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Clinical Study

Oxidative Stress Markers in Prostate Cancer Patients after HDR Brachytherapy Combined with External Beam Radiation

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Assessment of oxidative stress markers was perfomed in prostate cancer (PCa) patients subjected to high-dose brachytherapy (HDR) with external beam radiotherapy (EBRT). Sixty men with PCa were subjected to combined two-fraction treatment with HDR (tot. 20 Gy) and EBRT (46 Gy). Blood samples were taken before treatment, immediately afterwards, after 1.5–3 months, and approx. 2 years. Control group consisted of 30 healthy men. Erythrocyte glutathione peroxidase activity in the patients was lower than in healthy subjects by 34% ($P < 0.001$), 50% ($P < 0.001$), 30% ($P < 0.05$), and 61% ($P < 0.001$), respectively, at all periods. No significant differences were found by comparing superoxide dismutase and catalase activity in PCa patients with that of the controls. After 2 years of the end of treatment, the activity of studied enzymes demonstrated a decreasing tendency versus before therapy. Blood plasma thiobarbituric acid reactive substances (TBARS) concentration was higher than in the controls at all periods, while erythrocyte TBARS decreased after 2 years to control levels. The results confirm that in the course of PCa, imbalance of oxidant-antioxidant processes occurs. The therapy did not alter the levels of oxidative stress markers, which may prove its applicability. Two years is too short a period to restore the oxidant-antioxidant balance.

1. Introduction

Prostate cancer (PCa) is among the most frequently diagnosed malignancies in men [1]. Depending on the clinical advance, grade of histopathological differentiation, and baseline prostate-specific antigen (PSA) levels, as well as the patient's general condition and preferences, different PCa therapeutic approaches have been adopted. Among basic radical methods are surgical treatment and radiotherapy. Within radiotherapy, radiation delivery is obtained using external beam (EBRT) or brachytherapy (BT); the latter employing sources of ionizing radiation inserted into the tumour [2]. Usually, in temporary BT, high-dose rate (HDR) is used in contrast to permanent BT in which low-dose rate is applied (LDR) [3]. Interstitial HDR brachytherapy is utilized in PCa therapy both as an independent method and in combination with teleradiotherapy [3]. Oncology centres adopt different criteria of assessing patients' eligibility for HDR-BT alone or combined radiotherapy.

The source of many diseases, including tumours, lies in an increased generation of reactive oxygen species (ROS) resulting in an oxidative stress. States of prostatic hyperplasia and enlargement are often concomitant with inflammation accompanied by increased generation of ROS, reactive nitrogen species (RNS), and oxidizing halogen derivatives [4]. Increased ROS occurrence induces damage to lipids (by peroxidation), nucleic acids, and proteins [5]. DNA damage may in turn lead to alterations in transcription and replication,

Table 1: Patient characteristics.

	Mean	SD
Age (years)	67.4	7.41
PSA (ng/mL)	11.12	7.25
Gleason score	5.67	1.37
TNM	1.66	0.63
Haemoglobin (g/dL)	13.63	1.3

induction of signal transduction pathways, and genomic instability, which constitutes the basis for carcinogenesis [6]. Human organism is protected against the harmful ROS activity by a complex antioxidant system. Enzymatic antioxidant shield comprises, among other elements, superoxide dismutase (SOD), which catalyses superoxide anion dismutation, as well as catalase (CAT) and glutathione peroxidase (GPx), which decompose hydrogen peroxide [7, 8].

In search of effective methods of PCa therapy, oncology centres introduce their own therapeutic procedures. These may affect the ongoing cellular oxidation-reduction processes in different ways. There are very few reports on oxidative stress in the circulating blood of PCa patients undergoing HDR-BT combined with EBRT. The aim of this study was to assess the activity of SOD, CAT, and GPx, as well as the concentration of the thiobarbituric acid reactive substances (TBARS) as products of lipid peroxidation in the blood of PCa patients subjected to HDR-BT combined with EBRT. We also aimed to determine the direct effect of radiotherapy on oxidative stress levels and compare the results obtained in the patients with those of the control group composed of healthy men with no malignancies.

2. Materials and Methods

2.1. Patients. The study included 60 men aged 53–80 with prostate cancer limited to prostate gland ($T_{1ABC}N_0M_0$, $T_{2ABC}N_0M_0G_x$, and $T_{1ABC}N_0M_0G_x$), treated at the Department of Oncology and Brachytherapy, Collegium Medicum of Nicolaus Copernicus University, Toruń, Poland (Franciszek Łukaszczyk Oncology Center in Bydgoszcz, Poland). The patients were administered a combined treatment employing two-fraction interstitial HDR brachytherapy and radiation with external beams.

