European Journal of Therapeutics

DOI: 10.5152/EurJTher.2019.190052

Original Research

Role of Serum HMGB1 in Prostate Cancer

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ABSTRACT

Objective: 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.

Methods: Patients followed up for histopathologically verified diagnosis of prostate carcinoma and prostatitis in 2014-2017 at the Medicalpark Hospital Urology Clinic were included. HMGB1 measure-ment 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 pros-tate 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 distri-bution. 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 an-tigen (tPSA) levels were conducted automatically with the electro chemiluminescent method. A mod-erate 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 carcino-ma, 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 prevalent type of cancer affecting men in the USA (1). Early stage prostate carcinoma can be treated with radical surgery or definitive radiotherapy. Despite these treatments, local or remote relapse can occur in the patients. Approximately 10%-20% of patients with prostate carcinoma are estimated to present metastatic disease (2). Therefore, early diagnosis of prostate carcinoma in men is essential.

Prostate specific antigen (PSA) is crucial for the diagnosis of prostate carcinoma. Findings of abnormal digital rectal examination and high serum PSA levels are the most important indicators of prostate carcinoma (3). Increased serum PSA levels are associated with carcinoma, bacterial prostatitis, prostatic inflammation, benign prostate hyperplasia (BPH), and urinary system infection.

The prevalence of prostatitis is 8.2% in men, and the prevalence of acute bacterial prostatitis (ABP), a pyogenic infection of the urinary system, is 5% in patients with prostatitis (4). ABP is frequently caused by microorganisms such as *Escherichia coli, Enterococcus, Proteus, Pseudomonas,* and *Klebsiella*. Organisms such as *Serratia* also cause prostatitis, though less frequently. Prostatitis can cause edema in the prostate, leading to urinary retention which may cause serious complications (5). In the early stage of

How to cite: Solakhan M, Çiçek H, Benlier N, Yıldırım Z, Sever ÖN, Orhan N, et al. Role of Serum HMGB1 in Prostate Cancer. Eur J Ther 2020; 26(2): 107–10.

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Received: 30.05.2019 • **Accepted:** 19.08.2019

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treatment, parenteral antibiotics such as ceftriaxone, aminoglycosides, and fluoroquinolone are administered and, if the patient cannot perform intravenous hydration and urination, drainage is performed with catheter. PSA values are usually high in patients with ABP and, sometimes, unexpected high values are detected, leading to unnecessary prostate biopsies.

High mobility group box (HMGB) are non-histone nuclear proteins with several 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 highly prevalent and continues in adulthood (6). HMGB1 acts as a chromatin binder factor. It binds to the small groove of DNA, thereby modifying the interaction with DNA of certain transcription factors including p53 and steroid hormone receptors. It plays a vital role in mechanisms such as DNA repair, transcription, differentiation, extracellular signaling, and somatic recombination. Different agents including HMGB1 receptor antagonist, HMGB1 signaling inhibitors, HMGB1 RNA inhibitors, antibodies, vagus nerve stimulators, among others, have been used for the inhibition of the expression and activity of HMGB1 (7). In addition to its nuclear functions, it acts an extracellular signaling molecule through passive and active secretion by necrotic and normal cells, respectively, playing an important role in inflammation.

In this study, we aimed to investigate the diagnostic role of serum HMGB1 level in prostatitis and prostate carcinoma, its differential diagnosis, and the relationship between HMGB1 and PSA.

METHODS

Patient Selection

Patients diagnosed with prostate cancer and prostatitis between 2014 and 2017 at the Sanko University Hospital Urology Clinic were included in the study. Written consent was obtained from all patients included in the study. Patient files were screened, and information such as age, gender, and routine laboratory tests were retrospectively collected. Patients were categorized according to Gleason score and PSA levels in the following groups: 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 of patients were collected for routine checks before initiating the first-line systemic chemotherapy. The blood samples were centrifuged for 15 min at 1.000 g within 1 h of sample collection. The resulting sera were transferred as aliquots into microtubes and immediately preserved at -80°C. The samples were transferred to a refrigerator (4°C) the night before the measurements were to be performed. Serum samples were kept at room temperature for 2 h before being analyzed by the enzyme-linked immunosorbent assay method. The samples were vortexed, and measurement procedures were appropriately followed.

Main Points:

- The diagnostic role of HMGB-1 in prostate cancer.
- Relationship between HMGB1 and cancer aggressiveness.
- The distinctive role of HMGB between inflammation and cancer.
- A new marker in prostate cancer.

HMGB1 and PSA Measurement

The serum HMGB1 levels were measured using Rel Assay commercial kits (Rel Assay Diagnostics® Mega Tıp Ltd, Turkey) as per the manufacturer's instructions. Sandwich enzyme immunoassay technique was employed, and each sample was analyzed twice. Standard curves (concentration *vs.* absorbance) and calculations were performed using Biotek_ELx808 device (Winooski, Vermont, USA). The sensitivity of the test was 0.06 ng/mL, with a detection range of 1–32 ng/mL. Intra- and inter-assay variation coefficients were estimated to be 5.7% and 6.3% respectively. The electrochemiluminescent method was used to automatically measure serum total PSA values using Hitachi Modular Analytics E 170 device (Roche Diagnostics GmbH, Germany).

