Changes of free radical oxidation and of antioxidant defense system in peripheral blood and fluid discharged from prostate draining lodge in large transbladder adenomectomy

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Abstract

Background: This study is dedicated to determination of the peculiarities of the lipid peroxidation and antioxidant system processes in the patients with prostate adenoma (PA), and the correlation between blood serum and fluid draining from lodge after adenomectomy with the aim of highlighting the risk factors of this pathology.
Material and methods: There were studied 79 men aged 50-75 years, divided into two specific groups: group 1 – 49 men with PA treated surgically by transvezical method, modified by us and group 2 – 30 healthy men.
Results: The results showed that in PA the processes of lipid oxidation with production of reactive oxygen species increased, confirmed by marked increasing of hydroperoxides of lipids, keto-diene conjugates and carbonyl compounds. There had been an impressive increase of the malondialdehyde level in both blood serum at pre- and postoperative stages and in the drained fluids from the lodge in patients of group 1. A deficiency of antioxidants was demonstrated by total antioxidant reduced activity and decreased functionality of the main antioxidant enzymes – superoxide dismutase, glutathione peroxidase, catalase in pre- and post-operative periods, as well as in fluids from the drained lodge in group 2.
Conclusions: It was found that oxygen species manifested an imbalance between reactive oxygen species generation and antioxidant protective system and they are an important pathogenic cause in self-maintenance and exacerbation of inflammatory response that can influence the pre- and postoperative evolution of PA, a predictive factor in developing treatment strategies, postoperative management.
Key words: prostate adenoma, urology, oxidative and antioxidant system.

Background

One of the main problems of vital importance in the treatment of the prostate adenoma (PA) for complex intraoperative and postoperative monitoring of the patients and prophylaxis of the complications is the maintenance of body homeostasis and hemostasis in the lodge after adenomectomy.

The reactive oxygen species (ROS) have a pivotal role in maintaining homeostasis of the organism, and in modulation of the regulatory processes. Currently is known the role of ROS in signaling and control of all aspects of life, including energy regulation, cytoskeletal structure, transport of substrates, proliferation, differentiation, and apoptosis, immune modulation and inflammatory responses, etc. [8]. ROS are largely responsible for the cellular and molecular particularities of the affected tissues by various pathologies.

The biological effects of ROS are determined by the balance between their production and their inactivation rate

by antioxidant defense system (AOS). Enzymatic and non-enzymatic antioxidants act in the human organism, and their high efficiency consists in the synergy of their action, each of them acting by different mechanisms on the ROS chains in the different levels (membrane, cytoplasm, extracellular fluids, etc.) [2, 10, 11].

In recent years it has been eloquently demonstrated that activation of lipid peroxidation (LP) involves significant damage in the cell membranes and other lipid-containing structures, which may disrupt its functions. Therefore intensification of LP in pathological condition is accompanied by accumulation of biochemical products such as lipid hydroperoxides, conjugated dienes (CD), carbonyl compounds (CC) and malondialdehyde (MDA) in the tissues and body fluids [7; 4] and are hazardous for living organisms and damage all major cellular constituents.

The generation of free radicals and nonradical reactive species and the intensity of LP formation is managed and maintained within the physiological limits under the influence of AOS compounds classes with specific hydrophilic antioxidant activity in the cytosol and body fluids and with antioxidant hydrophobic activity in biological membranes. It is well known that antioxidant enzymes such as: superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPO), γ-glutamyl transpeptidase (γ-GTP), etc., play a critical role in maintaining biological redox balance and homeostasis and thus cell integrity and functions [16; 6].

According to the data from the literature the role of oxygen radicals, LP and AOS is frequently mentioned in the pathogenic mechanisms of PA onset and development. According to some authors PA development may be an alternative way to prostate carcinogenesis due to increased prostate disorders promoted by oxidative stress and inflammatory mediators [19; 12; 21]. The hypothesis is based on the role of inflammation in stimulating prostate carcinogenesis [1]. This increasing trend can result from perturbation of the DNA repairing systems and increased nitrogenous bases injuries caused by elevated levels of ROS in the prostate cells [3, 13]. Ageing, significant imbalance of oxidation/reduction (redox) status, infection and inflammation are recognized as major risk factors for the development of benign prostatic hyperplasia and prostate cancer.

