Free Radical Research

ISSN: 1071-5762 (Print) 1029-2470 (Online) Journal homepage: http://www.tandfonline.com/loi/ifra20

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To cite this article: Ferhat Hanikoglu, Aysegul Hanikoglu, Ertan Kucuksayan, Murat Alisik, Ahmet Anil Gocener, Ozcan Erel, Mehmet Baykara, Aurora Cuoghi, Aldo Tomasi & Tomris Ozben (2016): Dynamic Thiol/Disulphide Homeostasis Before and After Radical Prostatectomy in Patients with Prostate Cancer, Free Radical Research, DOI: 10.1080/10715762.2016.1235787

To link to this article: http://dx.doi.org/10.1080/10715762.2016.1235787

Accepted author version posted online: 13 Sep 2016.
Published online: 13 Sep 2016.

Dynamic Thiol/Disulphide Homeostasis Before and After Radical Prostatectomy in Patients with Prostate Cancer

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ABSTRACT

Thiol groups are important anti-oxidants and essential molecules protecting organism against the harmful effects of reactive oxygen species (ROS). The aim of our study is to evaluate thiol-disulphide homeostasis with a novel recent automated method in patients with localized prostate cancer (PC) before and six months after radical prostatectomy (RP). 18 patients with PC and 17 healthy control subjects were enrolled into the study. Blood samples were collected from the controls subjects and patients before and six months after RP. Thiol-disulphide homeostasis was determined using a recently developed novel method. Prostate specific antigen (PSA), albumin, total protein, total thiol, native thiol, disulphide and total antioxidant status (TAS) were measured and compared between the groups. Native thiol, total thiol and TAS levels were significantly higher in the control group than the patients before RP (p values < 0.001). There was a non-significant increase in the native thiol, total thiol and TAS levels in the patients six months after RP in comparison to the levels before RP (p values 0.3, 0.3 and 0.09 respectively). We found a significant negative correlation between PSA and thiol levels. Our study demonstrated that the decreased thiol and TAS levels weakened anti-oxidant defence mechanism in the patients with PC as indicated. Increased oxidative stress in prostate cancer patients may cause metabolic disturbance and have a role in the aetiopathogenesis of prostate cancer.

KEYWORDS: Prostate cancer; dynamic thiol-disulphide homeostasis; antioxidant; ROS;

Introduction

Prostate cancer (PC) is the second most common cancer and the second most common cause of death in male cancer patients [1,2]. Radical prostatectomy (RP) is the main treatment modality for patients with localized (non-metastatic) PC [3]. Previous studies demonstrated that ageing, genetic predisposition, androgen hormone levels, dietary factors, inflammation and oxidative stress play crucial role in the initiation and development of PC. Oxidative stress in the development of PC has been under intensive research [4].

Oxidative stress due to the excessive production of reactive oxygen species (ROS) causes damages to DNA, protein and lipids. Oxidative protein damage causes irreversible modifications in serum and tissue proteins. The structure and activity of oxidized proteins and biomolecules change markedly in comparison to their native forms. Oxidative modification of DNA and proteins may affect a variety of cellular functions, and cause cell damage and death or mutations and carcinogenesis [5,6]. Thiols are essential and potent anti-oxidant molecules containing a functional sulfhydryl group protecting organism against the harmful effects of oxidative stress damage. Thiol groups in cysteine, homocysteine, glutathione, albumin and other proteins, constituents of the antioxidant defence mechanism, are oxidized by ROS, giving rise to reversible disulphide bonds. The disulphide bonds can be reversibly reduced to thiol groups by a number of antioxidants [7]; in this way the thiol/disulphide homeostasis is maintained. Dynamic thiol-disulphide homeostasis has a crucial role in redox states such as antioxidant defence, oxidation of proteins, apoptosis, activity of enzymes, and cellular signal transduction mechanism. Thiol-disulphide homeostasis has been measured since 1979 only in one direction, but the novel method recently developed by Erel and Neselioglu allows the levels of both variables to be measured separately, as well as jointly [8].

In our study, we measured plasma native thiol, total thiol, and disulphide levels, then calculated the ratios of disulphide/native thiol, disulphide/total thiol, and native thiol/total thiol in the plasma of prostate cancer patients before and six months after radical prostatectomy operation using a novel, automated method in order to determine the level of dynamic thiol/ disulphide homeostasis. According to the results of our literature search, thiol/disulphide homeostasis has not been studied and measured using such a novel method in patients with prostate cancer, thus our study provides original data on this important issue.