Statistical Analysis

Statistical analysis was performed with Statistical Package for the Social Sciences for Windows 15.0 software package (SPSS Inc., Chicago, ILL, USA The variables were investigated using visual (histogram and probability graphs) and analytical methods (Kolmogorov–Smirnov/Shapiro–Wilk test) to determine whether or not they are normally distributed. In the Kolmogorov–Smirnov test, a p value greater than 0.05 indicates normal distribution. Because normal distribution was observed, differences between the two groups were analyzed by paired student's t-test. Differences between patients with prostatitis or prostate carcinoma and the healthy individuals were analyzed by the Friedman test. The patient and healthy individual groups were compared using the Mann-Whitney U test when variables were not normally distributed. The difference between the groups was investigated using the Kruskal–Wallis test when the variables did not show normal distribution in more than two independent groups. Pairwise comparisons were made by the Mann–Whitney U test and evaluated using the Bonferroni correction. The relationship between HMGB1 and PSA measurements were evaluated by Spearman's correlation. Total type-1 error was set at 5% as the level of statistical significance.

RESULTS

A total of 78 patients participated in this study. Among these patients, 30 (38.5%) had prostatitis, 25 (32%) had prostate cancer, and 23 (29.5%) were healthy volunteers. The mean age of patients with prostatitis was 60.9±11.2, whereas that of patients with prostate carcinoma 70.2±6.3. The age difference between the two groups was statistically significant (p=0.001) (Table 1).

Mean PSA detected was 45.2±102 (range: 4.9-385) in the prostatitis group and 141.2±222.7 (range: 6-1200) in the prostate carcinoma group. The difference between the PSA of the two groups was evaluated by the Mann–Whitney U test because the PSA variable was not normally distributed. A significant difference in PSA levels was observed between the two groups (p=0.02). PSA level was found to be higher in patients diagnosed with prostate cancer in comparison with those diagnosed with prostatitis (Table 2).

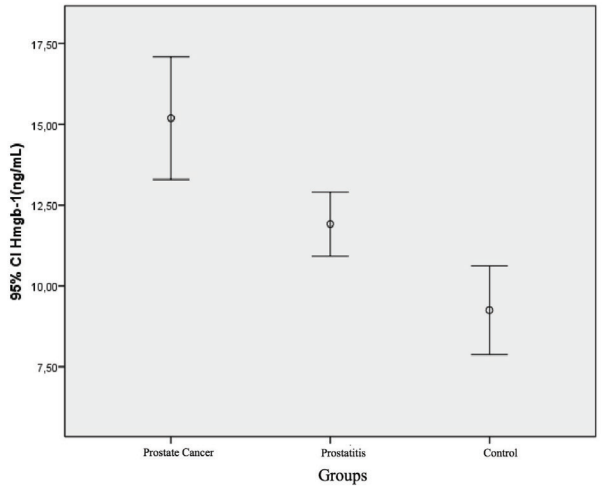
Table 1. Comparison of ages and serum t PSA levels

	Prostate Cancer (n:25)	Prostatitis (n:30)	Control Group (n:23)	p
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. Serum 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 (Hmgb-1 levels)



Mean HMGB1 was 11.9±2.6 (range: 6.718.4) ng/mL in the prostatitis group, 15.1±4.5 (range: 8.4–24.8) ng/mL in the prostate cancer group, and 9.2±3.1 (range: 4.7–18.7) ng/mL in the healthy individuals group. The difference between the groups was investigated by Friedman test because the HMGB1 variable did not show a normal distribution. Significant differences were found between the groups ($p<0.001$) (Figure 1). When a pairwise comparison was performed between the groups, a significant difference was identified between the prostatitis group and the healthy individuals group ($p=0.001$); between the prostate cancer group and the healthy individuals group ($p<0.001$); and between the prostatitis group and the prostate carcinoma group ($p=0.006$).

The correlation between PSA and HMGB1 was investigated by the Spearman test because the variables did not follow a normal distribution. A moderate but highly significant positive correlation ($r=0.276$, $p=0.009$) was found between PSA and HMGB1.

When the patients with prostate carcinoma were evaluated based on the Gleason score and PSA, 3 (12%) had low risk, 8 (32%) had moderate risk, and 14 (56%) had high risk. The relationship between the HMGB1 and prostate cancer risk groups was investigated by the Kruskal–Wallis test. Significant differences were not observed between the three groups ($p<0.352$).

