Comparison of Serum Gamma Glutamyl Transferase Levels between Prostate Cancer Patients and Their Healthy Peers

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Abstract

Background: Prostate cancer (PCa) is the most common cancer affecting men, apart from cutaneous cancers. Serum prostate specific antigen (PSA) levels are frequently used to predict prostate cancer diagnosis. However, many causes (e.g., prostatitis, benign prostate obstruction, urethral catheterization) may cause elevated PSA, in addition to PCa. We aimed to investigate the gamma glutamyl transferase (GGT) levels, a serum biomarker not affected by situations other than cancer causing elevated PSA.

Methods: The study evaluated male patients with prostate biopsy due to high serum PSA levels and/or abnormal digital rectal examination (DRE) examined in Ordu University Education and Research Hospital, Ordu/Turkey urology clinic from April 2019 to April 2021. The patient group in the study included 261 men with PCa diagnosis and the control group included 245 healthy men with normal PSA levels, and no PCa and/or benign prostate obstruction (BPO). The two groups were compared in terms of serum GGT levels.

Results: GGT was significantly low in the PCa group and might be a predictor in terms of PCa (*P*=0.000). In the malignant (PCa) group, the GGT cut-off value was identified as 21.5 (sensitivity 68.6%, specificity 54.4%).

Conclusion: Serum GGT levels might assist in diagnosis of PCa. However, diagnostic power is weak due to low specificity. There is a need for studies investigating the efficacy of GGT levels for prediction of PCa diagnosis and assessing other parameters alongside GGT.

Keywords: Gamma glutamyl transferase; Prostate cancer; Biomarker; Prostate specific antigen

Introduction

Prostate cancer (PCa) is a common cancer around the world. PCa is the most frequently diagnosed cancer in men in the United States of America, similar to most European countries (1). It is thought to be the second most frequent cause of deaths linked to cancer among men (2). The etiology is not fully known, but causes re-

ceiving most attention include age, genetic factors, diet, sexually transmitted diseases and environmental factors.

Currently, the most frequently used biomarker among PCa screening tests is the serum prostate specific antigen (PSA) value. However, diagnostic sensitivity is not sufficiently high as with other

prostate cancer biomarkers. Many causes like benign prostate obstruction (BPO), acute prostatitis, and urologic instrumentation may cause increases in serum PSA values along with PCa. To increase the diagnostic sensitivity of PSA, a variety of methods like PSA derivatives, PSA kinetics and prostate health index (PHI) are used. In spite of these methods, PCa diagnosis is made with pathologic investigation of tissue obtained by prostate needle biopsy. In spite of the intense use of clinical biomarkers when directing the biopsy process in patients, the cancer detection rates for first biopsy are 25-30% (3-7). For this reason, there is a need for new studies to lower the number of unnecessary prostate biopsies and about more sensitive clinical biomarkers for PCa.

The carcinogenesis mechanism in PCa is still not fully understood, but many studies in recent times reported that chronic inflammatory processes play an important role in PCa development. Inflammation is found in tissues obtained from prostate biopsies, radical prostatectomy specimens and BPO resection tissues (8,9). Many factors are thought to comprise the basis for the association between inflammation and cancer. For example, inflammatory compounds like free oxygen radicals and cytokines released into this environment cause DNA injury and may affect the cancer development process (10,11). The oxidant/antioxidant balance disrupted in favor of oxidants in the carcinogenesis process may contribute to this pathologic process. Changing the oxidant/antioxidant balance in favor of antioxidants is proposed to have protective effects against cancer and many antioxidant compounds are used with this aim (12).

Gamma glutamyl transferase (GGT) is an enzyme regulating the glutamic acid and cysteinyl glycine conversion stage of glutathione included in the gamma glutamyl cycle. GGT activity in prostate gland fluids is much higher than serum GGT activity (13). GGT has a protective role against free oxygen radicals forming in many cancer types (14). For this reason, studies investigating the correlation between GGT levels and cancer development have an important place in the literature (15-20). We planned this study due to the low sensitivity of PSA levels for PCa identification and the low cancer detection rates on first biopsy. To the best of our knowledge, the number of prospective controlled studies investigating this topic in the literature is limited (21). In this prospective-controlled study, we compared the serum GGT levels between healthy men and PCa patients. Thus, we aimed to assess the usefulness of serum GGT level as a parameter assisting PCa diagnosis.

