitney U-test are presented in Table 3 & 4, respectively.

Table 5 contains important parameters ("Specificity" and "Accuracy"), which reflect the possibilities that Rb and Zn concentrations as well as RbZn concentration multiplication in prostate fluid can aid the diagnosis of PCa (an estimation is made for "PCa or normal, CP, and BPH"), if the level of "Sensitivity" was chosen as 100%.

Table 6 also contains important parameters ("Sensitivity" and "Accuracy"), which reflect the possibilities that Rb and Zn concentrations as well as RbZn concentration multiplication in prostate fluid can aid the diagnosis of PCa (an estimation is made for "PCa or normal, CP, and BPH"), if the level of "Specificity" was chosen as 100%.

Table 7 presents optimal ("Sensitivity" is not below 80%) levels of "Sensitivity", "Specificity" and "Accuracy" when Rb and Zn concentrations as well as RbZn concentration multiplication in prostate fluid are used for the diagnosis of PCa (an estimation is made for "PCa or normal, CP, and BPH").

Individual data sets for the Rb and Zn concentration and also for RbZn concentration multiplication ratio investigated in EPF samples of normal, inflamed, benign hyperplastic and cancerous prostates are shown in Figure 1.

Table 1 Mean values of the Rb and Zn concentrations (mg/L) and also RbZn concentration multiplication in prostate fluid of normal (N), inflamed (CP), benign hyperplastic (BPH) and cancerous prostate (PCa)

Parameter	Prostate fluid			
	N	**CP**	**BPH**	**PCa**
	41-82 years (n=38)	**37-65 year (n=33)**	**52-75 year (n=51)**	**47-77 year (n=24)**
	Mean±SD(SEM)	**Mean±SD(SEM)**	**Mean±SD(SEM)**	**Mean±SD(SEM)**
Rb	1.16±0.52(0.10)	2.32±1.13(0.30)	1.70±1.41(0.23)	0.53±0.38(0.11)
Zn	598±207(34)	382±275(48)	488±302(42)	62.0±98.3(20.1)
RbZn	684±374(69)	1239±1130(302)	1077±1337(217)	19.2±34.2(9.9)

Mean, arithmetic mean; SD, standard deviation; SEM, standard error of mean

Table 2 Median and range of the Rb and Zn concentrations and also RbZn concentration multiplication in prostate fluid of normal (N), inflamed (CP), benign hyperplastic (BPH) and cancerous prostate (PCa)

Parameter	Prostate fluid			
	N	**CP**	**BPH**	**PCa**
	41-82 years	**37-65 year**	**52-75 year**	**47-77 year**
	(n=38)	**(n=33)**	**(n=51)**	**(n=24)**
	Median(Range)	**Median(Range)**	**Median(Range)**	**Median(Range)**
Rb	1.03(0.376-2.45)	1.75 (0.730-4.54)	1.46 (0.210-5.04)	0.422(0.013-1.39)
Zn	560(253-948)	295(62.0-1051)	427(45.0-977)	21.6(2.82-371)
RbZn	568(209-1716)	854(93.0-3183)	514(37.5-4763)	3.26(1.12-114)

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Table 3 Ratio of means and the difference (Student's t-test) between mean values of the Rb and Zn concentrations and also RbZn concentration multiplication in prostate fluid of normal (N), inflamed (CP), benign hyperplastic (BPH) and cancerous prostate (PCa)

Parameter	CP and N		BPH and N		PCa and N		PCa and CP		PCa and BPH	
	Ratio	p≤	Ratio	p≤	Ratio	p≤	Ratio	p≤	Ratio	p≤
	CP/N		BPH/N		PCa/N		PCa/CP		PCa/BPH	
Rb	2.00	**0.002**	1.47	**0.031**	0.46	**0.001**	0.23	**0.001**	0.31	**0.001**
Zn	0.64	**0.001**	0.82	**0.044**	0.104	**0.001**	0.16	**0.001**	0.13	**0.001**
RbZn	1.81	0.095	1.57	0.092	0.028	**0.001**	0.015	**0.001**	0.018	**0.001**

Bold significant differences

Table 4 Ratio of medians and the difference (Wilcoxon-Mann-Whitney U-test) between mean values of the Rb and Zn concentrations and also RbZn concentration multiplication in prostate fluid of normal (N), inflamed (CP), benign hyperplastic (BPH) and cancerous prostate (PCa)

Parameter	CP and N		BPH and N		PCa and N		PCa and CP		PCa and BPH	
	Ratio	*p*	Ratio	*p*	Ratio	*p*	Ratio	*p*	Ratio	*p*
	CP/N		BPH/N		PCa/N		PCa/CP		PCa/BPH	
Rb	1.7	**<0.01**	1.42	**<0.01**	0.41	**<0.01**	0.24	**<0.01**	0.29	**<0.01**
Zn	0.53	**<0.01**	0.76	**<0.01**	0.039	**<0.01**	0.073	**<0.01**	0.051	**<0.01**
RbZn	1.5	>0.05	0.9	>0.05	0.0057	**<0.01**	0.0038	**<0.01**	0.0.0063	**<0.01**