DISCUSSION

PSA is the most frequently used biochemical marker for the diagnosis of prostate carcinoma. PSA levels are employed during treatment evaluation and follow-up (10). PSA can be detected in the serum of patients with bacterial prostatitis at highly varying levels. Many studies have demonstrated that PSA levels normalized in prostatitis patients after treatment (11). In this study, we demonstrate that the HMGB1 measured in serum can play a vital role in the differential diagnosis of prostate carcinoma and prostatitis.

HMGB are non-histone nuclear proteins with different functions in the cell. HMGB proteins were first purified from the nucleus in the 1970s. They were named so because of their fast movement in sodium dodecyl sulfate polyacrylamide gel electrophoresis on their initial discovery (6). HMGB1, HMGB2, and HMGB3 are members of the HMGB protein family. HMGB2 and HMGB3 have limited expression, whereas HMGB1 has a prevalent expression pattern, which can be regulated by environmental factors. HMGB2 is highly expressed during embryogenesis, which is in contrast with HMGB1, and its expression in adulthood is limited to the lymphoid organs and the testis. Knowledge about the common and differential functions of HMGB1 and HMGB2 proteins with reference to pathological processes can assist in the treatment of cancer (12). HMGB1 binds to the small groove of the DNA, and it modifies the interaction with the DNA of certain transcription factors including p53 and steroid hormone receptors. It plays a role in mechanisms such as the repair of DNA, transcription, cellular differentiation, extracellular signaling, and somatic recombination. HMGB-1 is considered an essential facilitator in diseases such as sepsis, atherosclerosis, cancers, arthritis, acute lung injury, epilepsy, myocardial infarction, and local and systemic inflammation. Modulation of HMGB1 levels in the human body provides a means of management of these diseases (13). HMGB1 has a high bonding affinity for certain receptors, and it is passively secreted by necrotic cells but actively secreted by inflammatory cells. These receptors include receptor for advanced glycation end products (RAGE) and Toll-like receptors (TLR)-2, TLR-4, TLR-9 (14).

The relationship between prostate carcinoma and HMGB1 expression has been investigated previously. Li et al. (15) observed a relationship between HMGB1 mRNA and protein expression in prostate carcinoma cell cultures using polymerase chain reaction and Western blotting, respectively. Using immunohistochemistry (IHC), they showed HMGB1 expression in the tumor tissue

samples of 168 patients with prostate carcinoma obtained by prostatectomy. They detected a high HMGB1 protein expression in the tissue samples in their study, which correlated with the high Gleason score and the pre-operative PSA concentration.

Gnanasekar et al. (16) investigated the role of HMGB1 in the development of prostate carcinoma. They demonstrated that HMGB1 plays a crucial role in the expression and upregulation of androgynous receptors in patients with prostate carcinoma. Further, Zhang et al. (17) reported a high HMGB1 expression in metastatic prostate cancer samples, which enhanced the aggressiveness of PC3 cells. HMGB1 promotes epithelial–mesenchymal transition and upregulates the expression of MMP-1, -3, and -10 by activating RAGE/NF-κB signaling in PC3 cells, thus facilitating cancer metastasis.

These studies strongly indicate that HMGB1 has a vital role in the progression of prostate carcinoma. However, Zhao et al. (18) investigated HMGB1 and RAGE expression in tissue samples taken after prostatectomy of 85 patients with prostate cancer by IHC. They did not observe any relationship between HMGB1 and RAGE expression using Gleason score. Although they asserted that HMGB1 expression was a poor prognostic marker, they stated that it could be used as a novel prognostic marker for prostate carcinoma. We did not observe any relationship between serum HMGB1 levels and risk groups guiding the prostate carcinoma treatment in our study.

There are few studies that investigated HMGB1 expression in patients with prostatitis. Xue et al. (19) compared HMGB1 expression in tissue samples of BPH patients with prostatitis and BPH patients without prostatitis. They detected a higher HMGB1 expression in the BPH patients with prostatitis.

The limitations of our study include its small sample size and the retrospective study design.

CONCLUSION

In this study, we demonstrated that high serum PSA and HMGB1 levels were highly correlated, and that HMGB1 level was apparently higher in patients with prostate carcinoma. We beleive that HMGB1 level in serum could be a useful marker for the differentiation of prostatitis and prostate carcinoma in the early diagnosed prostate carcinoma cases.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Sanko University 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.

Author Contributions: Concept – M.S., M.Y., H.Ç.; Design - M.S., H.Ç.; Supervision - M.Y., H.Ç., Resource – M.S., M.Y., H.Ç., Z.Y., O.S., N.O.; Data Collection and/or Processing - N.B., M.S., N.O.; Analysis and/or Interpretation - M.Y., H.Ç., N.B.; Literature Search - O.S., Z.Y., M.S.; Writing - M.S., H.Ç.; Critical Reviews – M.Y.

Conflict of Interest: Authors have no conflicts of interest to declare.

Financial Disclosure: The authors declared that this study has received no financial support.

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