The study was authorized by Collegium Medicum Bioethical Committee of Nicolaus Copernicus University (No. KB/605/2007). The patients provided their written informed consent to participate in the scientific study. They were treated according to the adopted procedures. Characteristics of the examined cohort are presented in Table 1. Control group was composed of 30 men with no detected cancerous diseases, aged 48–78 (avg. 61.9 ± 12.4 years of age).

2.2. Study Design: Radiotherapy. HDR-BT was applied in two fractions, before and after external beam radiation. The volume and stadium of the tumour were preliminarily assessed, which permitted us to appropriately plan the positioning of needle drivers, so that radiation would cover the entire prostate gland and its sheath. BT was administered to the patients under lumbar anaesthesia and in gynaecological position, by inserting TRUS-controlled needle driver into prostate gland. Subsequently, a moving radiation source with iridium 192 was attached using a perforated template with multiple holes, delivering a dose of 10 Gy. During the entire surgery, lasting for approx. 2 hours, two dosimeters recorded radiation levels in the urethra and anus. The patients had also a Foley catheter inserted into the bladder prior to the surgery and removed on the following day. Postoperative hospitalization lasted 2 days and was followed by 4-field technique of external beam radiation (23 sessions delivering a total dose of 46 Gy) for 4-5 weeks. EBRT therapy was then followed by another BT administration, which increased the final delivered dose to 66 Gy (biological dose of 90 Gy).

Blood was taken for analysis from the patients' basilic vein four times: on the day of admittance to the ward prior to therapy administration, immediately after therapy administration, after 1.5–3 months of the end of therapy and finally after approx. 2 years (during follow-up visits). Ten patients participated in the last analysis. The control group members had their blood taken once, following the same procedure as in the patients.

2.3. Assay of Antioxidant Enzyme Activity in Erythrocytes. SOD activity was determined using a method based on enzymatic inhibition of adrenaline autoxidation to adrenochrome in basic medium [9]. Absorbance measurements were taken at $\lambda = 480$ nm, and SOD activity was expressed in U/g Hb. Catalase activity was determined following the method by Beers and Sizer [10] based on a decreasing absorbance of H_2O_2 solution decomposed by the enzyme. The quantity of H_2O_2 decomposed over specified time was calculated using the molar absorbance coefficient. Absorbance was measured at $\lambda = 240$ nm and catalase activity was expressed in IU/g Hb. GPx activity was determined following Paglia and Valentine [11], that is, using the oxidation of reduced glutathione (GSH) by H_2O_2 catalysed by GPx. Oxidized glutathione is then reduced by exogenous glutathione reductase. This causes NADPH, a coenzyme in this reaction, to become oxidized into $NADP^+$ which induces a change in the absorbance at $\lambda = 340$ nm. GPx activity was expressed in U/g Hb.

2.4. Assay of TBARS Concentration in Blood Plasma and Erythrocytes. TBARS concentration was determined using a method based on coloured complexes formed by the products of lipid peroxidation and thiobarbituric acid at 100°C in acidic medium [12, 13]. To prevent the occurrence of peroxidation products during the reaction, 0.01% solution of 3,5-dibutyl-4-hydroxytoluene (BHT) was added to the reaction tubes. Extinction was measured at $\lambda = 532$ nm. The main product of lipid peroxidation process, which reacts with thiobarbituric acid is malondialdehyde (MDA). Therefore, for the sake of simplicity, the levels of all TBARS were transformed into mol MDA and expressed

Table 2: Markers of oxidative stress in blood of prostate cancer patients and in control group.

	Control	Patients			
		Before therapy	After radiotherapy		
			Immediately	1.5–3 months	2 years
SOD (U/g Hb)	1017.9 ± 253.5	1292.7 ± 534.6	1377.1 ± 646.1	1237.7 ± 542.1	789.9 ± 113.3
CAT (10^4 IU/g Hb)	58.62 ± 10.04	62.48 ± 13.79	63.31 ± 16.19	61.16 ± 14.2	58.1 ± 11.4
GPx (U/g Hb)	12.2 ± 6.3	8.1** ± 3.9	6.1** ± 3.1	8.5*[a] ± 4.2	4.8** ± 1.9
$TBARS_{eryth.}$ (nmol MDA/g Hb)	24.1 ± 8.8	43.0** ± 19.7	38.5* ± 19.7	41.0** ± 16.3	29.8 ± 11.4
$TBARS_{plasma}$ (10^{-2} nmol MDA/mL)	28.5 ± 8.8	44.6** ± 19.7	45.7** ± 19.7	43.2** ± 16.3	46.4* ± 15.6