Bold significant differences

Table 5 Parameters of the importance ("Specificity" and "Accuracy") of Rb and Zn concentrations and also RbZn concentration multiplication in prostate fluid for the diagnosis of PCa (anestimation is made for "PCa or normal, CP, and BPH prostate"), if level of "Sensitivity" was chosen as 100%

Rb and Zn concentration or RbZn concentration multiplication	Upper limit for PCa	Sensitivity %	Specificity %	Accuracy %
Rb	1.39mg/L	100	49±6	55±5
Zn	371mg/L	100	60±5	66±5
RbZn	115	100	89±4	90±3

Table 6 Parameters of the importance ("Sensitivity" and "Accuracy") of Rb and Zn concentrations and also RbZn concentration multiplication in prostate fluid for the diagnosis of PCa (an estimation is made for "PCa or normal, CP, and BPH prostate"), if level of "Specificity" was chosen as 100%

Rb and Zn concentration or RbZn concentration multiplication	Upper limit for PCa	Sensitivity %	Specificity %	Accuracy %
Rb	0.20 mg/L	17±11	100	89±3
Zn	45.0 mg/L	63±10	100	94±2
RbZn	37	83±11	100	98±2

Table 7 Optimal levels of "Sensitivity", "Specificity" and "Accuracy" when Rb and Zn concentrations and also RbZn concentration multiplication in prostate fluid for the diagnosis of PCa (an estimation is made for "PCa or normal, CP, and BPH prostate")

Rb and Zn concentration or RbZn concentration multiplication	Upper limit for PCa	Sensitivity %	Specificity %	Accuracy %
Rb	0.92mg/L	90±8	72±5	74±5
Zn	120mg/L	92±6	92±3	92±2
RbZn	57	90±8	93±3	92±3

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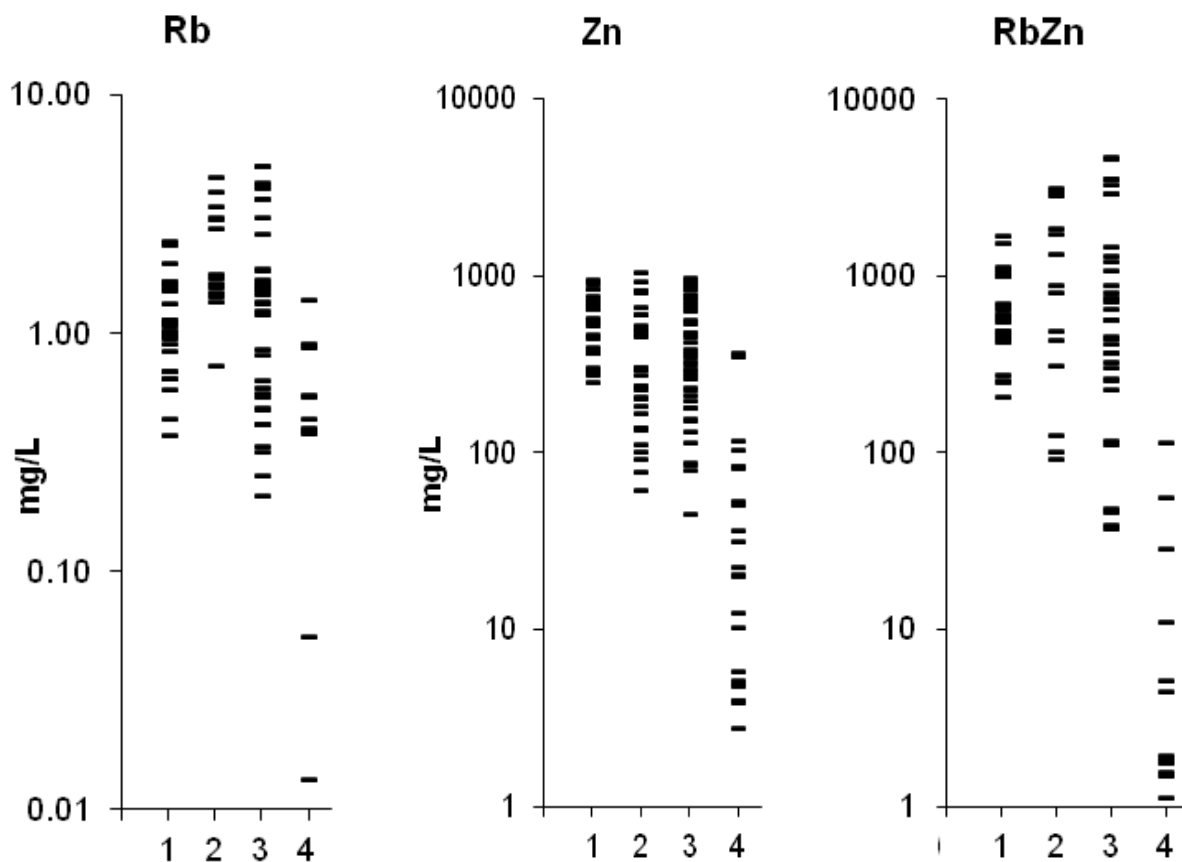Copyright:
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Figure 1 Individual data sets for Rb and Zn concentrations (mg/L), and for RbZn concentration multiplication in prostate fluid of normal (1), inflamed (2), benign hypertrophic (3) and cancerous prostate (4).

