 DOI: https://doi.org/10.5114/ceji.2017.72285

Is oxidative stress measured by thiol/ disulphide homeostasis status associated with prostate adenocarcinoma?

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Abstract

Aim of the study: *We aim to examine the relation between thiol/disulphide homeostasis and transrectal ultrasound guided prostate biopsy (TRUS-Bx) results and evaluate whether it was effective on the distinction of benign and malign prostate disease.*

Material and methods: *The study included 29 men histopathologically diagnosed as prostate adenocancer (Pca) (group 1), 30 men having benign prostate hyperplasia (BPH) (group 2) and age match 30 healthy individuals in the control group (group 3). Thiol/disulphide homeostasis was measured using a novel automatic and spectrophotometric method.*

Results: *Among the three groups, a statistically significant difference was detected among native thiol, total thiol levels and disulphide/total thiol, disulphide/native thiol and native thiol/total thiol ratios which are thiol/disulphide homeostasis parameters apart from disulphide ($p < 0.001$, $p < 0.001$, $p < 0.001$, $p < 0.001$, $p < 0.001$ respectively). Additionally, no significant difference was detected in albumin and total protein levels among the groups ($p = 0.223$, $p = 0.316$ respectively). Serum native and total thiol levels were high and disulphide level was low in group 1 when compared to the group 2 ($p = 0.003$, $p = 0.007$, $p = 0.265$ respectively). In addition, serum native thiol, total thiol and disulphide levels were low in group 1 when compared to the group 3, but while low native and total thiol levels were significant, low disulphide levels were not found significant ($p < 0.001$, $p < 0.001$, $p = 0.331$, respectively).*

Conclusions: *Thiol/disulphide homeostasis was found to be disturbed in Pca patients detected with TRUS-Bx. This is suggesting serum native thiol, total thiol level and ratios provides a novel biomarker for the role for oxidative stress in disease etiopathogenesis.*

Key words: *dynamic thiol-disulphide homeostasis, prostate cancer, benign prostatic hyperplasia, oxidative stress.*

(Centr Eur Immunol 2018; 43 (2): 174-179)

Introduction

Prostate cancer (PCa) constitutes a source of concern in developed countries where the ratio of elder males is higher. Pca is the most common extracutaneous cancer in elder males (> 70) in Europe and the second most common cause for cancer-related deaths in Australia [1, 2]. Biopsies guided by serum Prostate Specific Antigen (PSA) levels, Digital Rectal Examination (DRE) and Transrectal Ultrasonography (TRUS) are routinely used for scanning, detection and diagnosis of prostate cancer [1, 3]. Transrectal ultrasound guided prostate biopsy (TRUS-Bx) is one of the most common interventional procedures made by urologists. Despite the developments in prostate imaging area, it is used as the standard procedure for prostate cancer diagnosis [3].

Studies made in recent years show that increased free oxygen radicals and lipid peroxidation play a role in the pathogenesis of many patients [4, 5]. Free radicals cause structural deterioration of cells by effecting all important components such as lipid, protein, DNA and carbohydrates. Free oxygen radicals in biological systems and

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Submitted: 8.06.2017; Accepted: 28.07.2017

other free radicals constitute one of the most important causes of oxidative stress [6]. Increase in free radicals and oxidative stress may cause tumor development by causing DNA damage and increasing the mutation and oncogenic transformation speed. They can also cause cancer and metastasis development by effecting cellular functions such as cell proliferation, cellular remodeling, apoptosis and aging [7]. There are several studies showing that oxidative stress plays role in the etiology of prostate cancer [8, 9].

Thiol is an organic compound containing the sulphydryl group which has a critical role in preventing the formation of any oxidative stress condition in the cells. Thiol groups are important members of antioxidant cascade. Oxidation reaction of thiols with oxidizing molecules causes the formation of reversible disulphide bonds. This transformation is the earliest observable symptom of radical-mediated protein oxidation. Disulphide bond structures forming when oxidative stress condition ends can be reduced to the thiol groups again and thus dynamic thiol disulphide balance which has a vital importance for the organism is continued [10]. Dynamic thiol/disulphide balance condition has critical roles in organizing anti-oxidant defense, detoxification, apoptosis and enzyme activities, transcription and cellular signal transfer mechanisms. While this double-sided balance could be measured on one side as total thiol in the past, all components can be measured separately today using a new developed method and so can be evaluated both individually and as a whole [10-14]. Abnormal thiol-disulphide balance is detected in many diseases such as diabetes mellitus, cardiovascular diseases, cancer and kidney failure [5, 11-13].