Prostate chronic inflammatory processes generate significantly elevated levels of reactive oxygen species, nitrogen and halogen compounds. Oxidative stress causes changes of the DNA that can lead to genome instability and may initiate carcinogenesis. However, it was shown that oxidative damage is not sufficient to initiate this process. Peroxidation products induced by ROS appear to take part in the epigenetic mechanisms regulating genome activity.

Thus, we consider fully argumented the initiation of the detailed research on evaluation of peculiarities of intensity of LP and AOS in patients with PA and the correlation of data from blood serum and peripheral fluid from drained lodge after adenomectomy in co-report with normal particularities for revelation of the possibilities of modern diagnosis, treatment and risk factors of this disease. Despite the diagnostic performance, wide variation in surgical tactics remains quite problematic developments in the postoperative period, often triggered by various complications, which justified the need for a study on the evaluation of free radical oxidation processes in the PA.

The aim of this study was to evaluate the processes of free radical peroxidation, the state of serum antioxidant system in pre- and post-operative patients with PA in serum and fluid draining from the lodge after adenomectomy in the early post-operative stage for the assessment of their role in the pathogenesis of the lesion development and evolution in PA.

Material and methods

The research is based on a study of 79 men aged 50-75 years, and according to the aim they were divided into two specific groups:

· Group 1 (G1), study group – patients with confirmed diagnosis of PA (n = 49)

· Group 2 (G2), control group – healthy people (donors of blood) (n = 30)

49 patients from the first group with diagnosis of PA were operated on in the Department of Urology of Holy Trinity Municipal Hospital by transvezical method in our modification, with endo-urethral drainage of the lodge and Foley catheter with a triple step No 20 into sutured bladder [5].

Material for the study were 5 ml of serum collected from cubital vein preoperative one hour before surgery and 24 hours after surgery intervention and 5 ml of fluid from the lodge obtained by endo-urethral method.

Lipid peroxidation intensity was assessed by determining the level of lipid hydroperoxides, conjugated dienes, and carbonyl compounds in the polar and nonpolar phases, and malondialdehyde.

Antioxidant defense system particularity was studied by evaluating the level of following indicators: total antioxidant activity (TAA) with ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid), and TAA with DPPH (2,2-diphenyl-1-picrylhydrazyl) in both phases – hexane (lipophilic) and isopropanol (hydrophilic), and the activity of such antioxidant enzymes as: superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPO), γ-glutamyl transpeptidase (γ-GPT). Assessment of the level of total proteins was performed by biuret method with the set of Eliteh reagents, France.

The correlation of the radical oxidation processes and antioxidant system in the blood serum with the fluid eliminated from the postoperative lodge was examined by assessment of the indices of pro- and anti-oxidant systems in the supernatant obtained after centrifugation of the liquid from the lodge.

Techniques adapted for application on multi-modal microplate hybrid reader Synergy H1 (Reader Hydride) (BioTek Instruments, USA) were used for examination of free radical oxidation processes and AOS.

Biochemical investigations were conducted in the Scientific Biochemical Laboratory of Nicolae Testemitsanu State University of Medicine and Pharmacy.

StatsDirect softwear (StatsDirect Ltd, UK) (6.0 Statistics. Soft Inc., 2002) was used for calculation of t-Student criteria.

Results and discussion

Evaluation of the results of lipid peroxidation and antioxidant system in blood and in fluids from the lodge of enrolled patients are shown in the tables 1 and 2.

According to the data presented in table 1, in patients with large PA (group 1) before and after the surgery, the blood concentration of LHPO, CD and CC in both phases – hexane (lipophilic) and isopropanol (hydrophilic) significantly ($p<0.05$) exceed these values in control group. The values of malondialdehyde, the final product of lipid peroxidation processes, doubled during all the stages of the study, with serum variations from 20.9±3.52 preoperatively to 21.6±4.38 postoperatively and 22.5±5.24 in the lodge in comparison with 10.06 ± 0.148 in the group 2.

In this study, lipid peroxidation products, namely the MDA proved to be significantly increased in patients with PA at preoperative stage. Thus we concluded that MDA level was significantly higher in patients from G1 at both stages in comparison with the control one.