Discussion

As was shown by us[27–39] results from the use of CRM IAEA H-4 as certified reference materials for the analysis of samples of EPF is acceptable. Good agreement of the Rb and Zn contents, analyzed by the [109]Cd EDXRF method, with the certified data of reference materials indicates an acceptable accuracy for the results obtained in the study of two TEs of the EPF presented in Table 1–7.

From Table 3 & 4, it is observed that in the EPF of inflamed prostates the mean/median values for the RbZn multiplication do not differ significantly from normal levels, while the value of Rb is higher and Zn is significantly lower.

In the EPF from hyperplastic prostates the mean/median values for the RbZn multiplication do not differ significantly from normal levels, while the value of Rb is higher and Zn is significantly lower (Table 3 & 4). In the EPF of cancerous prostates the mean/median values for the Rb, Zn and RbZn are significantly lower than normal level. Moreover, these differences also exist when EPFs of cancerous prostates are compared with EPFs of inflamed or hyperplastic prostates (Table 3 & 4).

Thus, from Table 3 & 4, as well as from individual data sets (Figure 1), it is observed that measurements of the Rb and Zn concentrations and also RbZn concentration multiplication in EPF could become a powerful diagnostic tool when seeking PCa. To a large extent, continuation of the search for new methods for early diagnosis of PCa was due to experience gained after a critical assessment of the limitations of the current PSA blood serum tests.[5–11,41] In addition to the PSA test and morphological study of needle-biopsy cores of the prostate, the development of highly precise less invasive testing methods will clearly be very useful. The proportion of subjects with of normal, inflamed, benign hyperplastic and cancerous prostates in the present study reproduced the Urological Department's usual patient proportions in the MRRC. Thus, our data allow us to evaluate adequately the importance of Rb and Zn concentrations and also RbZn concentration multiplication in EPF for the diagnosis of PCa. As is evident from Table 3 & 4, as well as from individual data sets (Figure 1), the RbZn concentration multiplication in EPF is potentially the most informative parameter for a differential diagnosis of PCa.

The equations for calculation of such important characteristics of the diagnostic test as "Sensitivity",

"Specificity/Selectivity" and "Accuracy" are well known.[42] They comprise:

$$Sensitivity = \left\{ True\ Positives\ (TP) / \left[TP + False\ Negatives\ (FN) \right] \right\} \times 100\%$$

$$Specificity = \left\{ True\ Negatives\ (TN) / \left[TN + False\ Positives\ (FP) \right] \right\} \times 100\%$$

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$$Accuracy = \left[\left(TP + TN \right) / \left(TP + FP + TN + FN \right) \right] \times 100\%$$

For example, if "Sensitivity" of a new method of PCa diagnosis is set equal to 100% the resulting values for "Specificity/Selectivity" and "Accuracy" are presented in Table 5. If "Specificity/Selectivity" of a new method of PCa diagnosis is set equal to 100% the resulting values for "Sensitivity" and "Accuracy" are presented in Table 6. The data for "Sensitivity", "Specificity/Selectivity" and "Accuracy" can be more balanced if a level of ≥80% for "Sensitivity" is acceptable (Table 7). It should be noted that the number of people (samples) examined was taken into account for calculation of confidence intervals of data presented in Table 5–7.[42]

From data in Table 5–7 and Figure 1 it follows, after comparison, that levels of "Sensitivity", "Specificity/Selectivity" and "Accuracy" for the Zn and RbZn in EPF used as PCa biomarkers are better than results from the PSA level blood serum test. Among them the most informative tumor marker is the RbZn concentration multiplication (Figure 1). In other words, if level of RbZn concentration multiplication in EPF sample is lower than 57, one could diagnose a malignant tumor with an accuracy 92±3%. Thus, using the level of RbZn in an EPF sample as a tumor marker makes it possible to diagnose cancer in the 90±8% of cases (sensitivity) with specificity/selectivity 93±3%. The high level of specificity/selectivity, 93±3%, means that this test results in a significant decrease in the number of unnecessary biopsies, because on average only 7% (100%-93%=7%) of prostate biopsies fail to detect PCa (i.e. are false negative) in men who have prostate cancer. Thus, using the proposed test will reduce the number of true negatives after biopsy.

