The Diagnostic Value of Ischemia-modified Albumin in Prostate Cancer

Prostat Kanserinde İskemi-modifiye Albümin'in Tanısal Değeri

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──── **Abstract** ────

Aim: The aim of this study was to investigate whether serum ischemia-modified albumin (IMA) levels have a diagnostic value in prostate cancer (Pca).

Methods: Thirty primary Pca patients and 30 age-matched healthy male subjects were included in this prospective case-control study. The patients were selected from the urology clinic of a tertiary university hospital. Healthy men included in the control group were selected from hospital staff and patient relatives. Patients with severe chronic diseases and other malignancies were excluded. Serum IMA level was measured using the colorimetric method. The results were reported in absorbance unit (ABSU).

Results: Serum IMA levels were significantly higher in the study group when compared to the control group (0.843±0.76 and 0.443±0.49 ABSU, respectively; p=0.002). The mean IMA value in patients with a Gleason score ≥7 (1.08±0.053 ABSU) was significantly higher than in patients with Gleason score ≤6 (IMA 0.418±0.64 ABSU). According to the receiving operating characteristic (ROC) analysis, when the cut-off value was ABSU=0.57, the sensitivity and specificity were 80% and 56.7%, respectively.

Conclusion: Serum concentrations of IMA are significantly elevated especially in clinically significant Pca patients. Its high sensitivity (80%) in ROC analysis suggests that IMA can be used an ancillary biomarker in diagnosis.

Keywords: IMA, prostatic cancer, diagnosis, bio-marker

──── **Öz** ────

Amaç: Bu çalışmada Prostat kanserinde (Pca) serum iskemi modifiye albümin (İMA) düzeylerinin tanısal bir rolü olup olmadığının araştırılması amaçlandı.

Yöntemler: Bu prospektif olgu kontrol çalışmasına 30 primer Pca hastası ve kontrol grubuna 30 sağlıklı erkek birey (yaşla eşleştirilmiş) dahil edildi. Hastalar, üçüncü basamak bir üniversite hastanesindeki üroloji kliniğinden seçildi. Kontrol grubuna alınan sağlıklı erkekler ise hastane çalışanlarından ve hasta yakınları arasından seçildi. Ciddi kronik hastalıkları ve başka malignitesi olan hastalar çalışma dışı bırakıldı. Serum IMA seviyesi kolorimetrik yöntem ile ölçüldü ve sonuçlar absorbans birimi (ABSU) olarak verildi

Bulgular: Çalışma grubundaki hastalarda ortalama IMA düzeyi kontrol grubu ile karşılaştırıldığında istatiksel olarak anlamlı oranda artmış olduğu tespit edildi. (Çalışma grubu=0,843±0,76 kontrol grubu=0,443±0,49 ABSU p=0,002) Gleason skoru (GLS) ≥7 olan hastaların ortalama İMA (1,08± 0,053 ABSU) düzeyleri, GLS ≤6 olan hastalara (İMA=0,418±0,64 ABSU) göre istatiksel olarak anlamlı oranda yüksek bulundu. Alıcı işletim karakteristiği (ROC) analizine göre eğri altında kalan alan %73,1 bulundu. Cutt-off değeri 0,57 ABSU alındığında sensivite %80 ve spesifite %56,7 bulundu.

Sonuç: Verilerimiz özellikle klinik olarak anlamlı Pca hastalarında serum İMA seviyelerinin istatiksel olarak anlamlı oranda yüksek olduğunu göstermektedir. Aynı zamanda İMA'nın ROC analizine İMA senstivitesinin %80 görülmesi tanıda yardımcı bir biyobelirteç olarak kullanılabilirliğini desteklemektedir.