The relation between thiol/disulphide homeostasis and prostate biopsy result has not yet been investigated. Our aim for this study was to examine the relation between thiol/disulphide homeostasis and biopsy result in the patients who had TRUS-Bx and evaluate whether thiol/disulphide homeostasis was effective on the distinction of benign and malign.

Material and methods

Study design and groups

This study was made by Necmettin Erbakan University Meram Faculty of Medicine Urology Clinic. Consent according to Helsinski declaration was taken from Necmettin Erbakan University Meram Faculty of Medicine ethics committee before the study (no. 2017/821).

Patients who referred to urology polyclinic with high PSA, abnormal DRE and/or prostate cancer suspicion in the radiological evaluation were included to study. The study included 89 men: 29 histopathologically diagnosed as Pca (group 1), 30 histopathologically diagnosed as BPH (group 2) and 30 age macth healthy individuals. Conditions such as being newly diagnosed and not having surgical treatment, chemotherapy and radiotherapy were demanded in the patient group. Study exclusion criteria were determined as dietary application and medicine use that may affect biochemical analysis results, presence of any systemic and chronic diseases, smoking and alcohol use.

Blood sampling and measurement of dynamic thiol/disulphide homeostasis

In all subjects blood sample was taken in tubescontaining ethylenediaminetetraacetic acid and serum samples were immediately separated by centrifugation at 1500 rev for 10 min. The separated serums were kept in –80°C after coding. Thiol/ disulphide homeostasis tests were measured using a novel automatic and spectrophotometric method developed by Erel and Neselioglu (10). First, free functional thiol groups (–SH) were extricated by decreasing disulphide bonds (–S–S–) by sodium borohydride (NaBH4). The unused NaBH4 remnants are completely removed by formaldehyde. So this prevents further reduction of 5,5′-dithiobis- 2-nitrobenzoic acid (DTNB) as well as any disulfide bonds resulting from the reaction with DTNB. Total thiol groups involving reduced and native thiol groups were determined after reaction with DTNB. The disulfide parameter is a value which can be calculated automatically as half of the native thiol content and total thiol content. Disulphide/total thiol, disulphide/native thiol and native thiol/total thiol ratios were calculated as percentages. Prostate specific antigen (PSA) was measured by chemiluminescense method on the ADVIA Centaur analyzer (Siemens Diagnostics). Free PSA measurements were performed by immunassay method using the Immulite 2000 system (Diagnostic Products Corporation).

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

Data for a total of 89 patients including 59 TRUS-Bx patients (29 malign, 30 benign) and 30 Control patients

Table 1. Demographic information, biochemical and pathological results of the groups

Parameter	Group 1	Group 2	Group 3	*p* value[α]
Number	29	30	30	
Age (years)	69.5 ±9.1	69.7 ±8.7	66.57 ±8.5	0.12
BMI	26.2 ±2.1	26.8 ±2.7	25.9 ±1.9	0.09
PSA (ng/ml)	23.6 ±9.6	7.59 ±6.16	2.05 ±1.07	< 0.001
Free PSA (ng/ml)	4.12 ±3.8	1.38 ±1	1.06 ±0.8	< 0.001
TRUS-Bx result	Malign	Benign	–	
Histology	Prostate adenocarcinoma Gleason score 6: 10 Gleason score 7: 9 Gleason score 8: 3 Gleason score 9: 5 Gleason score 10: 2	BPH	–	
Metastasic disease (%)	4 (13.7%) Abdominal: 2 Bone: 2	0	–	

[α]*:Kruskal -Wallis Test*

Table 2. The thiol/disulphide homeostasis parameters, albumin and total protein levels of the groups

Parameters	Group 1	Group 2	Group 3	*p* value[α]
Total protein (g/dl)	7.7 ±0.4	7.5 ±0.3	7.9 ±0.4	**0.316**
Albumin (g/dl)	3.94 ±0.3	3.78 ±0.4	3.98 ±0.3	**0.223**
Native thiol (SH) (µmol/l)	303.46 ±48.5	258 ±49	424 ±35.6	< 0.001
Total thiol (µmol/l)	345.05 ±56.7	301.8 ±51.9	463 ±35.8	< 0.001
Disulphide (SS) (µmol/l)	19.63 ±7.19	21.93 ±6.02	19.78 ±9.8	**0.157**
Disulphide/native thiol	6.5 ±2.5	8.7 ±2.9	4.7 ±2.5	< 0.001
Disulphide/total thiol	5.6 ±2.1	7.3 ±2.1	4.2 ±2.04	< 0.001
Native thiol/total thiol	88 ±4.3	85.3 ±4.2	91.5 ±4.1	< 0.001

[α]*: Kruskal-Wallis Test*

were evaluated in this study. Age, PSA, free PSA values and TRUS-Bx results are available in Table 1. No statistically significant age difference was detected among the three groups ($p = 0.12$). A significant difference was detected in PSA and free PSA values among all groups ($p < 0.001$, $p < 0.001$, respectively).