Characteristically, elevated or deficient levels of TEs and electrolytes observed in the EPF of cancerous prostates are discussed in terms of their potential role in the initiation, promotion, or inhibition of prostate cancer. In our opinion, abnormal levels of TE contents and Zn/TE ratios in the EPF of cancerous prostates could be the consequences of malignant transformation. Compared to other fluids of the human body, the prostate's secretion contains higher levels of Rb and Zn and some other TEs. These data suggest that these elements could be involved in prostatic function. The suppressed prostatic function can be both a cause and a consequence of CP or BPH. Malignant transformation is accompanied by a drastic loss of tissue-specific functional features, which leads to a significant reduction in the content of some elements associated with functional characteristics of the human EPF, including such TE as Rb and Zn.

It is necessary to keep in mind that biochemical, or in other words functional, changes in prostatic cells are present from the earliest development of malignancy, which precedes any histopathological indication of malignancy, and these biochemical changes persist during progression of the malignancy and remain present in advanced prostate cancer. Thus, Rb and Zn depletion is an early step in the cancer proliferation process and Rb and Zn depletion in EPF precedes the morphological transformation of cells from being histopathologically normal to cancerous.

In our study the portable device we used for EDXRF analysis, with its [109]Cd source for the excitation of X-ray fluorescence in the EPF sample, was developed by ourselves. More powerful devices for EDXRF analysis with X-ray tubes, including "the total reflection" version (TRXRF) of the method, allow reliable determinations of the Rb, Zn and many other TE concentrations in a drop of a human body fluid within 10min.[43] EDXRF is a fully instrumental and non-destructive method because a drop of EPF is investigated without requiring any sample pretreatment or its consumption. Moreover, it is well known that among the most modern analytical technologies, EDXRF is one of the simplest, fastest, most reliable and efficient of the available techniques for TE determination.[43] There are many different kinds of EDXRF and TRXRF device on the market and technical improvements are frequently announced.

The routine screening for PCa has generally included invasive (by a venipuncture) testing of PSA level in blood serum. The method presented here for PCa screening is a noninvasive and safe procedure because only requires a drop of EPF. This is obtained during a digital rectal examination using prostate gland massage. Many urologists have successfully and easily obtained EPF this way, so it seems likely that others will be able to do likewise for most of their patients.

All of these advantages, including the elimination of CP and BPH as confounding conditions when screening for PCa, along with its "Sensitivity", "Specificity" and "Accuracy" for PCa identification all exceeding 90%, favor the EDXRF of TEs and their concentration combination in EPF over the use of PSA levels for this purpose. Also these results suggest a strong possibility that it can replace the PSA level determination in screening for PCa. In our opinion, obtaining the levels of TEs and their combination in a drop of EPF, using EDXRF, is a fast, reliable, and non-invasive diagnostic tool that can be successfully used by physicians, who are not urologists, at the point-of-care for highly effective PCa screening and as an additional test before prostate gland biopsy. Its advantages have been outlined above. Further we believe it is superior to the PSA level determination for this purpose.

Conclusion

There is a critical need for a highly reliable, accurate, simplified biomarker and procedure for the screening for prostate cancer, or as an adjunct for the PSA test during the urological examination of patients as candidates for prostate biopsy. In the present work, Rb and Zn concentration measurements were carried out in the EPF samples from normal, inflamed, hyperplastic, and malignant prostates using the non-destructive, instrumental [109]Cd EDXRF micro method developed by us. It was shown that this method is an adequate analytical tool for the non-destructive determination of Rb and Zn concentrations as well as for calculation of RbZn concentration multiplication in the EPF samples of human prostate in normal and some pathological conditions. It was observed that in the EPF of cancerous prostates, levels of Rb and Zn concentrations and also RbZn concentration multiplication were significantly lower in a comparison with those in the EPF of normal, inflamed, and hyperplastic prostates. It was shown that "Sensitivity", "Specificity" and "Accuracy" of PCa identification using the Zn and RbZn levels in the EPF samples was significantly higher than that using PSA levels in blood serum. It was concluded that study of the Rb and Zn levels and their multiplication in an EPF drop, obtained by using EDXRF, is a fast, reliable, and non-invasive diagnostic tool that can be successfully used by physicians at the point-of-care for highly effective PCa screening and as an additional test before a the prostate gland biopsy.

Acknowledgments

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Conflicts of interest

The authors declare that there no conflict of interest.

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