Role of native Thiol, total Thiol and dynamic Disulphide in diagnosis of patient with prostate cancer and prostatitis

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ABSTRACT

Background: Our study investigates whether Native Thiol, Total Thiol and disulphide levels measured in serum of patients with prostate cancer and prostatitis and of healthy subjects, have any role in differential diagnosis.

Materials and Methods: Patients followed up for histopathologically verified diagnosis of prostate cancer and prostatitis in 2016-2017 at the Medicalpark Gaziantep Hospital Urology Clinic were included in the study. Native Thiol (NT), Total Thiol (TT), Dynamic Disulphide (DD) levels in serum were measured by a novel automated method.

Results: NT, TT, DD, NT / TT ratios, DD / TT ratio and DD / NT ratio were measured as 118.4 ± 36.8μmol / L, 150.3 ± 45.3μmol / L, 15.9 ± 7μmol / L, 78.8 ± 7μmol / L, 10.5 ± 3.5μmol / L, 13.8 ± 5.8μmol / L respectively in patients with prostate cancer; as 116.4 ± 40.5μmol / L, 147.5 ± 50.1μmol / L, 15.5 ± 8.7μmol / L, 79.7 ± 9μmol / L, 10.1 ± 4.5μmol / L, 13.5 ± 7.2μmol / L in patients with prostatitis and as 144.1 ± 21.2μmol / L, 191 ± 32.3μmol / L, 23.4 ± 10.1μmol / L, 76.1 ± 98.3μmol / L, 11.9 ± 4.1μmol / L, 16.4 ± 6.9μmol / L in healthy subjects. Significant difference was detected between groups of NT, TT and DD levels (p = 0.008, p = 0.001, p = 0.002). No significant difference was detected in terms of the NT / TT, DD / TT and DD / NT rates (p = 0.222, p = 0.222, p = 0.222).

Conclusions: Serum NT, TT, DD levels in patients with prostatitis and prostate cancer were found significantly lower compared to the control group. This indicates that just as inflammation, prostate cancer also increases oxidative stress on tissues.

INTRODUCTION

Prostate cancer is the second most prevalent type of cancer in men and is the second most prevalent cause of death from cancer in men. Past studies have shown the important role of age, genetic predisposition, androgen hormones, diet-related factors, inflammation and oxidative stress in the development of this disease (1). It was reported that oxidative stress can play a significant role in the development of prostate cancer through lipid per-oxidation and similar mechanisms (2).
It was reported that oxidative stress resulted from the disruption of the balance of anti-oxidants and reactive oxygen radicals and that this caused various systemic diseases. Over-production of reactive oxygen types (ROS) causes damage in proteins and lipids. Oxidative damage is irreversible in serum and tissue proteins and significant changes occur in the structure and activity of proteins and biomolecules. Oxidative modifications in DNA and proteins can impact certain cellular functions, resulting in cell death, death or mutation and carcinogenesis formation.

Thiols are essential and strong anti-oxidant molecules in the sulfhydryl group, consisting of hydrogen atom and sulfur atom bonded to a carbon atom. The disulfide bond in their structure is a covalent bond and is also named as SS-bond or disulfide bridge. They play an important role in protecting oxidant stress from harmful effects. The leading thiols found in plasma are low molecule-weight thiols including albumin thiols, protein thiols and cysteine, cysteinylglycine, glutathione, homocysteine and γ-glutamylcysteine. The thiol groups are oxidized with disulfide bonds getting reversibly oxidized by ROS. This mechanism mediates their anti-oxidant effects. The created disulfide bonds can again be reduced to thiol groups. Dynamic thiol-disulfide homeostasis plays an important role in anti-oxidant defense, detoxification, apoptosis, arranging enzymatic activities, and cellular signal transmission.

When oxidative stress occurs, it has been noted that reduced thiol concentration increases and disulfide values increase in correlation. The study was conducted on patients his-topathologically diagnosed with prostate cancer or prostatitis and followed up by the Gaziantep Medicalpark Hospital Urology Clinic in 2014-2017 and the results were published in a prostatic biopsy indication in recent years. Increased levels of serum PSA are associated not only with cancer but also with bacterial prostatitis, prostatic inflammation, benign prostate hyper trophy and urinary system infection (8).

Prostatitis is a disease that is observed at a rate of 8% (12.3-2.7%) in men. Acute bacterial prostatitis (ABP) is a pyogenic urinary system infection of the urinary system. It is observed at a rate of 5% among general prostatitises (8), Escherichia coli is the most frequent cause of acute bacterial prostatitis. Enterococci, Proteus, Pseudomonas, Klebsiella and Serratia are factors less frequently responsible. ABP can cause urinary retention causing edema in the prostate. It can also cause serious complications from prostate abscess to urosepsis (8). Its treatment is usually performed according to clinical symptoms. Parenteral anti-biotics and hydration are performed in the early stage. Catheter and drainage are implemented if unable to urinate. Serum PSA values are usually high in ABP (9). Additionally, high levels of erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) and white blood cell count in full blood count accompany the situation.