Methods and Materials

Study subjects

The study was conducted in accordance with the Declaration of Helsinki, and approved by the local ethical committee; written informed consent was received from the patients and control subjects before being included into the study. The patients and control cohorts were recruited at the Urology Department, Medical Faculty, Akdeniz University.

18 patients newly diagnosed with localized (non-metastatic) prostate cancer and 17 healthy, age and body mass index (BMI)-matched male control subjects having normal PSA levels and no prostate disease were enrolled into the study. Subjects who had a history of liver diseases, chronic ischemic diseases, systemic inflammatory diseases or using antioxidant supplements, lipid lowering drugs, cigarette and alcohol were excluded from the study.

Laboratory analysis

Fasting morning venous blood samples were collected from the patients before and six months after the RP operation and from the controls into both EDTA containing and serum

separating tubes. After centrifugation, separated plasma and serum samples were frozen at -80°C until analysis. Samples with significant absorptions in the red region of the visible spectrum, which equates to extracellular haemoglobin levels of greater than approximately 0.3 g/l, were excluded from the study analyses. Thiol-disulphide homeostasis was determined with a novel method described previously [8]. Albumin was measured by the bromocresol green method; total protein by enzymatic colorimetric method; prostate specific antigen (PSA) by electrochemiluminescense. Total antioxidant status was determined using an automated colorimetric method, developed by Ozcan Erel as described previously[9].

Statistical analysis

Statistical analysis was performed using SPSS Statistics (SPSS Inc. Chicago, IL, USA). Quantitative data were given as mean ± SD or medians (interquartile ranges, IQR). Normal distribution and differences between variances were determined using Kolmogorov-Smirnov and Levene tests, respectively. For comparisons between two groups, Student's *t* test and Mann Whitney *U* test were used as appropriate. For subgroup analyses, Kruskal-Wallis test was used to determine significant differences. Mann-Whitney *U* test was used to determine differences among groups if a significant difference was found in Kruskal-Wallis test. Spearman correlation test was performed between the variables. *p* value <0.05 was considered statistically significant.

Results

Laboratory findings of the patients and control group including disulphide/native thiol, disulphide/total thiol and native thiol/total thiol ratios are shown in Table 1. There was no significant difference in terms of age (65.5 ± 5.1 year vs 66.7 ± 5.2 year, p=0.5) and body mass index (26.81 ± 2.32 kg/m² vs 26.48 ± 2.18 kg/m², p=0.6) between patients and control subjects. Native thiol and total thiol levels in all of the groups are shown as whiskers graphs in Figure 1 and 2. Native thiols, total thiols, disulphide and TAS levels were significantly higher in the control group compared to the patient group before RP (p values <0.001, 0.0001, 0.02 and 0.0001, respectively). As expected, PSA levels were higher in the patients before RP compared to the controls and patients after RP (both p<0.0001). There was a significant negative correlation between the PSA levels and native and total thiol levels in the control subjects and patient group before RP (p=0.01, r= -0.4 and p=0.001, r= -0.51 respectively).

Patients six months after RP in comparison to the patients before the operation had higher native thiol levels (364.3 ± 46.88 µmol/L vs 350.7 ± 46.35 µmol/L, p= 0.3) and higher total thiol levels (392 ± 44.2 vs 378.8 ± 46.52, p= 0.3), but these increases were not statistically significant (p= 0.3, p= 0.3, respectively). Similarly, the decreases in total protein and albumin levels (p=0.09 and p=0.1, respectively) and the increase of TAS level (1.22 ± 0.22 mmol Trolox Equivalent/L vs 1.09 ± 0.21 mmol Trolox Equivalent/L, p=0.09) were not significantly different in patient group after RP compared to patients before RP. Native thiols, total thiols, disulphide, TAS and albumin levels were significantly higher in the control group compared to the patients after RP (p values 0.002, 0.0004, 0.007, 0.02 and 0.007, respectively). No other significant differences in the measured parameters were found among the groups.

Discussion

Previous studies have shown that impaired oxidant-antioxidant status in the prostate plays a role in the initiation and progression of PC [10]. The relation between oxidative stress and prostate diseases such as benign prostate hyperplasia (BPH), prostate inflammation, and prostate cancer has been investigated in various studies [6,11]. No study has been performed so far to elucidate the dynamic of thiol/disulphide levels in blood of PC patients. Glutathione (GSH) level was found lower in PC patients compared with the controls [12]. Only a small thiol fraction is found among low molecular weight compounds, such as cysteine (Cys), cysteinylglycine, GSH, homocysteine and γ-glutamylcysteine; the main part is found in albumin and other plasma proteins [13,14].