Materials and Methods

Study Design

Our study was prospectively designed and assessed the patient population in the central Black Sea region. The study prospectively assessed male patients with prostate biopsy due to high serum PSA levels and/or abnormal digital rectal examination (DRE) findings examined in Ordu University Education and Research Hospital, Ordu/Turkey urology clinic from April 2019 to April 2021. The study included a patient group of 261 men with PCa diagnosis after biopsy and a control group of 245 men with normal PSA levels and without PCa and/or benign prostate obstruction (BPO). Patient data were prospectively recorded by a urologist specialized in the topic. Every patient included in the study provided informed consent for use of personal data. Permission for the study was provided by the local Ethics Committee of Ankara Lokman Hekim University (decision no: 2020/24).

Blood samples were taken after eight hours fasting in the morning. Suspicious DRE finding and/or PSA value >4 ng/mL were accepted as biopsy indications. Benign causes of PSA elevation like urinary tract infection (UTI), urethral instrumentation and constipation were excluded. Additionally, the accuracy of PSA elevation was checked 2-3 weeks later in all patients. All patients with planned prostate biopsy were given the required information and provided written consent. Antibiotic prophylaxis was administered routinely before the procedure (ciprofloxacin 500 mg, 2 doses, oral). The biopsy was performed by

a single experienced clinician, accompanied by TRUS and with 12 cores. Exclusion criteria in the study included factors that may change GGT levels including cholestasis, alcohol use, cholelithiasis, hepatitis, cirrhosis, and hepatotoxic medication use.

The study included a total of 506 patients, including 261 men with prostate cancer (Group 1) and 245 men in the control group (Group 2, PSA <3 ng/mL). The demographic data for cases are summarized in Table 1. The groups were compared in terms of age, age at first complaint, height, weight, waist circumference, body mass index (BMI), PSA and testosterone levels, fasting blood sugar (FBS), BUN, creatinine, ALT, AST, GGT, ALP, LDH, CRP and ESR levels.

Statistical Analysis

All statistical analyses in the study used SPSS v26 (IBM Inc., Chicago, IL, USA) software. The student t test was used for data analysis. Before analysis, the Levene and Kolmogorov-Smirnov tests were used to check the variance equality and normal distribution. If these assumptions were not met, data were analyzed using the Welch t test. The two-way chi square test was used to assess differences in frequency distribution between the study groups for categoric variables. To determine the diagnostic model and cut-off value for the structural model, receiver operator characteristic (ROC) curve analysis was used. The ROC analysis method assesses the use of a variable (continuous variable) as diagnostic test in a defined diagnostic interval. With this method, the values belonging to the patient and control groups are ranked from small to large to create tables of ranked values and sensitivity and specificity values are calculated for a variety of positivity cut-off values. ROC curves are drawn using sensitivity and specificity coordinate values obtained for ten separate positivity cut-off values. The area under the curve and 95% confidence interval are determined. If the 0.5 value (theoretical lack of difference) is outside the confidence interval, a statistically significant diagnostic value is considered. In our study, the ROC analysis method was used with the aim of calculating the efficacy of GGT levels for PCa diagnosis. For all statistical tests, significance level was determined as 95% ($P<0.05$).

Results

Overall, 506 cases were assessed including 261 PCa patients in the malignant group and 245 healthy men in the control group. The mean age of patients was 67.65±9.08 years in the malignant group and 62.16±8.25 in the control group. The demographic characteristics of the groups are summarized in Table 1. There were no significant differences identified between the groups in terms of age, age of first complaint, height, weight, waist circumference, BMI, testosterone level, FBS, BUN, creatinine, ALT, AST, ALP and LDH levels ($P>0.05$). The mean age and PSA values for patients in the malignant group were significantly high compared to the control group ($P<0.001$). The mean CRP levels and ESR values were significantly higher for malignant group patients compared to the control group ($P<0.01$). The mean ALP in the malignant group was significantly high compared to the control group ($P<0.05$). The mean GGT levels for patients in the malignant group were significantly low compared to the control group ($P<0.001$). The mean ALT values for patients in the malignant group were significantly low compared to the control group ($P<0.05$).

Table 1: Distribution of the patients' demographic characteristics

Characteristics	Control group (n = 245)	Malign group (PCa) (n = 261)	P value
Age (yr)	62.16±8.25	67.65±9.08	0.000***
Waist Circumference (WC)	103.48±59.73	98.56±13.07	0.225
BMI (kg/m²)	42.31±20.8	28.02±9.96	0.290

Welch's t-test, *:<0.05, **:<0.01, ***:< 0.001

Table 2 shows the rates for chronic alcoholism, smoking, previous surgery and comorbid diseases for patients. There were no significant differences between the groups in terms of chronic alcoholism, smoking, diabetes mellitus (DM), heart disease, hypertension (HT), pulmonary disease and family PCa history ($P>0.05$).

