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Original Research

Role of Serum HMGB1 in Prostate Cancer

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ABSTRACT

Purpose: In our study the diagnostic role of HMGB1 levels measured in serum were investigated in prostatitis and prostate carcinoma diagnosis and in the differential diagnosis of these two diseases.

Material Method: Patients followed up for histopathologically verified diagnosis of prostate carcinoma and prostatitis in 2014-2017 at the Urology Clinic were included. HMGB1 measurement in serum was performed with the ELISA method.

Results: A total of 78 subjects were included in the study, consisting of 30 (38.5%) prostatitis patients, 25 (32%) prostate carcinoma patients and 23 (29.5%) healthy subjects. HMGB1 was detected as 11.9±2.6 (Range 6.7-18.4) ng/ml in the prostatitis group, 15.1±4.5 (Range 8.4-24.8) ng/ml in the prostate carcinoma patients and as 9.2±3.1 (Range 4.7-18.7) ng/ml in the control group. The difference between the groups were investigated using the Friedman test as HMGB1 did not show normal distribution. Significant difference was detected between the three groups ($p<0.001$). When the groups were compared in pair, significant difference was detected between the prostatitis group and the control group ($p=0.001$). Significant difference was again detected between the prostate carcinoma group and the control group ($p<0.001$). Significant difference was detected between the prostatitis group and the prostate carcinoma group ($p=0.006$). Measurement of serum total prostate specific antigen (tPSA) levels were conducted automatically with the electro chemiluminescent method. A moderate level of ($r=0.276$) but a highly significant ($p=0.009$) positive correlation was found between PSA and HMGB1.

Conclusion: In our study we showed that high PSA and high HMGB1 were highly correlated. HMGB1 measured in serum could be a useful marker in the differentiation of prostatitis and prostate carcinoma, in the early diagnosis of suspected prostate carcinoma and that HMGB1 value was significantly high in prostate carcinoma patients.

Keywords: Diagnosis, HMGB1, prostate carcinoma, prostatitis, PSA

INTRODUCTION

Prostate carcinoma is the most frequently seen cancer in men in the USA (1). Early stage prostate carcinoma can be cured with radical surgery or definitive radiotherapy. Despite these treatments local or remote relapse can occur in the patients. 10-20% of prostate carcinoma patients are presented with metastatic disease (2). Therefore, early diagnosis of these patients is important.

Prostate specific antigen (PSA) has an important role in prostate carcinoma diagnosis. Abnormal digital rectal examination findings and high serum PSA levels are the most important indicators that cause prostate biopsy indication (3). Increased levels of serum PSA are associated with carcinoma, bacterial prostatitis, prostatic inflammation, benign prostate hyperplasia (BPH) and urinary system infection.

Prostatitis is observed at a rate of 8.2% in men. Acute bacterial prostatitis (ABP) is a pyogenic infection of the urinary system and is seen at a rate of 5% among overall prostatitis patients (4). ABP is most frequently caused by *Escherichia coli; Entero-*

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coccus, Proteus, Pseudomonas, Klebsiella and *Serratia* organisms also cause it less frequently. Prostatitis can cause oedema in the prostate and result in urinary retention and may cause serious complications (5). In early stage treatment, parenteral antibiotics (such as ceftriaxone, aminoglycosides, fluoroquinolone) are given and if the patient cannot perform intravenous hydration and urination then drainage is performed with catheter. PSA values are usually high in case of ABP. Sometimes unexpected high values are detected. This may lead to unnecessary prostate biopsies.

High mobility group box (HMGB) are non-histone nuclear proteins with many functions in the cell. While the expression of HMGB3 and HMGB2 is limited to certain periods of life and to certain cells, the expression of HMGB1 is very prevalent and continues in adulthood (6). HMGB1 acts as a chromatin binder factor. It binds to the small groove of the DNA and modifies the interaction with the DNA of certain transcription factors including p53 and steroid hormone receptors. It plays a role in DNA repair, transcription, differentiation, extra-cellular signalization and somatic recombination. Various strategies, such as HMGB1-receptor antagonists, inhibitors of its signalling pathway, antibodies, RNA inhibitors, vagus nerve stimulation etc. have been used to inhibit expression, release or activity of HMGB1 (7). In addition to these nuclear functions, it also functions as an extra-cellular signaling molecule by being passively secreted from necrosis cells and actively secreted from cells playing a role in inflammation (8).

