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Research Article

Preliminary Study of Prostatic Fluid Levels of Zinc Concentration as the Biodosimeter of Males Exposure to Ionizing Radiation

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Abstract

Among different bio dosimeters, there is a need in methods that can rapidly and accurately determine individual exposure to ionizing radiation during examination a big cohort of people, particularly men. In present study contents of trace elements in the prostatic fluid were investigated to search for a new biological dosimeter. Prostatic fluid samples were obtained from 15 men aged 38 - 77 years suffering from bladder cancer. All patients were treated with external (distant) gamma-ray therapy. Fractionated tumour irradiation was used with a total dose of 65 Gy. The tumour was daily irradiated with the dose ranged from 6 to 12 Gy. The prostate radiation dose was calculated according to the distribution of isodose levels within patient's body during the simulation of external local gamma-ray irradiation of bladder tumour. Transrectal digital massage was used to obtain expressed prostatic fluid samples before and 2 - 4 times during radiation therapy for the energy dispersive X-ray fluorescent analysis. A day after the first irradiation of the prostate with the average dose 4.6 Gy to prostate concentration of zinc in the prostatic fluid was about three times lower the metal level before gamma-ray therapy. The dose detection threshold was calculated up to 0.1 Gy. The exponential function was the best for approximation the "dose-effect" dependence of the zinc level in prostatic fluid from the radiation exposure during external gamma-ray therapy of patients with bladder cancer.

Keywords: Prostate; Prostatic Fluid; Biomarkers; Trace Elements; Zinc; Biological Dosimetry

Abbreviations

TE: Trace Element; EPF: Expressed Prostatic Fluid; IR: Ionizing Radiation; EDXRF: Energy Dispersive X-Ray Fluorescent Microanalysis.

Introduction

The increase usage of radionuclides in medicine, meteorology, agriculture, industry and army as well as the expansion of nuclear power stations is directly associated with the increasing probability of nuclear and radiation emergencies. At present, there is also a great risk of terrorist nuclear attacks. The occupational workers always wear personal radiation dosimeters while at the time of the radiation accident with individuals within the population the exposed persons do not have any dosimeter. Biodosimetry helps in ascertaining the exposure and quantifies the radiation dose using biological indicators of radiation when either physical dosimeters become unreliable or not available.

The principle of bio dosimetry is to utilize changes induced in the individual by ionizing radiation to estimate the dose and, if possible, to predict the biological consequences of the dose or the clinically relevant response [1]. There are three basic types of bio dosimetry with different and often complementary characteristics: (a) based on qualitative and quantitative changes in gene activation or chromosomal abnormalities; (b) based on quantitative changes in viability of cells and biochemical parameters of tissues and fluids; (c) based on physical changes in tissues. However, there is no single biological indicator which can fulfill all the requirements to meet challenges of dose estimation, owing to complexities of exposure scenarios [2]. Thus, among different bio dosimeters there is a need in methods that can rapidly and accurately determine individual exposure to ionizing radiation during examination a big cohort of people [3,4]. For example, such methods can be useful in identification of those who need medical assistance and guiding appropriate medical treatment in an emergency response to nuclear accidents and large-scale radiological emergencies.

In our previous studies the significant involvement of zinc (Zn), calcium (Ca), magnesium (Mg), rubidium (Rb) and some other trace elements (TEs) in the function of the prostate was found [5-15]. Moreover, it was demonstrated that the changes of Zn content and Zn relationships with other TE in the prostate tissue can be used as biomarkers of prostate disorders [16-39].

One of the main functions of the prostate gland is the production of prostatic fluid [40]. 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 [41]. After analyzing EPF expressed from the prostates of eight apparently healthy men, aged 25 - 55 years, it was found that Zn concentrations varied from 300 to 730 mg/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 [42-60].

It is well known that ionizing radiation (IR) could inevitably damage normal function of human body organs and tissues that usually induce such effects as lowering of the immune response, injury to the hematopoietic system, gastrointestinal system and central nervous system and also induce many other disturbances [61-63]. It is especially concerns the reproductive organs of man which functions are very sensitive to IR. There are a lot of papers and reviews focused on the disturbances in hormonal status (testosterone and gonadotrophin levels), on the causative effects in spermatogenesis (gravity of the oligospermia or azoospermia), on the testicular volumes decline and others abnormalities following total body irradiation in adults [63-67]. However, published data about the IR impact on the levels of TEs, including Zn, in EPF were not found.