In our study, serum t PSA level, TT, DD levels were measured in patients diagnosed with prostate cancer and in healthy subjects at the Medicalpark Gaziantep Hospital Urology Clinic and Dişileşik / NT, Disilphide / TT, NT / TT ratios were calculated to find Dynamic Thiol / Disulphide homeostasis levels for the purpose of investigating whether it has predictive value in differentiating Prostatitis-Prostate Cancer and diagnosing Prostate Cancer and its value in predicting prognosis.

MATERIAL AND METHODS

Patient Selection

The study was conducted on patients his-topathologically diagnosed with prostate cancer or prostatitis and followed up by the Gaziantep Medicalpark Hospital Urology Clinic in 2014-2017 and on healthy subjects compatible with the patients in terms of age. Patients previously treated and with metastasis, persons who were smoking and/or using alcohol, had chronic disease, acute or chronic infection or using anti-hyperlipidemic, antibiotic or anti-oxidant drugs were excluded from the study. We have taken informed consent form from the patients and healthy subjects to participate on the study. Socio-demographic characteristics, known diseases, medical history information such as personal and family history characteristics, used drugs etc., data such as routine laboratory tests were obtained retrospectively and recorded.

Sampling and Measurement of NT, TT, DD

Blood samples of the patients were collected prior to starting the medication. Non-anticoagulant venous bloods of the subjects taken into tubes after 12 hours of fasting were centrifuged at 3500 rpm for 30 minutes during the first 2 hours and then split into Eppendorf Microtubes and stored at -80°C. These samples were kept at 4°C temperature one night before measurement and put into room temperature 2 hours prior to the study and then the samples were mixed with a vortex and measurement was performed twice for each sample.

A novel automated assay method was used to measure dynamic thiol / disulphide homeostasis. The principle of this method is based on reducing the disulfide bonds of proteins to compose disulfides in oxidative medium. Sodium borohydride (NaBH4) is added for reduction of disulfide bonds into thiol groups again. The remaining NaBH4 and DTNB (5,5'-dithio-bis (2-nitrobenzoic acid) are removed by formaldehyde. In this way, the amount of native and reduced thiol groups are de-fined separately. The difference between the TT and the NT is divided by two to calculate the quantity of the DD bonds. Also, we calculate the NT, TT, DD / TT, NT / TT and DD / NT percent ratio.

Measurement of serum t PSA levels were conducted using the E170 device (Roche Diagnostics GmbH, Germany). Patients were classified into risk groups according to Gleason score and t PSA values. Split into risk groups was as follows: low risk prostate cancer: T1-T2a stage and Gleason score ≤ 6 and t PSA ≤ 10, moderate risk prostate cancer: T2b stage and the Gleason score ≤ 7 and ≤ 10 or t PSA ≤ 20 and high-risk prostate cancer: ≥ T2c stage or Gleason score 8-10 or t PSA ≥ 20 (11).

Statistical analysis

Statistical analyses were performed using the SPSS for Windows 15.0 package software. Comparison of the various to normal distribution was examined using visual (histogram and probability graphs) and analytic methods (Kolmogorov-Smirnov / Shapiro-Wilk tests). In the Kolmogorov-Smirnov test cases with p value greater than 0.05 were accepted as normal distribution. Differences between prostate cancer, prostatitis and the control groups in terms of NT, TT and DD were computed using the unilateral ANOVA test, while these variables showed normal distribution. The homogeneity of the variations was evaluated using the Levene test. Cases where the p value was lower than 0.05 were evaluated as statistically significant results. In cases with significant differen-ces between the groups, post-hoc pair comparisons were performed using the Tukey’s Test. NT / TT, DD / TT and DD / NT ratios were detected to not show normal distribution. The differences between these variables between prostate cancer, prostatitis and control groups were compared using the Kruskal-Wallis test. Pair comparisons were performed using the Mann-Whitney U test and evaluated using the Bonferroni correction. Total type-1 error level was used as 5% for statistical significance.

RESULTS

A total of 80 subjects were included in the study, consisting of 30 (37.5%) prostatitis patients, 25 (31.3%) prostate cancer patients and 25 (31.3%) healthy subjects. Patients diagnosed with prostatitis had a mean age of 60.5 ± 12.8 (range 31-83). Patients diagnosed with prostate cancer had a mean age of 70.6 ± 6 (range 58-82). The age difference between the two groups was evaluated using the Student t test as they had normal distribution. Statistically significant difference was detected between the two groups in terms of age (p = 0.001). Patients diagnosed with prostatitis consisted of younger patients.

Total Prostate Specific Antigen was detected as 139 ± 257.8 (range 4-1200) in patients with prostatitis and as 51.3 ± 112 (range 3.9-405) in pa-


In our study we considered that serum NT, TT and DD levels could be a good marker in differentiating patients with high PSA values and normal patients. In this study we aimed to show the change in thiol/disulfide values in two different diseases with acute and chronic progression occurring on the same tissue.