SOD: superoxide dismutase; CAT: catalase; GPx: glutathione peroxidase; TBARSs: thiobarbituric acid reactive substances.
Data expressed as mean $\overline{x}$ ± SD; *$P < 0.05$; **$P < 0.001$ compared with control group.
[a]$P < 0.05$ immediately after radiotherapy.

in nmol MDA/g Hb for erythrocytes and in nmol MDA/mL for blood plasma.

2.5. Statistical Analysis. Statistical analysis was conducted using the ANOVA (analysis of variance) method (*STATISTICA v. 9.1*). A hypothesis of the equality of two means was tested. Differences at a significance level of $P < 0.05$ were assumed as statistically significant. Dependencies between the analysed parameters were assessed using correlation matrices. A statistical hypothesis of the significance of the correlation coefficients was tested.

3. Results

3.1. Erythrocyte Antioxidant Enzymes Activity. No statistically significant differences were found by comparing SOD and CAT activity in the erythrocytes of PCa patients before brachytherapy with that of the control group or by comparing SOD and CAT activity in the patients at different times of analysis (Table 2). After approx. 2 years of the end of therapy, the activity of both enzymes demonstrated a decreasing tendency, and CAT activities decreased to control levels.

GPx activity in the erythrocytes of PCa patients prior to therapy administration was lower by approx. 34% ($P < 0.001$) than the control (Table 2). Once radiotherapy was concluded, the activity of the enzyme decreased by a statistically insignificant value of 25% compared to that determined before therapy administration, whereas after 1.5–3 months of the end of therapy, the activity increased by 39% ($P < 0.05$) in comparison to that measured immediately after treatment. GPx activity measured in the patients immediately after brachytherapy conclusion and 1.5–3 months later was approx. 50% ($P < 0.001$) and 30% ($P < 0.05$) lower than in the control group, respectively. GPx activity in the patients after approx. 2 years of the end of therapy was lower by approx. 61% ($P < 0.001$) than in the control group.

3.2. TBARS Concentration. TBARS concentration in the erythrocytes of PCa patients was significantly higher at all times of analysis than that in the control group, with the exception of lipid peroxidation product levels 2 years after treatment (Table 2). Erythrocyte TBARS concentration was higher by 78% ($P < 0.001$) prior to therapy administration, 60% ($P < 0.05$) immediately after the end of therapy, and 70% ($P < 0.001$) after 1.5–3 months of the end of therapy. Approx. 2 years after treatment, erythrocyte TBARS concentration in the patients decreased to a level not differing statistically from the concentration of lipid peroxidation products in the control group. Blood plasma TBARS concentration in PCa patients before radiotherapy, immediately afterwards, as well as 1.5–3 months and approx. 2 years after treatment, was higher than in the control group. The differences were 56% ($P < 0.001$), 60% ($P < 0.001$), 52% ($P < 0.001$), and 63% ($P < 0.05$), respectively. No statistically significant changes in erythrocyte and blood plasma TBARS concentrations in the treated patients were reported. However, a series of statistically significant correlations between the analysed parameters was determined (Table 3).

4. Discussion

TBARS concentrations in blood plasma and erythrocytes of prostate cancer patients before treatment were found to be higher than that in the control group, which indicates a disturbance in the oxidant-antioxidant balance induced by this tumour, as well as intensification of lipid peroxidation processes. The presence of oxidative stress in PCa patients has been confirmed in other studies, which, similarly to our study, report higher TBARS concentrations in blood plasma [14] or both blood plasma and erythrocytes [15] in the patients, as compared to the control group. The authors also reported lower SOD, CAT, and GPx activities in patients' blood, insignificantly lower levels of vitamins E and C, as well as significantly lower concentration of reduced glutathione [15]. On the other hand, Battisti et al. [14] observed higher SOD activity and lower CAT activity in PCa patients' blood as compared to the control. Our study, described in this paper, did not reveal any differences in SOD and CAT activities in the erythrocytes of PCa patients prior to therapy administration and the control group. Only GPx activity was lower than that in the control group, which may be due to GSH deficiency. In Nigerian PCa patients, for example, with PSA concentration of 11–20 ng/mL and

Table 3: Statistically significant correlation coefficients between measured parameters.