Anahtar Sözcükler: İMA, prostat kanseri, tanı, biyomarker

Introduction

Prostate cancer (Pca) is currently accepted as one of the most important health problems affecting males. Pca is the second most common cancer with more than 1.1 million new cases occurring worldwide. This accounts for approximately 15% of all diagnosed cancers in men (1). Pca is followed by lung and colorectal cancers respectively. Moreover, Pca is the second most common cause of cancer deaths in men. (2-4). Prostate-specific antigen (PSA) test is a molecular test discovered 40 years ago and is the most commonly used to detect the disease and evaluate recurrence. Although this test is very important in early diagnosis, it is recently criticized for being not specific cancer, revealing false positive results and leading often to overdiagnosis (5,6). Today, PSA value is determined by visual methods and histopathological scoring. However, all these methods are still unsatisfactory to determine the individual prognosis. This sometimes leads to unnecessary treatment and sometimes obstacle to necessary treatment (7). Recently, trials tend to investigate biomarkers that are safe to solve these clinical problems (8).

There are many evidences that demonstrate effect of reactive oxygen species (ROS) in the development of cancer. ROS consist of hydroxyl radicals, superoxide anion radicals and lipid peroxide radicals. High concentrations of ROS may cause both tissue damage and DNA damage by inducing oxidative stress. ROS is claimed to act as secondary messengers in intracellular signaling cascades in the cell and to provide cancer cell viability (9-12).

Ischemia-modified albumin (IMA) is an altered type of serum albumin generated by ROS (13,14). IMA is accepted as a reliable biomarker of oxidative stress (15). Elevated serum IMA is demonstrated in various diseases associated with inflammation and oxidative stress. Moreover, serum IMA level has been shown to increase in stomach cancer, soft tissue cancer, neuroblastoma and benign prostatic hyperplasia (BPH) (16-18).

In this study, we aimed to compare serum IMA levels between patients with Pca and controls and investigated the value of serum IMA levels in the diagnosis of Pca.

Methods

Study Design

This study was approved by our local ethics committee [Van Yüzüncü Yıl University, Faculty of Medicine (approval no: 01)]. Thirty primary Pca patients and 30 age-matched healthy male subjects were included in this prospective case-control study. The patients were selected from the urology clinic of a tertiary university hospital. Patients with a high serum PSA value (≥4 ng/mL) and/or abnormal digital rectal examination findings underwent transrectal

ultrasound (TRUS)-guided biopsy. All patients underwent a 12-core biopsy protocol, including six parasagittally and six laterally targeted cores covering the base, mid-zones, and apex. The biopsy samples were numbered separately and analyzed by an uropathologist. Patients who were diagnosed with primary Pca after TRUS-guided biopsy were included in the study. Healthy men included in the control group were selected from hospital staff and patient relatives. All volunteers in the control group were screened for Pca, BPH, other malignancies and chronic diseases. Patients with preoperative severe comorbidities, such as severe cardiac and neurological diseases, other malignancies, acute and chronic infectious diseases, acute organ failure, chronic obstructive pulmonary disease and other ischemic and immunosuppressive diseases, were excluded from the study. Moreover, individuals with abnormally low or high serum albumin levels (<20 or >55 g/L) were also excluded from the study.

Demographic characteristics and clinic and histopathologic data of both groups were recorded and analyzed comparatively. Written informed was obtained from all the participants.

Blood samples were drawn from the antecubital vein into EDTA and NA-citrate blood tubes and centrifuged at 3000 rpm for 10 minutes. The serum and plasma specimens were stored at -80 °C until the biochemical analysis.

Measurement of IMA

Serum IMA level was measured with colorimetric analysis that is developed by Bar-Or et al. (19). To measure serum IMA, 50 lL cobalt chloride is added to 200 µL of serum, vigorously mixed and the mixture solution is incubated for 10 minutes to ensure cobalt binds to albumin appropriately. Next, 50 uL (1.5 mg/mL) dithiothretol (DTT) solution (Sigma-Aldrich, Germany) is added as coloring agent and mixed vigorously then incubated at room temperature for 2 minutes. Finally, 1.0 mL of 0.9% NaCl is added. One colorimetric control is prepared for each sample. 50 µL of distilled water is used for control samples instead of 50 µL of 1.5 mg/mL DTT. Resultant color complex is measured with spectrophotometric method in wavelength of 470 nm. Results are reported in absorbance units (ABSU).