MDA is a derived product of polyunsaturated fatty acids peroxidation. Evaluation of serum MDA is a non-invasive biomarker of oxidative stress. Our data showed an increased MDA in the patients with PA which had a positive correlation with PSA levels. Therefore we concluded that this index could be considered a useful marker of lipid peroxidation and prostate inflammation and could be a significant predictive factor of these processes.

Highly reactive aldehydes (4-hydroxynonenal, MDA), lipid peroxidation products are able to change the structure of DNA and proteins, making them mutagenic, cytotoxic and genotoxic. We therefore believe that a high level of MDA and of other reactive aldehydes can explain the changes in nitrogenous bases of DNA not only in prostate cancer, but also in glandular epithelium of PA.

High values of oxidative stress (OS) indices in the fluids from postoperative lodge which had small difference in comparison with the values recorded in the blood at pre- and postoperative stages was another feature documented in patients of G1.

It is known that hydroperoxides have the ability to oxidize thiol group-containing compounds (cysteine, glutathione, lipoic acid). But the SH (thiol) groups are not the only unique site of peroxide oxidation. Interaction of LP and proteins results in development of specific complexes with covalent links between NH2 (amino) groups of aminoacids and aldehydes or carboxylic groups of lipoperoxides. Thus, we can conclude that MDA has a dominant role in the formation of insoluble protein polymers through the creation of links between polypeptide chains involving lysine amino-groups. Likely, that certain hydroperoxide interaction with proteins determines the biological effects of lipoperoxides on the cells, including cytolytic effect.

The biochemical investigation of the markers listed above, for the assessment of antioxidant processes peculiarities in

Table 1

Changes in lipid peroxidation processes in the serum and in the fluids from the lodge in patients with PA

Parameters	General group of study			
	G1 – study group (n=49)			(G2) Control group (n=30)
	In blood serum		Fluids of the lodge	
	Before surgery (first 60 min)	After surgery at 24 hours	After surgery at 24 hours	
Age (years)	**68,6±1,05**			**68,9 ±0,80**
MDA, nM/L	20,9±3,52* (207%)	21,6±4,38* (215%)	22,5±5,24* (224%)	10,06±0,148 (100%)
In hexane, lipophilic nonpolar phase				
LHPO, cu/ml	10,75±1,14** (279%)	10,25±1,27* (270%)	11,41±1,39** (297%)	3,84±0,09 (100%)
CD, cu/ml	7,33±0,86** (277%)	6,15±0,82* (233%)	6,40±0,77* (242%)	2,64±0,12 (100%)
CC, cu/ml	5,31±0,67*** (352%)	4,4±0,55*** (291%)	2,82±0,44** (187%)	1,51±0,02 (100%)
In isopropanolic, hydrophilic polar phase				
LHPO, cu/ml	10,67±2,03* (189%)	9,64±1,16* (170%)	11,37±2,18** (207%)	5,65±0,04 (100%)
CD, cu/ml	5,71±0,86** (198%)	4,40±0,63** (153%)	6,95±0,95*** (241%)	2,88±0,03 (100%)
CC, cu/ml	2,28±0,37* (158%)	2,30±0,26** (160%)	3,84±0,51*** (268%)	1,44±0,06 (100%)
Total protein, g/L	53,4±4,22** (78%)	49,8±6,04** (73%)	51,7±6,27* (75%)	68,6±2,45 (100%)

Note: LHPO – lipid hydroperoxides; conjugated dienes (DC), carbonyl compounds (CC) and malondialdehyde (MDA); cu - conventional units.
Statistically significant differences:
a) compared to the control group * - $p<0.05$; ** - $p<0.01$; *** - $p<0.001$;
b) compared to the group before surgery and after surgery, # - $p<0.05$; ## - $p<0.01$; ### - $p<0.001$.