Gleason score was reported as 6 in 10 among 29 patients with malign TRUS-Bx result, 7 in eight patients, 8 in three patients, 9 in five patients and 10 in two patients. Presence of metastatic disease was detected in four patients (13.7%) in the scanning tests made in patients with prostate cancer after biopsy.

Among the three groups, a statistically significant difference was detected among native thiol, total thiol levels and disulphide/total thiol, disulphide/native thiol and native thiol/total thiol ratios which are thiol/disulphide homeostasis parameters apart from disulphide ($p < 0.001$, $p < 0.001$, $p < 0.001$, $p < 0.001$, $p < 0.001$ respectively). Additionally, no significant difference was detected in albumin and total protein levels among the groups ($p = 0.223$, $p = 0.316$ respectively).

The thiol/disulphide homeostasis parameters (native thiol, total thiol, disulphide levels and disulphide/total thiol, disulphide/native thiol and native thiol/total thiol ratios), albumin and total protein levels of the groups are summarized in Table 2.

When subgroup analysis was made in malign and benign group (group 1 and group 2) patients, it was detected that native and total thiol levels were high in malign group compared to the benign group and disulphide level was low. But while high native and total thiol levels were significant, low disulphide levels were not found significant ($p = 0.003$, $p = 0.007$, $p = 0.265$, respectively).

When subgroup analysis was made in malign and control group (group 1 and group 3) patients, it was detected that native and total thiol and disulphide levels were low in malign group compared to the control group. But while low native and total thiol levels were significant, low

disulphide levels were not found significant ($p < 0.001$, $p < 0.001$, $p = 0.331$, respectively). Details on subgroup analyses among groups are available in Table 3.

No significant correlation was detected between thiol/disulphide homeostasis parameters and PSA and free PSA levels in the correlation analysis.

Table 3. Details on subgroup analyses on thiol/disulphide hemostasis

Parameters	Group 1/Group 2 *p* valueβ	Group 1/Group 3 *p* valueβ
Native thiol (SH) (μmol/l)	0.003	< 0.001
Total thiol (μmol/l)	0.007	< 0.001
Disülphide (SS) (μmol/l)	0.265	0.331
Disulphide/native thiol	0.007	0.001
Disulphide/total thiol	0.005	0.001
Native thiol/total thiol	0.042	< 0.001

β: *Mann Whitney U Test*

Discussion

Development of efficient therapies requires a better understanding of the mechanisms underlying prostate carcinogenesis. Numerous epidemiological, experimental and clinical studies have provided supportive evidence that oxidative stress is associated with the development of PCa [15-18].

Oxidative stress occurs following an increase in the reactive oxygen species (ROS) production and/or a simultaneous impairment of the antioxidative capacity of a cell [19]. PCa cells, in comparison to healthy cells, are characterized by innate oxidative stress, a hallmark of the aggressive phenotype of this disease [16, 17]. In PCa, increased ROS production caused by various processes leads to oxidative stress, including intrinsic conditions such as metabolic alterations, androgen receptor activation and mutation-induced mitochondrial dysfunctions, and also extrinsic environmental factors such as inflammation, xenobiotic metabolism and hypoxia [15].

ROS have very short half-lives and low concentrations that make their direct measurement very difficult in cells and tissues or body fluids. Therefore, to quantify the status and potential damage of oxidative stress, other indicators are used, such as biomarkers that measure tissue destruction (e.g., lipid peroxidation, protein and DNA damage) and the concentration of antioxidants in the blood [20]

Thiol metabolism and its role in oxidative stress have received recent attention as potential biomarkers of oxidative stress. Thiol groups of proteins are not just antioxidants buffers; they also regulate the redox system. Oxidative protein damage is characterized by an increase in protein carbonyl levels and a decrease in protein thiol levels. The decreased levels of thiol groups of proteins are associated with decreased serum antioxidant power [21-23]. So, thiol/disulphide homeostasis may already be regarded as an oxidative stress marker like lipid hydroperoxide, total antioxidant/oxidant status and paraoxonase. Therefore changes in thiol/disulphide balance caused by oxidative stress provide valuable information about various abnormal biochemical processes. 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 [10-14].

In Erel's study, while disulphide levels were found to be higher in inflammatory diseases such as obesity, smoking, pneumonia, diabetes mellitus and bronchiolitis compared to control group; in proliferative diseases such as renal cell carcinoma, colon carcinoma, urinary bladder carcinoma and multiple myeloma disulphide levels were found to be considerably low [10]. Furthermore, the lowest disulphide levels were observed in aggressively growing tumors, whereas in relatively slow growing tumors like renal carcinoma and basal cell carcinoma the decrease was observed to be in subnormal level [24].