The aim of the present study was to estimate the changes of Zn concentration in EPF during the course of external gamma therapy of patients with the bladder cancer and to assess possible application of this metal level changes for bio dosimetric purposes.

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

Samples

Specimens of EPF were obtained from 38 men with apparently normal prostates (N, mean age ± Standard Deviation - 59±11 years, range 41-82 years) and from 15 patients with bladder cancer (BC, mean age 51 ± 12 years, range 38 - 77 years) in the Urological Department of the Medical Radiological Research Centre (MRRC) using a standard rectal massage procedure. The diagnosis of each bladder condition was made by qualified urologists. The BC stage ranged from T1N0M0 to T3N0M0 and prostate glands were intact. In all cases the N classification of prostate were confirmed by clinical examination and by cytological and bacteriological investigations of the EPF samples. The diagnosis of BC had been confirmed by clinical examination and morphological results obtained during studies of biopsy and resected materials. Healthy subjects were asked to abstain from sexual intercourse for three days preceding the procedure. Patients with BC were treated with external (distant) gamma-ray therapy. Fractionated tumour irradiation was used with a total dose of 65 Gy. As a rule, patients were daily treated with the dose ranged from 6 to 12 Gy in seven fractions. Direct irradiation to the bladder tumor was, in lower doses, affected the prostate. The radiation doze absorbed by prostate depends on the bladder tumor location and individual anatomical characteristics of bladder and prostate. Individual doses were calculated according to the distribution of isodose levels within the body during the simulation of external local gamma-ray irradiation to the bladder tumour. Specimens of EPF of patients with BC were obtained before the impact of IR and from 1 to 4 times during radiation therapy.

Specimens of EPF were obtained in sterile containers, which were appropriately labeled. 20 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 20 µL sample of the EPF was dropped on a 11.3 mm 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 4 µm gage Dacron film and centrally pulled onto a Plexiglas cylindrical frame [45].

Standards and certified reference material

To determine concentration of the Zn by comparison with known standards, aliquots of solutions of commercial, chemically pure compounds were used for calibration [68]. 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 11.3 mm 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 4 µm 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 [45].

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Instrumentation and method

The facility for the radionuclide-induced EDXRF included an annular 109Cd 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 109Cd source arrived at the surface of the specimen inducing fluorescent Kα X-rays from the Zn. The fluorescence reached the detector after passing through a 10 mm diameter collimator. Then the X-ray's arrival was recorded [45]. The duration of the measurements of Zn concentration was 5 min for each sample obtained from healthy persons as well as from patients with BC before and after 1 or 2 fractions of radiation therapy. The duration of the Zn measurements was 60 min for each sample obtained from patients with BC after 3 and more fractions of radiation therapy. The intensity of the Kα-line of Zn for EPF samples and standards was estimated from a calculation of the total area under the corresponding photopeak in the spectra.

Computer programs and statistic

All EPF samples for EDXRF were prepared in duplicate and mean value of Zn content was used in the final calculation. Using the Microsoft Office Excel programs (Windows 10), some statistical characteristics, such as arithmetic mean (M), standard deviation (SD), standard error of the mean (SEM), minimum and maximum values (Range), and median were calculated for Zn concentrations in the EPF of healthy persons and patients with BC. For the construction of diagram illustrating individual data set for changes of Zn concentrations in the EPF of patients with BC during radiation therapy, the Microsoft Office Excel software (Windows 10)was also used.

Results and Discussion

Significant agreement of the Zn content, analyzed by the ^{109}Cd EDXRF method, with the certified data of reference materials (Table 1) indicates an acceptable accuracy for the results obtained in the study and presented in (Figure 1 and Tables 2 and 3).