Table 2

Changes of antioxidant system in serum and in fluids from the lodge in patients with PA

Parameters	General group of study			
	G1 – study group (n=49)			(G2) Control group (n=30)
	In blood serum		Fluids form the lodge	
	Before surgery (first 60 min)	After surgery in 24 hours	After surgery in 24 hours	
Age (years)	**68,6±1,05**			**68,9 ±0,80**
TAA - ABTS, mM/L	0,36±0,06 (75%)	0,37±0,04 (77%)	0,36±0,08 (75%)	0,48±0,04 (100%)
TAA - DPPH, mM /s.L, lipophilic, nonpolar phase	2,66±0,31* (78%)	2,71±0,37 (87%)	2,65±0,41 (85%)	3,42±0,19 (100%)
TAA - DPPH, mM/ s.L, hydrophilic, polar phase	1,78±0,32* (70%)	1,92±0,64 (76%)	1,91±0,77 (76%)	2,54±0,11 (100%)
CAT, nkat/L	45,9±3,72*** (67%)	44,5±4,86** (65%)	56,2±6,33 (82%)	68,5±0,85 (100%)
SOD, cu	614,8±71,3*** (55%)	795,8±68,1*** (71%)	736,9±80,9*** (66%)	1124,0±40,5 (100%)
GPx, nkat/L	84,2±9,4* (61%)	97,6±10,6 (71%)	88,4±9,1* (64%)	138,1±15,3 (100%)
γ-GPT, IU/L	29,20±3,24* (136%)	22,06±2,62 (103%)	32,04±3,64* (149%)	21,5±2,13 (100%)

Note: LP – lipid hydroperoxide; diene conjugates (CD), carbonyl compounds (CC) and malondialdehyde (DAM);
cu - conventional units; IU – international units.
Statistically significant differences:
a) compared to the control group * - p<0.05; ** - p<0.01; *** - p<0.001;
b) compared to the group before surgery and after surgery, # - p<0.05; ## - p<0.01; ### - p<0.001.

patients with PA (G1) proved a deficiency of antioxidants, by significant decrease of total antioxidant activity (TAA-DPPH) in both phases – hexane (lipophilic) and isopropanol (hydrophilic) (table 2).

It is worth mentioning that the main activity of antioxidant enzymes (SOD, CAT, and GPO) significantly decreased in serum by 33-45% at pre- and postoperative stages in patients with PA. The only exception is the important increase of γ-GPT activity at preoperative stage.

Therefore, it is to note that the cause of increased oxidative stress may be the over-production of free radicals or the decrease of the inactivation of free radicals by antioxidant enzymes SOD, CAT, GPO. Elevated levels of free radicals are also accompanied by high MDA level, a product of lipid peroxidation at preoperative stage in patients with PA in comparison with the control group.

The study results showed that oxidative stress in patients of group 1 with PA was significant in the pre-operative stage in comparison with control group G_2. Similar peculiarities were noted by other researchers. Also our results are in concordance with some studies which have established the presence of chromosomal aberrations in these patients [16].

According to Przybyszewski W.M. and Rzeszowska-Wolny J. [14] prostate inflammation can generate significantly elevated levels of ROS, reactive nitrogen species, and halogenated compounds. Neutrophils and macrophages provide a source of free radicals that can induce hyperplastic changes through oxidative stress (OS) in the prostatic tissue and DNA damage. OS can induce vascular tissue injury and damage to the structure and function of proteins, genomic damage that due to posttranslational modifications including those involved in DNA repair and apoptosis [15]. This can lead to oxidative damage of DNA by mutations, deletions or rearrangements and reduce DNA repair. OS also modifies the population of stem cells. Genomic changes result in modulation of an imbalance between cell proliferation and cell death. According to some opinions the change in normal ratio is regulated by programmed cell death that leads to hyperplasia or precancerous processes [9].

In this context, some scientific sources state that tissue of human prostate is vulnerable to DNA oxidative damage due to decreased level of DNA enzymes. In these cases OS may enable the transcription of nuclear transcription factor-kB (NF-kB) by tumor necrosis factor TNF-α pathway/AP-1 transduction and NF-kB-inducing kinase transduction pathways. It should be noted that NF-kB is known as a transcriptional regulator of the inflammatory processes and activator of the gene responsive for regulation of the immune response, inflammation, cell proliferation, cell migration and apoptosis. Thus, nuclear translocation of NF-kB can activate target genes involved in carcinogenesis. These features in particular NF-kB deregulation were proposed to be one of the supposed molecular mechanisms that induce chronic inflammation and cancer [9]. Some opinions state, that cell soluble proinflammatory mediators directly activate NF-kB and induce local production of proinflammatory factors in patients with PA, but not in the control group [22, 25].