In our study the diagnostic role of HMGB1 levels measured in serum were investigated in prostatitis and prostate carcinoma diagnosis and in the differential diagnosis of these two diseases and the relation between HMGB1 and PSA were examined.

PATIENTS AND METHOD

Patient Selection

Patients diagnosed with prostate cancer and prostatitis between 2014-2017 in the Urology Clinic were included in the study. Written consent was obtained from the patients. Patient files were screened and information such as age, gender and routine laboratory tests were retrospectively obtained. Patients were divided into risk groups according to Gleason score and PSA levels. Low-risk prostate cancer: T1-T2a stage and Gleason score ≤6 and PSA ≤10, moderate risk prostate cancer: T2b stage and / or Gleason score = 7 and 10≤PSA≤ 20 and high-risk prostate cancer: ≥ T2c stage or Gleason score 8-10 or PSA> 20 **(9).**

Sampling

Blood samples remaining from the blood samples of patients for routine checks were collected prospectively directly before the start of first-line systemic chemotherapy. They were centrifuged for 15 min at 1,000g within 1 hr of collection. The resulting sera were aliquoted into microtubes and either immediately frozen at -80 °C. These samples were placed into a refrigerator at 4 °C one night before the measurements. Serum samples were kept at room temperature for 2 hours before operating with the ELISA method. The samples were then mixed using vortex and measurement procedures were applied.

HMGB1 and PSA Measurement

HMGB1 serum levels were measured using Rel Assay Brand commercial kits and by complying with manufacturer's instructions (Rel Assay Diagnostics® Mega Tıp Ltd, Turkey). Analysis operations were performed by using sandwich enzyme immunoassay technique and by repeating twice for each sample. All concentration/absorption graphic curves of the test and calculations regarding the results were performed on the program of the Biotek_ELx808 (Winooski, Vermont, USA) device. The test was determined to have a sensitivity of 0.06 ng / mL and detection range of 1-32 ng / mL. Intra-assay and inter-assay variation coefficients were determined as 5.7% and 6.3% respectively. Serum total prostate specific antigen (tPSA) values were measured automatically with electrochemiluminescent method by using the Hitachi Modular Analytics E 170 device (Roche Diagnostics GmbH, Germany).

Statistical Analysis

Statistical analyses were performed using the SPSS for Windows 15.0 package software. Compliance of the variables to normal distribution was examined using visual (histogram and probability graphs) and analytic methods (Kolmogorov-Smirnov/Shapiro-Wilk tests). In the Kolmogorov-Simirnov test, cases with p value greater than 0.05 were accepted as normal distribution. Differences between the two groups were tested using the student t test as normal distribution was observed. Differences between the prostatitis, prostate carcinoma patients and the control group were examined using the Friedman test. The patient and control group were compared using the Mann-Whitney U test when normal distribution was not observed. The difference between the groups were investigated using the Kruskal-Wallis test when the variable did not show normal distribution in more than two independent groups. Pair comparisons were made using Mann-Whitney U test and evaluated by using Bonferroni correction. The relation between the HMGB1 and PSA measurements were evaluated with the spearman test. Total type-1 error level was used as 5% for statistical significance.

RESULTS

The study was completed with a total of 78 patients. In these patients, 30 (38.5%) patients had prostatitis, 25 (32%) patients had prostate cancer, 23 (29.5%) patients had healthy volunteers.

The mean age of patients with prostatitis was 60.9±11.2 whereas the mean age of patients with prostate carcinoma was 70.2±6.3. The difference between the two groups in terms of age was statistically significant (p=0.001) (Table 1).

PSA was detected as 45.2±102 (4.9-385) in the prostatitis group and as 141.2±222.7 (Range 6-1200) in the prostate carcinoma group. The difference between the two groups in terms of PSA was evaluated by using the Mann-Whitney U test as the PSA variable did not have a normal distribution. Statistically significant difference was observed between the two groups in terms of PSA levels (p=0.02). PSA value was found to be higher in patients diagnosed with prostate cancer with respect to patients diagnosed with prostatitis (Table 2).