The novel automated measurement method used in our study provided us with an opportunity to measure serum/plasma total thiol, native thiol, and disulphide levels. Recent studies have demonstrated an imbalance in thiol-disulphide homeostasis status involved in the aetiopathogenesis of diabetes mellitus [15], cardiovascular disease [16], cancer [17], rheumatoid arthritis [18], chronic kidney disease [19], Parkinson's disease [20] and liver disease [21]. Therefore, the determination of dynamic thiol disulphide homeostasis can provide valuable information in physiological and pathological biochemical processes. In previous studies Erel *et al*. showed that plasma disulphide levels were higher in smokers and in patients with, diabetes, obesity, and pneumonia; while plasma disulphide resulted lower in patients with proliferative diseases such as multiple myeloma, urinary bladder cancer, colon cancer and renal cancer. Aggressively growing tumours showed the lowest disulphide levels, while slowly growing ones showed subnormal values [8].

It was also previously shown that the increase of disulphide/native thiol ratio is positively correlated with age [22]. This show us that oxidative stress may increase with age [23] and disulphide/thiol homeostasis may shift towards disulphide. Further studies have confirmed the positive correlation between oxidative stress and age; while a negative correlation between native thiol level and both age and BMI has been determined [13]. As stated in the Study Subjects section, the controls and study group were matched for age and BMI, hence the correlation of thiol levels with BMI and age could not be carried out. Furthermore, a negative correlation with BMI has been shown for TAC levels [24]. In order to avoid the effect of age and BMI on oxidative stress and thiol levels, we enrolled age- and BMI-matched patients with localized (nonmetastatic) prostate cancer and healthy control subjects into our study.

While there are numerous studies reporting increased oxidative stress in PC patients, only a limited number of studies have demonstrated the level of antioxidant capacities. Oxidative stress biomarkers, such as thiobarbituric acid reactive substances, total oxidative status, malondialdehyde, plasma nitrite/nitrate levels, lipid peroxide activities were reported to be elevated in PC patients compared to the healthy controls [25,26]. In addition, decreased antioxidant enzymes such as catalase, manganese containing superoxide dismutase (MnSOD), copper and zinc containing superoxide dismutase (Cu,ZnSOD) [27], glutathione peroxidase (GPX) and impairment of oxidative stress/antioxidant status have been reported in PC patients [2]. Aryal *et al.* found decreased levels of antioxidant vitamins such as α-tocopherol and ascorbate levels in benign prostate hyperplasia patients [28]. Other studies reported a positive correlation between oxidative stress and progression to PC and anti-oxidants like vitamin E and selenium decreased risks for PC [29].

It is known that thiol groups have a crucial role in ROS detoxification and a thiol decrease leads to failure of the antioxidant defence mechanism. It has been proved that thiol disulphide

homeostasis and thiol oxidation have regulatory critical roles in protection against oxidative stress, detoxification, regulation of enzymes and essential cellular pathways such as signal transduction, pro-apoptotic, anti-apoptotic signalling [30]. As stressed above, there is no information available regarding native thiol and disulphide levels in PC patients. Therefore we performed our study to fill this gap and provide data on this important point, examining thiol and disulphide levels in PC patients just before and 6 months after radical prostatectomy.

A significant decrease in total thiol, native thiol, disulphide and TAS levels was found in PC patients before RP compared with the control group. The reason for the low disulphide levels in patients group before RP ($p=0.02$) lies in the dramatic native thiol loss ($p<0.001$) and the deteriorating capacity to resist oxidant stress ($p<0.0001$), when compared with control subjects. As a result, an impaired thiol/disulphide balance occurs in these patients before RP.

Native thiols, total thiols and TAC levels increased, while disulphide levels decreased, however in a non-significant manner six months after RP surgery, when compared to the preoperatory levels. These findings indicate that thiol/disulphide homeostasis begins to shift towards thiols following the operation; hence thiol levels and total antioxidant capacity increases following RP. Although the levels of native thiol and TAS increased in the patients after RP, they were still significantly lower than native thiols and TAS levels when compared with the control group ($p=0.002$, 0.02, respectively). One of the limitations of our study is the short follow-up time (six months) after RP. The observed increasing trend in native thiol and TAS, however non-significant, is expected to be significantly higher in a longer follow-up time.