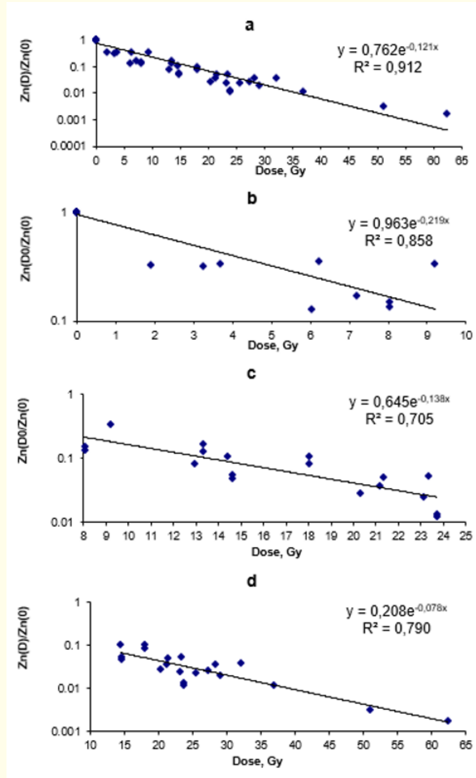

Figure 1: Individual data set for the Zn(D)/Zn(0) ratio, where Zn(0) and Zn(D) are concentrations of Zn in prostatic fluid samples obtained before (0) and during (D) fractionated radiation therapy of patients with bladder cancer: a – range of dose from 0 to 65 Gy, b - range of dose from 0 to 10 Gy, c - range of dose from 10 to 20 Gy, and d - range of dose from 20 to 65 Gy.

Certified value			This work results
Mean	95% confidence interval	Type	Mean ± SD
86	83 - 90	Certified value	90 ± 5

Table 1: EDXRF data Zn contents in the IAEA H-4 (animal muscle) reference material compared to certified values (mg/kg, dry mass basis).

Healthy men				Patients with bladder cancer	
This work results		Reference data [60]			
Mean	±SD	Median of Means	Range of Means	Mean	±SD
598	207	580	490 - 598	588	408

Table 2: Concentrations of Zn (mg/L) in the prostatic fluid of healthy men and patients with bladder cancer before radiation therapy.

Both mean value and the range of Zn concentration in the EPF of healthy males agree well with the reference data (Table 2). The Zn concentration in the EPF of patients with BC did not differ from that in healthy subjects (Table 2).

No	Fraction 1		Fraction 2		Fraction 3		Fraction 4		Fraction 5		Fraction 6		Fraction 7	
	D	Ratio	D	Ratio	D	Ratio	D	Ratio	D	Ratio	D	Ratio	D	Ratio
1	-	-	9.2	0.342	-	-	25.5	0.023	-	-	-	-	-	-
2	3.3	0.319	-	-	-	-	23.3	0.054	-	-	-	-	-	-
3	3.7	0.338	-	-	12.9	0.083	20.3	0.028	36.9	0.0122	-	-	-	-
4	1.9	0.327	-	-	13.3	0.168	-	-	32.1	0.0378	-	-	62.3	0.0017
5	-	-	-	-	13.3	0.129	-	-	-	-	51.0	0.0032	-	-
6	-	-	6.2	0.357	-	-	21.4	0.050	-	-	-	-	-	-
7	-	-	-	-	-	-	28.2	0.037	-	-	-	-	-	-
8	-	-	-	-	18.0	0.104	-	-	-	-	-	-	-	-
9	-	-	-	-	18.0	0.084	-	-	-	-	-	-	-	-
10	-	-	6.0	0.130	-	-	21.2	0.037	-	-	-	-	-	-
11	-	-			-	-	27.2	0.026	-	-	-	-	-	-
12	-	-	7.2	0.171	14.4	0.106	23.1	0.025	-	-	-	-	-	-
13	-	-	-	-	-	-	29.0	0.020	-	-	-	-	-	-
14	-	-	8.0	0.135	14.6	0.049	23.7	0.012	-	-	-	-	-	-
15	-	-	8.0	0.150	14.6	0.054	23.7	0.013	-	-	-	-	-	-
M	2.9	0.328	7.5	0.214	14.9	0.097	24.2	0.030	34.5	0.025	51.0	0,0032	62.3	0.0017
SD	0.9	0.009	1.2	0.105	2.0	0.039	2.9	0,014	3.4	0.018	-	-	-	-

Table 3: Impact of the dose (D, Gy) on the Zn(D)/Zn(0) ratio (Ratio), where Zn(0) and Zn(D) are concentrations of Zn in prostatic fluid samples obtained before (0) and during (D) fractionated radiation therapy of patients with bladder cancer. No – index of patient.

The experimental results show that during fractional radiation therapy the Zn concentration in the EPF of patients with BC drastically decreased and by the end of treatment (after 7th fraction) the Zn concentration in the EPF was almost three orders of magnitude lover than that before therapy (Figure 1 and Table 3). After the 1st fraction when the mean of dose to prostates of three patients was about 2.9 Gy the mean of Zn concentration in the EPF of these patients was 3 times lower than that before therapy (Table 3). After the fours fraction when the mean of dose to prostates of eleven patients was about 24 Gy their mean of Zn concentration in the EPF was 30 times lower than that before therapy (Table 3).