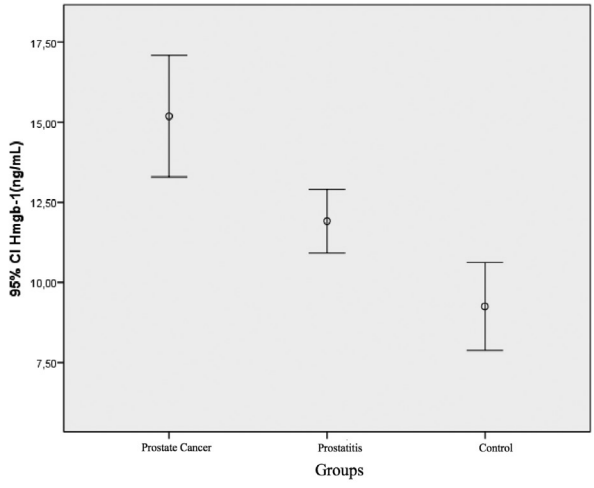
Table 1. Comparison of ages and serum t PSA levels

	Prostate Cancer (n:25)	Prostatitis (n:30)	Control Group (n:23)	P Value
Age(years) Mean±SD (min–max)	70.2±6.3 (55–80)	60.9±11.2 (34–78)	52.3±22.1 (44–76)	0.001
t PSA(ng/ml) Mean±SD (min–max)	141.2±222.7 (6–1200)	45.2±102 (4.9–385)	1.1±3.8 (0.6–3.3)	0.001

Table 2. HMGB 1 levels of groups

	Prostatitis (n:30)	Prostate Cancer (n:25)	Control Group (n:23)
HMGB1 ng/ml Mean±SD (min–max)	11.9±2.6 (6.7–18.4)	15.1±4.5 (8.4–24.85)	9.2±3.1 (4.7–18.7)

Figure 1. Differences between groups



HMGB1 was observed as 11.9±2.6 (Range 6.7-18.4) ng/ml in the prostatitis group, 15.1±4.5 (Range 8.4-24.8) ng/ml in the prostate cancer patients and as 9.2±3.1 (Range 4.7-18.7) ng/ml in the control group. The difference between the groups were investigated by using the Friedman test as HMGB1 did not show normal distribution. Significant difference was found between the groups (p<0.001) (Figure 1). When the groups were compared in pair, significant difference was identified between the prostatitis group and the control group (p=0.001). Significant difference was again observed between the prostate cancer group and the control group (p<0.001). Significant difference was observed between the prostatitis group and the prostate carcinoma group (p=0.006).

The correlation between PSA and HMGB1 was investigated by using the spearman test as the variables did not have normal distribution. A moderate level of (r=0.276) but a highly significant (p=0.009) positive correlation was found between PSA and HMGB1.

When the patients with prostate carcinoma were evaluated according to Gleason score and PSA, 3 (12%) had low risk, 8 (32%) had moderate risk and 14 (56%) had high risk. The relation between the HMGB1 and prostate cancer risk groups was investigated by using the Kruskal-Wallis test. Significant difference was not observed between the three groups (p<0.352).

DISCUSSION

PSA is the most frequently used biochemical marker in prostate carcinoma diagnosis. PSA levels are used in both treatment evaluation and in follow-up (10). Serum PSA levels can be detected at highly varying levels in bacterial prostatitis patients. Many studies have shown that PSA levels returned to normal after treatment of prostatitis (11). In our study we have shown that HMGB1 levels measured in serum can play a role in the differential diagnosis of prostate carcinoma and prostatitis.