Statistical Analysis

All data were analyzed using the SPSS version 20 (IBM SPSS Statistics for Windows, Version 20.0, IBM Corp., NY, USA). Continuous variables were expressed as mean ± standard deviation or median value with the range of values when a normal distribution or a non-normal distribution was present, respectively. Categorical variables were expressed as ratio and frequency. The difference in

the level of IMA between the groups was evaluated using the ANOVA. Multiple comparisons were made using the Tukey HSD test. The Student's t-test or Mann-Whitney U test was used in the binary comparison of the groups where appropriate. The capacity of serum IMA values in predicting presence of Pca was analyzed using ROC curve analysis. A p value of less than 0.05 was considered statistically significant.

Results

There was no significant difference in body mass index comorbidities, prostate volume and serum albumin level between the patients and controls (Table 1).

IMA levels were analyzed in study and control groups. The mean IMA level in Pca patients was statistically significantly higher than in controls (0.843±0.76 and 0.443±0.49 ABSU, respectively (p=0.002) (Figure 1).

The patients in study group were divided into two subgroups according to Gleason score (GS). The mean IMA level in patients with GS ≥7 (1.08±0.053 ABSU) was significantly higher than in patients with GS ≤6 (0.418±0.64 ABSU). The mean IMA level in patients with clinical stage 1 and stage ≥2 was 0.457±0.41 and 1.13±0.18 ABSU, respectively. The difference in IMA value between these two subgroups was statistically significant (p=0.002). On the other hand, when mean IMA was compared between patients with stage 1 disease and GLS score ≤6 and the control group no statistically significant difference was detected (p>0.05) (Table 2).

In the study, according to ROC curve analysis results (figure 2), the area under curve was 73.1%. When the cut-off value was ABSU=0.57, sensitivity and specificity were 80% and 56.7%, respectively (Figure 2).

When the correlation between PSA values and IMA values in patient group was investigated, approximately 70% positive correlation was observed (p<0.001) (Figure 3).

Discussion

Development of cancer is a multi-stage process. ROS-mediated DNA damage plays an important role, especially in the initial and advanced stages of this process (20). ROS consist of hydroxyl radicals, peroxide and superoxide and they are also generated by healthy cells. However, tissue or DNA damage develops if ROS is generated excessively (10). Hydroxyl radicals cause base modification and genomic instability by interacting with purines and pyrimidines (20). Moreover, it has been suggested that they activate intracellular signal pathways and increase the durability of cancer cells (10).

IMA is considered a biomarker of oxidative stress. Ischemic, inflammatory diseases and diseases such as stomach, colorectal and bladder cancers have been shown to increase serum IMA levels.

Many *in vitro* and *in vivo* studies showed that oxidative stress caused by overproduction of ROS or impaired anti-oxidant mechanisms play a key role in the pathogenesis
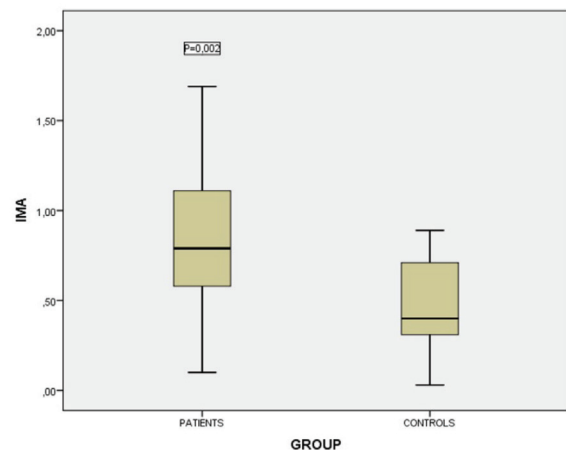