In our study, thiol levels were found high in the patient group of patients reported to have prostate adenocarcinoma according to the biopsy result compared to the benign patient group and disulphide levels were low and thiol/disulphide hemostasis was towards the thiol side. This situation may be explained by the proliferative characteristic of PCa. Also it may be foreseen that the increase in thiol levels in PCa group occur to increase antioxidant capacity in response to the increased ROS activity. In the recent studies it was reported that disulphide levels should be lower in tumors with aggressive growth and are in subnormal levels in tumors growing very slowly [10, 24]. In fact, since prostate cancer has a slow progression, disulphide levels in our study were detected a little lower than the control group.

Various studies reported that the increase in disulfide level showed oxidative stress and the increase in native thiol level showed the anti-oxidant mechanism responding to ROS [10-14]. But disulphide levels in our study were found lower than the control and BPH group and the thiol levels showing the antioxidant response were found higher than the control and BPH group. We think that this probably didn't cause a significant or fast increase in disulphide levels due to the slow progress of prostate cancer. But depending on the anti-oxidant response occurring in the organism in response to the oxidative stress exposed in many years, we think that thiol disulphide hemostasis deteriorated and thiol levels in PCa patients increased significantly. Additionally disulphide levels in BPH patients were found higher than PCa patients. The reason for this condition may be the accompanying prostatitis presentation subclinically causing high PSA in most patients.

There is a limited number of studies investigating the effects of thiol-disulphide hemostasis on cancer patients [13, 24-27]. Hanikoğlu *et al*. investigated the difference in thiol/disulphide hemostasis in the 6th month before and after radical prostatectomy in prostate cancer patients. They found that although there is no statistically significant difference in the sixth month after radical prostatectomy, the thiol level increased and disulphide level decreased. This situation may be explained with the decrease in oxidative stress and increase in antioxidant activity in response to the treatment. In this study, it was detected that thiol and disulphide levels were higher in the healthy control group compared to the cancer group [25]. These values are in line with our study.

Abnormal thiol/disulphide homeostasis probably plays a role in the carcinogenesis process and tumor progression [26]. Güney *et al.* reported that although thiol and disulphide levels are significantly low in multiple myeloma patients compared to the control group, there was no difference in thiol/disulphide hemostasis among the groups [27]. Dirican *et al.* reported that thiol and disulphide levels were significantly low in non-small cell lung cancer patients compared to the control group. In this study, it was also predicted that the decrease in thiol and disulphide levels is related to the increase in ROS levels [13]. Demirseren *et al.* showed that thiol disulphide homeostasis in patients with basal cell carcinoma alters as the decrease of disulphide and the increase of thiol [24]. These results are in line with our results on prostate cancer with slow progression such as basal cell carcinoma.

Thiol and disulphide levels were quite different in the studies on cancer patients. We think that this difference may be due to the different area of cancer, different biology and pathogenesis of every cancer type, increase in intra-cellular ROS and the difference in apoptosis, transcription and cellular signal transfer mechanisms. No information is available on ideal thiol and disulphide levels in PCa patients. But according to this study, oxidative stress accompanied by damaged thiol/disulphide hemostasis towards thiol is present in patients detected to have prostate cancer.

This is the first study investigating the oxidative homestasis in histopathologically-proven prostate cancer and benign patient group. Sampling size is the biggest restriction of our study. Evaluation of other oxidant and antioxidant parameters was not included in this study as oxidant and anti-oxidant parameters were evaluated in prostate cancer patients in many studies. Thiol/disulphide homeostasis has other cellular functions such as cellular signal mechanisms, apoptosis and signal transfer. So we believe that further studies on wider sampling groups and prostate cancer groups divided into scores and stages are required.

Conclusions

In patients who had prostate biopsy, the difference between patients who had malign and benign biopsy result for thiol/disulphide homeostasis may show that the change in oxidative stress balance may have a role in prostate cancer. The role of oxidative stress which can be demonstrated through dynamic thiol/disulphide hemostasis in patients in whom prostate adenocarcinoma was detected with transrectal ultrasound guided prostate biopsy. This situation presents the role of oxidative stress in disease etiopathogenesis. Since prostate cancer is a rather heterogeneous disease, studies to be done with larger patient groups by dividing them into homogeneous sub-groups would be more informative on this subject.

Consent according to Helsinski declaration was taken from Necmettin Erbakan University Meram Faculty of Medicine ethics committee before the study (no. 2017/821).

The authors declare no conflicts of interest.

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