High mobility group box (HMGB) are non-histone nuclear proteins with many different functions in the cell. HMGB proteins were first purified from the nucleus in the 1970s. They were named as such due to their fast movement in the sodium dodecyl sulfate polyacrylamide gel electrophoresis when they were first discovered (6). HMGB1, HMGB2 and HMGB3 are members of the HMGB protein family. HMGB2 and HMGB3 have limited expression while HMGB1 has prevalent expression and its expression can be regulated with environmental factors. HMGB2 is expressed at high ratios during embryogenesis and in contrast with HMGB1, its expression in adulthood is limited to the lymphoid organs and the testis. The common and differential functions of HMGB1 and HMGB2 proteins with reference to pathological processes, with a special focus on cancer (12). HMGB1 binds to the small groove of the DNA and modifies the interaction with the DNA of certain transcription factors including p53 and steroid hormone receptors. It plays a role in DNA repair, transcription, cellular differentiation, extra-cellular signalization and somatic recombination. HMGB-1 is considered as an essential facilitator in diseases such as sepsis, collagen disease, atherosclerosis, cancers, arthritis, acute lung injury, epilepsy, myocardial infarction, and local and systemic inflammation. Modulation of HMGB1 levels in the human body provides a way in the management of these diseases. Various strategies, such as HMGB1-receptor antagonists, inhibitors of its signalling pathway, antibodies, RNA inhibitors, vagus nerve stimulation etc. have been used to inhibit expression, release or activity of HMGB1 (7). In addition to these nuclear functions, it also functions as a signal molecule and acts as an extra-cellular signal molecule in inflammation, cellular differentiation, cell migration and tumor metastasis (13). HMGB1 has high bonding affinity to certain receptors by being passively secreted from necrotic cells and actively secreted from

inflammatory cells. These include RAGE (receptor for advanced glycation end products) and Toll-like receptors (TLR)-2, TLR-4, TLR-9 (14).

The relation between prostate carcinoma and HMGB1 expression was investigated in many studies. Li et al. showed both HMGB1 mRNA and protein expression with their polymerase chain reaction and western blotting method in carcinoma cell cultures in prostate (15). Using immunohistochemical (IHC) method they showed HMGB1 expression in tumor tissue cell samples of 168 prostate carcinoma patients obtained with prostatectomy. In their studies they detected that HMGB1 protein expression was high in tissue samples and that this high level was correlated with both the Gleason score and the pre-operative PSA concentration.

In their studies Gnanasekar et al. investigated the role of HMGB1 in the development of prostate carcinoma (16). In this study they showed that HMGB1 had an important role in the expression and upregulation of androgynous receptors in prostate carcinoma patients. In their studies Zhang et al. reported that HMGB1 was highly expressed in metastatic prostate cancer samples and able to enhance the aggressiveness of PC3 cells. HMGB1 promotes the EMT process and upregulates the expression levels of MMP-1, -3 and -10 by activating the RAGE/NF-κB signaling pathway in PC3 cells, thereby facilitating cancer metastasis. (17).

These studies strongly indicate that HMGB1 could have an important role in the progress of prostate carcinoma. Zhao et al. on the other hand investigated HMGB1 and RAGE expression using IHC in tissue samples taken after prostatectomy from 85 prostate cancer patients (18). In this study they could not show the relation of both HMGB1 and RAGE expression with Gleason score. However, they asserted that HMGB1 expression was a poor prognostic marker and could be used as a new prognostic marker for prostate carcinoma. In our study we could not show the relation between HMGB1 levels measured in serum and risk groups that mostly guide for prostate carcinoma treatment.

There are few studies investigating HMGB1 expression in prostatitis patients. Xue et al. compared HMGB1 expression using IHC method in tissue samples of BPH patients with prostatitis and BPH patients without prostatitis (19). In this study they detected HMGB1 extraction to be at a higher ratio in BPH patient.

The limitations of our study is that the number of samples was small and the study was conducted retrospectively.

In our study we showed that high PSA and high HMGB1 levels measured in serum were highly correlated and that HMGB1 value was apparently higher in prostate carcinoma patients. We consider that HMGB1 measured in serum could be a useful marker in the differentiation of prostatitis and prostate carcinoma, in the early diagnosis of suspected prostate carcinoma cases.

Abbreviations
HMGB1**:** High mobility group box-1
PSA: Prostate specific antigene
ABP: Acute bacterial prostatitis
DNA: Deoxyribonucleic acid
RAGE: Receptor for advanced glycation end products
TLR-2: Toll-like receptors-2
TLR-4: Toll-like receptors-4
TLR-9: Toll-like receptors-9
mRNA: mesenger ribonucleicacid
IHC: Immunohistochemical
BPH: Bening prostate hyperplasia

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Local Ethical Committee (04/29.03.2018).

Informed Consent: Written informed consent was obtained from patients who participated in this study.

Peer-review: Externally peer-reviewed.

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