Group	Parameters	r
Control		
	$CAT/TBARS_{plasma}$	-0.37^*
	$CAT/TBARS_{eryth.}$	0.39^*
	SOD/GPx	0.44^*
	$SOD/TBARS_{eryth.}$	0.37^*
	$GPx/TBARS_{eryth.}$	0.49^{**}
Patients		
	$SOD/TBARS_{plasma}$	0.39^{**}
	$GPx/TBARS_{eryth.}$	-0.30^*
	$TNM/TBARS_{eryth.}$	-0.30^*
Before therapy	Gleason score/GPx	0.27^*
	Age/ASA class	0.27^*
	Gleason score/$TBARS_{eryth.}$	-0.33^*
	$Hb/TBARS_{eryth.}$	-0.31^*
	Leukocyte count/$TBARS_{eryth.}$	-0.32^*
Immediately after radiotherapy	$SOD/TBARS_{eryth.}$	0.45^{**}
1.5–3 months after	$CAT/TBARS_{plasma}$	-0.37^*
2 years after radiotherapy	$CAT/TBARS_{plasma}$	-0.67^*

$^*P < 0.05$; $^{**}P < 0.01$.
SOD: superoxide dismutase; CAT: catalase; GPx: glutathione peroxidase; TBARSs: thiobarbituric acid reactive substances, ASA: American Society of Anesthesiologists score.

PSA >20 ng/mL, the reported GSH concentration was lower than in the control (PSA <3.0 ng/mL) [16]. Changes in GPx activity similar to those described in this paper were reported by Aydin et al. [17], who observed decreased GPx activity in the erythrocytes of PCa patients (with no metastases), as compared to two control groups: healthy men and patients with benign prostatic hyperplasia. The authors also demonstrated lower SOD activity in PCa patients in comparison to both of the control groups [17]. Apparently, the profile of the reported alterations in the levels of oxidant-antioxidant balance markers may change according to tumour stadium. The lack of differences in SOD and CAT activities between the patients prior to therapy administration and the control group, presented in this paper, may be explained by too low PSA levels (average of 11.12 ng/mL) and the homogeneity of patient group (only cases of locally advanced prostate cancer).

After radiotherapy, no changes in erythrocyte SOD and CAT activity, as well as plasma and erythrocyte TBARS concentrations were found. After approx. 2 years of radiotherapy conclusion, CAT activity in patients' blood decreased in a statistically insignificant way and reached values similar to those of the control group. Erythrocyte TBARS concentration was also similar to that of the control group, which may indicate a tendency to normalize the oxidation-reduction processes at the systemic level. However, plasma TBARS concentration did not decrease, which proves that a complete restoration of oxidant-antioxidant balance has not been obtained. Two years may not be a sufficient period for the oxidation-reduction processes to reach correct parameters. The results of prostatic HDR-BT combined with EBRT are probably more long lasting. It has been demonstrated that, as opposed to surgical treatment, radiation requires several to 20 months for PSA concentration to decrease, while normalization may often be observed as late as 2-3 years after treatment [18]. It may therefore be presumed that the so-called "correct prostate gland" after therapy administration is characterized by fibrosis, calcification, and atrophy. The occurring reconstruction processes are putatively related to the generation of free radicals and the functioning of antioxidant barrier, which may have systemic manifestations and explain a poorly pronounced tendency to normalize the oxidative stress markers determined in this study. Processes taking place in other systems and organs may also have an effect on oxidant-antioxidant balance as therapy side effects. It has been demonstrated, for example, that toxic effects in the gastrointestinal and genitourinary tract may remain for about 10 years after brachytherapy, external beam radiotherapy, or radical prostatectomy [19].

Conversely, the results obtained immediately after treatment may indicate a lack of significant effect of HDR-BT combined with EBRT on oxidant-antioxidant processes in PCa patients. Increased ROS generation induced by radiation may only occur in neoplastically transformed tissues not analysed in this study, while local processes may not be sufficiently intense to manifest systemically as statistically significant changes in the determined markers.

The results confirm that in the course of PCa, imbalance of oxidant-antioxidant processes occurs. HDR-BT combined with EBRT and used in PCa treatment limited to prostate

gland did not induce changes in the levels of oxidative stress markers at the systemic level, which may prove its applicability in assessing oxidation-reduction processes in the treatment of patients with tumour at this stadium. A tendency to normalize oxidation-reduction processes after 2 years of therapy conclusion is visible, although this is too a short period to restore the oxidant-antioxidant balance.

Conflict of Interests

The authors declare that they have no conflict of interests.

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