Many studies have been conducted on oxidative stress in urological patients regarding prostate cancer, benign prostatic hyperplasia or prostate inflammation (12-14). Some studies investigated oxidative markers in semen and urine (15). However,
there are very few studies regarding thiol / disulphide homeostasis in the prostate tissue (17). Normal oxidative stress markers have been used for a very long time. However, using the current method developed by Dr. Erel and Dr. Neselioglu, plasma dynamic thiol / disulphide homeostasis can be measured with faster, more inexpensive, practical and fully-automatic spectrophotometric examination (10).

Many recent studies have shown disorder in plasma thiol / disulphide homeostasis in the etiopathogenesis of diseases such as diabetes mellitus, cardiovascular disease, cancer, inflammatory diseases, Parkinson’s disease and lung diseases (18-21). Therefore, determining dynamic thiol / disulphide homeostasis can provide many valuable information to detect the physiologic and pathologic biochemical process of many diseases. In their study, Erel et al. detected high plasma disulphide values in patients who were smoking, had diabetes, obesity and pneumonia and detected low values in patients with diseases such as bladder cancer, colon cancer, kidney cancer and multiple myeloma. Disulphide values were detected as very low in rapidly growing tumors while as slightly lower than normal in slow-progressing diseases (10). In some studies, it was detected that DD / DT ratios had positive correlation with age while in some studies it was shown to have negative correlation (22, 23). In our study, a clear evaluation was not made on correlation with age because age did not have normal distribution between the groups. Studies have shown that oxidative stress biomarkers such as thiobarbituric acid reactive substances, total oxidative status, malondialdehyde, plasma nitrite / nitrate levels, lipid peroxide activities increased in prostate cancer patients compared to the control group (24). Some studies have reported decrease in antioxidant enzymes such as catalase, superoxide dismutase containing manganese, superoxide dismutase containing copper and zinc, glutathione peroxidase, disrupted oxidative stress / antioxidant status in prostate cancer patients (25). In another study it was determined that prostate cancer progression and oxidative stress had positive correlation and shown that anti-oxidants such as vitamin E and selenium reduced this risk (26). Thiol / disulfide homeostasis and thiol oxidation have critical importance in protecting cells against detoxification, arranging enzymes and important cellular pathways such as proapoptotic, signal transmission and antiapoptotic signalization (27).

PSA is the most commonly used marker in prostate cancer diagnosis. PSA value, tumor volume and Gleason score are the most important prognostic factors in the course of prostate cancer. High PSA values were present in both groups in our study. PSA level increases rapidly in the event of acute bacterial prostatitis. However, blood values such as CRP, ESR and White Blood Cell also rise.

Prostate cancer is a chronic disease (except for high-risk prostate cancer). PSA values usually rise slowly and can also be detected at extremely high values depending on tumor aggressiveness. In this study, we aimed to show the change in thiol / disulphide values in two different diseases with acute and chronic progression occurring on the same tissue. According to our findings, NT, TT and disulphide values were found to be significantly different in both prostatitis and the prostate cancer compared to the control group. However, a statistically significant difference was not observed in thiol values depending on tumor aggressiveness. In our study, a clear evaluation was not made on correlation with age because age did not have normal distribution between the groups. Studies have shown that oxidative stress process continues in cancer patients unless treated. In inflammation, the oxidative process returns to normal after the causative infectious event is remedied. Thios, which are an anti-oxidant structure in the serum, may have decreased upon exposure to severe oxidative stress because anti-oxidant defense weakens or oxidation increases in prostate cancer or prostatitis patients. Oxidation products more progressed than disulphides were formed as thios were subject to severe oxidation. As these are usually products of irreversible thiol oxidation, it is considered that disulphides are also low (10).

Earlier studies have not shown the relation between tPSA level and thiol / disulphide values. In our study tPSA values and thiol/disulphide values were compared in both groups. However, although tPSA values were high in both groups, it was detected to be statistically higher in the prostate cancer group. However, no difference was detected between both groups in Thiol / Disulfide ratios. High tPSA values are a direct indicator of prostate tissue damage.

The main limitations of the present study are its retrospective and non-randomized nature. In addition, the number of patients involved is small. These results need to be supported by prospective, randomized studies, and comprehensive patient series.

CONCLUSIONS

Calculating thiol / disulphide values, which is a new marker, is an easy, inexpensive and reliable method. Serum NE TT DD levels in patients with prostatitis and prostate cancer were found significantly lower compared to the control group. This shows that just as inflammation, prostate cancer also increases oxidative stress on tissues. We consider that NT, TT, DD levels measures in serum can be used in the differential diagnosis of these pathologies.

ABBREVIATIONS

NT = Native Thiol
TT = Total Thiol
DD = Dynamic Disulphide
ROT = Reactive Oxygen Types
PSA = Prostate Specific Antigen
tPSA = Total Prostate Specific Antigen
ABP = Acute Bacterial Prostatitis
ESR = Erythrocyte Sedimentation Rate
CRP = C-Reactive Protein
NaBH4 = Sodium Borohydride
DTNB = 5,5′-dithio-bis (2-nitrobenzoic acid)

REFERENCES


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