MDA is a final product derived from peroxidation of polyunsaturated fatty acids and lipids. In contrast free radicals, aldehydes are relatively stable and, therefore, have the oppor-

tunity to broadcast within or outside the cell to attack distant objects in the place of origin initiated by free radicals. MDA not only reflects the level of lipid peroxidation, but is also a by-product of cyclooxygenase. Platelet activity, and persistent platelet activation is a common feature of many clinical syndromes associated with increasing of lipid peroxidation. According to Kulinski V. I. [23] measuring the level of MDA in plasma or serum of blood provides a suitable condition *in vivo* index of lipid peroxidation and is a biomarker, often used to investigate the radical-mediated physiological and pathological conditions. Therefore we note that circulating levels of MDA proved to be significantly higher in patients with large prostatic adenoma in comparison to healthy donors (G2). Similar results were obtained by other researchers and they are strongly correlated with the levels of prostate-specific antigen [20]. Some studies also indicate the role of lipid peroxidation that also may be a trigger in the synthesis of prostaglandins by cyclooxigenase-2 activation [17].

The products of peroxide oxidation of lipids are eliminated by superoxide dismutase, glutathione peroxidase, catalase, and such vitamins-antioxidants as α-tocopherol and ascorbic acid.

The authors noted that assessing the level of antioxidants in patients with PA can help in good management and reduce morbidity. Romanda Duru et al. [18] state that in the management of patients with PA, antioxidants supplementation is needed, because deficiency in antioxidants may be associated with cell degeneration, pathologic process and poor prognosis.

Extent of oxidative damage ROS-induced can be exacerbated by low efficiency of antioxidant defense mechanisms. Imbalance between OS and antioxidant components of the cell also plays the important role in the development of PA. Some sources of literature reveal OS growth and decreased antioxidant mechanism in prostate diseases including PA. However, data is not univocal. Low antioxidant capacity was found in patients suffering from benign prostate hyperplasia compared to controls. Negative correlation between blood serum peroxides and antioxidant capacity was observed in these patients [24].

The studies demonstrated in patients an imbalance between the production of RLO and antioxidant substances. This imbalance occurs because of excessive RLO and natural antioxidants can't cope with it, and in result appears incapable of annihilating them, as evidenced by the decreased total antioxidant activity in both phases – hexane, hydrophobic, nonpolar and isopropanol, hydrophilic, polar, both at the pre- and postoperative stages.

We note that oxidative stress is manifested by the imbalance between generations of ROS, on the one hand and protective antioxidant systems, on the other hand, forms pathogenic elements important in the self-maintaining and exacerbating the inflammatory response and influencing the development of PA patients during the study. Therefore the obtained results about oxidative processes and antioxidant correlations that reflect the current research findings open the new possibilities of drawing up strategies of medical and surgical management in patients with PA.

Conclusions

1. In the case of prostate adenoma, the processes of oxidation with production of reactive oxygen species were increasing, manifested by marked increases of the lipid hydroperoxides, conjugated dienes, carbonyl compounds and malondialdehyde in the blood and in the fluids from the lodge at the pre- and postoperative stages. Compared with G1 the recorded indices of oxidative stress were significantly higher, in particular malondialdehyde (22.5±5.24 nM/L) in blood serum, similar to pre- and postoperative 20.9±3.52 and 21.6±4.38 nM/L, respectively.

2. Deficiency of antioxidants was found in the patients with PA, as demonstrated by the important reduction of total antioxidant activity, decrease of the activities of the main antioxidant enzymes (SOD, CAT and GPO) during pre- and postoperative stages.

3. Oxidative stress is manifested by imbalances between generation of reactive oxygen species, on the one hand, and antioxidant systems protection, on the other hand, the activity of enzymes is an important pathogenic and informative marker in the self-maintenance and exacerbation of inflammatory response influencing evolution of PA at pre- and post-operative stages. Oxidative stress is a predictive element in developing treatment strategies for patients with PA at intra-operative and post-operative stages.

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