Dose-dependent decrease of Zn concentration in the EPF was more ideally fitted by an exponential law than by a linear, polynomial, logarithmic or power law (Figure 1a):

$$Zn(D)/Zn(0) = 0.762.e^{-0.121D} \quad (1)$$

Where D is radiation dose to prostate in range from 0 up to 65 Gy, Zn(0) and Zn(D) are concentrations of Zn in EPF samples obtained before (0) and after (D) irradiation in dose D. The accuracy of approximation can be improve, if to use three range of dose: from 0 to 10 Gy, from10 to20 Gy, and from 20 to 65 Gy (Figures 1b, 1c, and 1d, respectively).

The ratio Zn (D)/Zn (0) in EPF has many advantages over other biodosimetric markers under conditions of acute exposure. For example, it is more sensitive than cytogenetic indices. For comparison, after irradiation of prostate in dose 1 Gy the Zn level in EPF decreased in 30% (Figure 1b), while changes in cytogenetic indices ranged between 10% and 25% depending on the test used [69]. A dose detection threshold determined by us as the 9% reduction of Zn content in EPF (over 3 total errors of Zn measurements) corresponded 10 cGy. Thus, concentration of Zn in the EPF is potentially sensitive In vivo assay for early radiation biological dosimetry in the wide range of doses from 0.1 to 65 Gy.

In present study the portable device used for 109Cd EDXRF analysis of Zn level in EPF samples was developed by us. More powerful devices for EDXRF analysis with X-ray tubes, including "the total reflection" version (TRXRF) of the method, allow reliable determinations of Zn in a drop of EPF with concentration of metal up to 1 mg/L within 10 min [70]. 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 [70]. There are many different kinds of EDXRF and TRXRF device on the market and technical improvements are frequently announced. Thus, it is need no more than 10 min for the determination of Zn in the EPF sample using the modern EDXRF and TRXRF device. For comparison, biodosimetry using intra- and inter-chromosomal aberrations detection in human lymphocyte takes about 3 - 4 days for radiation dose estimation [4].

Cytogenetic analysis of the peripheral blood lymphocytes is a long-established gold standard technique. It is a biomarker of IR exposure, but it requires large time and trained workers for analysis [71]. Moreover, it is the invasive method because needs in a venipuncture. The method presented here for biodosimetry overcame all these problems. It use a noninvasive and safe procedure because only requires a drop of EPF which obtained by 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. Determination of the Zn concentration in the EPF sample using the modern EDXRF and TRXRF device takes 10 min. EDXRF or TRXRF analysis of Zn concentration in the EPF sample is the full instrumental method that does not require any sample treatment and high trained workers. Thus, in our opinion, the Zn level assessment in a drop of EPF, using EDXRF, is a fast, reliable, and non-invasive tool for early radiation biodosimetry that can be successfully used by physicians, who are not urologists, or by paramedics.

This study has several limitations. To clarify the usefulness and possible application of the Zn level assessment in EPF as bio dosimeter after radiation exposure, future studies should be directed toward receiving answers about (1) individual variations of the Zn level in the EPF of healthy males from day to day, (2) effect of race, nationality, diet, sexual and physical activity, climate and other factors on the Zn level in the EPF of healthy males, (3 changes of the Zn level in the EPF after total body irradiation, (4) the recovery dynamics of prostate function after total body irradiation.

Conclusion

It is a general consensus that there is yet no perfect biomarker of radiation exposure in the field of biological radiation dosimetry. The present work is a preliminary study regarding the application of Zn concentration in EPF as a biomarker for the assessment of prostate radiation exposure during external (distant) gamma-ray therapy of patients with bladder cancer. The study has demonstrated that Zn concentration in EPF is the very sensitive marker of radiation exposure. Thus, the Zn level assessment in a drop of EPF, using EDXRF, is a fast, reliable, and non-invasive tool for early radiation biodosimetry that can be successfully used by paramedics. The future studies need to clarify a possibility of using the Zn level in EPF as the radiation dosimeter for both local and total body irradiation, acute and chronic radiation exposure, and retrospective dosimetry.

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