Figure 1. IMA levels in groups

IMA: Ischemia-modified albumin

Table 1. Demographic and clinical characteristics of the patients			
	Working group	Control group	p
Age (years), mean ± SD	63.4±8.6	61.8±10.5	>0.05
BMI (kg/m²), mean ± SD	27.8±6.02	27.08±2.9	-
PSA (ng/mL), mean ± SD	18.07±15.3	2.1±0.12	<0.001
Prostatic volume, mean ± SD	73.7±46.8	71.3±31.9	>0.05
Comorbidities (DM-dyslipidemia CVD), n (%)	17 (56.6%)	15 (50.0%)	>0.05
Albumin (g/L)	29.6±5.2	28.94±6.2	>0.05
Gleason score ≤ 6, n (%)	11 (36.6%)	-	-
Gleason score ≥7, n (%)	19 (63.4%)	-	-
Clinical stage 1, n (%)	14 (46.6%)	-	-
Clinical Stage 2-4, n (%)	16 (53.33%)	-	-
SD: Standard deviation. BMI: Body mass index. DM: Diabetes Mellitus. CVD: Cardiovascular disease. PSA: Prostate specific antigen			

Table 2. Comparison of IMA value according to histopathological classification			
	Study group (Average IMA level, ABSU)	Control group Average IMA level, ABSU	p
Stage 1	0.457±0.41	0.443±0.49	>0.005
Stage 2,3,4	1.13±0.18		<0.001
GL score ≤6	0.418±0.64		>0.05
GL score ≥7 (7-10)	1.08±0.053		<0.001
Total patients	0.843±0.76		p=002
IMA: Ischemic human albumin, ABSU: Absorbance units, GL: Gleason			

of Pca (21,22). In this study, we aimed to reveal out the relationship between IMA, a biomarker of oxidative stress, and Pca. We analyzed serum IMA in patients with primary Pca. We demonstrated that IMA was significantly higher in patients with Pca than in control group. On the other hand, we observed that IMA levels were significantly higher in patients with GS ≥7. Moreover, it was observed that IMA levels were higher in patients with advanced stage (II,III,IV) Pca. We also found that IMA had a high positive correlation with PSA values in Pca patients. All
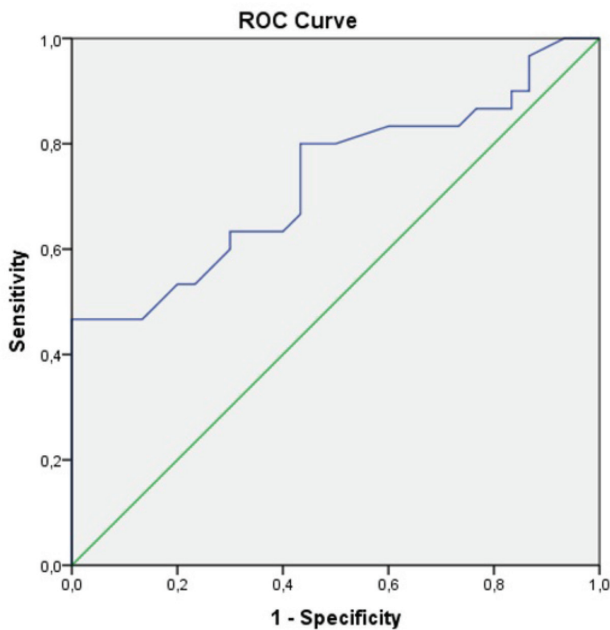

Figure 2. ROC Anaysis by IMA values in Pca patients

ROC: Receiver operating characteristic, IMA: Ischemia-modified albumin, Pca: Prostate cancer
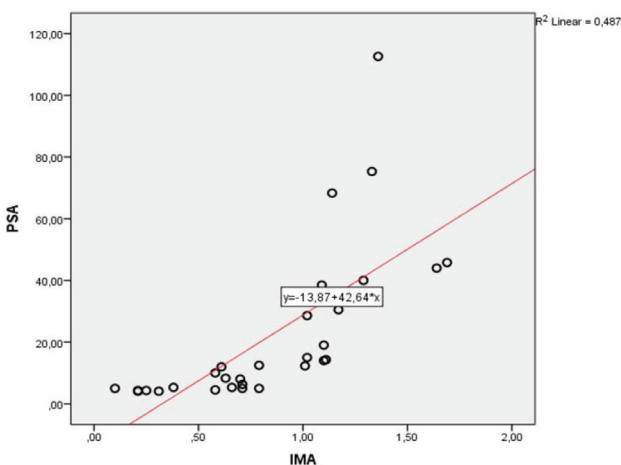