PSA is the most common used biomarker for PC diagnosis, and at the same time PSA, tumour volume and Gleason score are the main prognostic factors related to the severity of PC. Kato et al demonstrated a direct correlation between PSA levels and tumour volume in patients with localized PC [31]. Thus, PSA levels are of crucial importance for estimating tumour volume at the diagnosis. In our study, a significant negative correlation between PSA levels and both, native thiols and total thiols was found comparing the control group with patient before RP ($p=0.01$, $r= -0.4$ and $p=0.001$, $r= -0.51$ respectively). Thus, decreased thiol levels might be considered as a marker to indicate prostate tissue tumour volume and disease severity.

In a recent study, researchers compared glutathione to glutathione disulphide ratios in malignant and non-malignant human prostate cell lines. The results demonstrated that the more aggressive phenotype of prostate cancer cells shows adaptation to increased oxidative stress via up-regulation of glutathione turnover [32]. To the extent of our knowledge, there is no literature evaluating the relationship between thiol and PSA levels. We can hypothesize that decreased thiol levels associated to increased tissue damage result in PSA increase. We are aware that the small number of patients recruited in our study may pose a limit to our interpretation; further studies assessing thiol and PSA levels are needed to fully clarify the relation of decreased thiol levels with increased PSA levels as a prognostic marker in this relevant health problem.

Conclusion

PC patients have a decreased ability to cope with oxidative stress, as demonstrated by the decrease of TAS and native thiol levels. If the decrease in total and native thiol levels is the cause or result of prostate cancer development in the patients merit further investigations. We hypothesize that impaired balance in dynamic thiol/disulphide homeostasis and weakened

TAS is directly related to an increased oxidative damage in prostate tissue, playing an important role in progression to PC.

Conflict of interest: The authors declare that they have no conflict of interest regarding the present study.

Research funding: None declared.
Employment or leadership: None declared.
Honorarium: None declared.

Table Legend

Table 1: Laboratory findings of the control group and patient groups before and six months after RP. PSA levels were expressed as mean (median), other data were expressed as mean ±SD. $P < 0.05$ was considered significant for statistical analyses and only significant statistics were shown in the table. a: Control group compared to the patient group before RP, b: Control group compared to the patient group after RP, c: Patient group before RP compared to the patient group after RP.

Figure Legends

Figure 1. Box-and-whisker plot of native thiol levels in the control group (Median: 436.3 µmol/L),patient group before RP (Median: 338.3 µmol/L) and patient group after RP (Median: 367.5 µmol/L) groups (Whiskers: 10 and 90 percentile). The middle lines, upper and lower margin of boxes represent medians.

Figure 2. Box-and-whisker plot of total thiol levels in the control group (Median: 467.2 µmol/L), patient group before RP (Median: 363.1 µmol/L) and patient group after RP (Median: 393.2 µmol/L) (Whiskers: 10 and 90 percentile). The middle lines, upper and lower margin of boxes represent medians.

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Table 1. Laboratory findings of the control group and patient groups before and six months after RP.

	Control N=17	**Patients (Before R.P) N=18**	**Patients (After R.P) N=18**	**P**
PSA (ng/mL)	0.64 ± 0.58	6.04 ± 3.92	0.005 ± 0.017	**< 0.0001 a,b,c**
Total protein (g/dl)	7.6 ± 0.4	8 ± 0.5	7.7 ± 0.4	
Albumin (g/dl)	4.86 ± 0.2	4.74 ± 0.3	4.59 ± 0.2	**0.007 b**
TAS (mmol Trolox Equivalent/L)	1.36 ± 0.1	1.09 ± 0.21	1.22 ± 0.22	**< 0.0001 a, 0.02 b**
Native thiol (µmol/L)	419.8 ± 54.87	350.7 ± 46.35	364.3 ± 46.88	**< 0.001 a, 0.002 b**
Total thiol (µmol/L)	462.3 ± 61.52	378.8 ± 46.52	392 ± 44.20	**< 0.0001 a, 0.0004 b**
Disulphide (µmol/L)	21.25 ± 6.4	14.03 ± 10.54	13.87 ± 8.7	**0.02 a, 0.007 b**
Disulphide/native thiol (%)	5.06 ± 1.34	4.16 ± 3.36	3.98 ± 2.81	
Disulphide/total thiol (%)	4.57 ± 1.1	3.67 ± 2.7	3.57 ± 2.2	
Native thiol/total thiol (%)	90.8 ± 2.2	92.6 ± 5.5	92.8 ± 4.5	

PSA: Prostate spesific antigen; TAS: Total antioxidant status.

PSA levels were expressed as mean (median) and other datas were expressed as mean ±SD.

P < 0.05 was considered significant for statistical analyses and only significant statistics were shown in the table.

a: Control group compared to the patient group before RP,

b: Control group compared to the patient group after RP,

c: Patient group before RP compared to the patient group after RP.