Figure 3. IMA and PSA correlation in Pca patients

IMA: Ischemia-modified albumin, Pca: Prostate cancer

these data show that IMA can be associated with especially aggressive and high-grade Pca.

According to current European guidelines, there are several evidence-based treatment options available for these patients. Reliable biomarkers are urgently needed to guide the pre-treatment decision process for PCa patients (23). The fact that sensitivity of IMA (when the cut-off value is 0.57) is 80% in the diagnosis of PCa according to ROC analyses may indicate that this test can be used as a helpful biomarker for the diagnosis of PCa in larger future studies.

Fidan et al. (16) reported elevation in serum IMA, malondialdehyde (MDA)-an oxidative stress marker--and oxidative stress index in patients with stomach cancer and claimed that this condition was related to the impaired balance between oxidative and antioxidant processes. In a more recent study, Huang et al. (24) have identified significant elevation in serum IMA concentrations in patients with preoperative stage III stomach cancer. The authors claimed that serum IMA levels could be an independent prognostic factor in operable advanced gastric cancer patients. In another study, Ellidag et al. (25) detected that serum IMA, total oxidation status and oxidative stress index were significantly higher in patients with colorectal cancer than in controls (p<0.0001). Satoh et al. (26) reported that serum IMA levels in patients with colorectal cancer positively correlated with severity of the disease. In the literature, we found only one study investigating the relationship between prostatic diseases and IMA. In their study, Mastella et al. (17) investigated serum IMA in 27 patients with myocardial infarction (MI), 122 patients with benign prostatic hyperplasia (BPH) and 84 patients with PCa. Serum IMA levels were significantly higher in patients with MI and BPH than in control group. Although serum IMA levels were also higher in patients with PCa when compared to controls, the difference was not statistically significant. In this study, no subgroup analysis of Pca patients, nor clinical stage and GS score were mentioned. In another study, Ellidag et al. (27) found that serum IMA levels in patients with urinary bladder cancer were significantly high. Moreover, in their study including pediatric patients with soft tissue sarcomas and neuroblastoma, Stachowicz-Stencel et al. (18) reported that serum IMA levels were higher in the patient group compared to the control group.

Study Limitations

The fact that our study was single-centered, the number of patients was relatively low and the other oxidative stress parameters were not considered could be considered a significant limitation. However, although there are many studies on the relationship

between cancer and IMA in the literature, we think that it will contribute to the literature since there are very little research on the relationship between Pca and IMA. Moreover, we believe that higher IMA level in aggressive and advanced stage Pca and high sensitivity according to ROC analysis are important data. Future larger and multi-center studies may reveal more clearly whether IMA is a new biomarker and/or it has a prognostic value for Pca. This study may contribute to investigations on this matter.

Conclusion

Our data show that serum IMA levels are statistically significantly higher in especially advanced and aggressive Pca patients. Also, it was observed that serum IMA levels were positively correlated with PSA levels in Pca patients. Meanwhile, due to its high sensitivity (80%), IMA can be used as an ancillary biomarker in diagnosis.

Authorship Contributions

Concept: R.A., M.S. Design: R.A., R.E., M.S. Data Collection or Processing: R.A., R.E., M.S., M.D., K.T. Analysis or Interpretation: R.E., M.S., M.D., K.T. Literature Search: R.A., R.E. Writing: R.A., M.S.

Conflict of Interest: No conflict of interest was declared by the authors.

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