Table 2: Patients' chronic alcoholism, smoking status, previous surgeries and comorbid diseases

Patient Number (Ratio %)	Control group N(%)	Malign group (PCa) N(%)	P value
Chronic Alcoholism	23 (9.5)	32 (12.5)	0.272
Smoking	124 (51.5)	154 (59.5)	0.072
Heart disease	58 (23.8)	61 (23.4)	0.916
HT	90 (36.9)	103 (39.6)	0.529
DM	56 (22.9)	47 (18.0)	0.176
Family history of PCa	11 (78.6)	42 (91.3)	0.194
Presence of lower urinary tract symptoms	117 (47.8)	140 (53.6)	0.186

Welch's t-test, *:<0.05, **:<0.01, ***:<0.001

ROC curve analysis was performed and the area under the curve (ACU), sensitivity, specificity and 95% confidence interval (CI) were calculated. The ROC curves for GGT are presented in Fig. 1. As seen in Table 3, GGT was a predictor for the malignant group ($P<0.005$). The AUC for the ROC curve of GGT use for PCa diagnosis was 0.679 (95% CI: 0.625-0.733). The GGT cut-off value was identified as 21.5 for the malignant group (sensitivity 68.6%, specificity 54.4%). (Table 4).
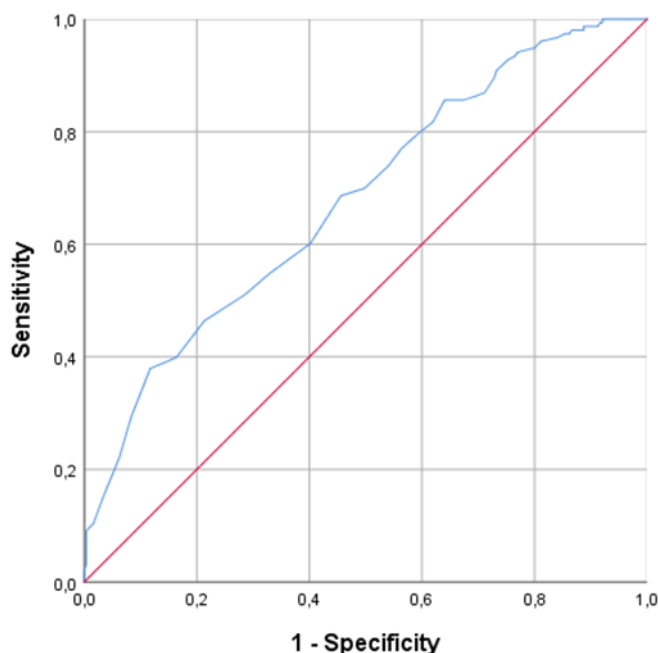
Fig. 1: The ROC curves of GGT

Table 3: Patients' laboratory results and group comparisons

Results	Control group	Malign group (PCa)	P value
PSA	1.43±1.08	52.80±15.24	0.000***
Total Testosterone	6.00±2.18	6.04±7.62	0.948
Creatinine	1.82±11.63	1.37±6.00	0.636
CRP	0.23±0.46	1.58±6.60	*0.002**
ESR	15.16±9.00	23.93±27.44	*0.007**
GGT	28.28±18.24	19.33±9.08	*0.000***
ALT	23.00±13.28	19.98±16.52	0.044*
AST	21.57±9.77	20.06±9.87	0.126
ALP	74.57±22.67	97.12±125.16	*0.044*
LDH	186.77±42.73	185.38±34.98	0.768

Welch's t-test, *:<0.05, **:<0.01, ***:<0.001

Table 4: Area under the curve (AUC) and 95% confidence interval (CI) for GGT

Cut-off value	Sensitivity (%)	Specificity (%)	AUC (95% CI)	P
21.5	68.6	54.5	0.679 (0.625-0.733)	0.001***

***<0,001

Discussion

Serum GGT levels are a part of routine biochemical analysis frequently used in our clinical practice. GGT is an enzyme regulating the glutamic acid and cysteinyl glycine conversion stage of glutathione included in the gamma glutamyl cycle (22). The reason for frequent use of this enzyme in clinical practice is that it increases in a variety of hepatobiliary pathologies led by cholestasis. At cellular level, GGT plays a role in protecting cells from oxidative stress due to its role in glutathione (GSH) transport (23,24). GSH is continuously expelled from cells. GGT-mediated metabolism

allows GSH to continuously re-enter cells and basically permits the main source of reactive oxygen species (ROS) along the electron-transport chain of mitochondria (25). For this reason, increasing GGT levels may reflect high GSH cycle in response to intracellular oxidative stress. Additionally, low but stable levels of GGT show the pro-oxidative role as an ROS source. Low pro-oxidant levels may encourage proliferation and modulate other functions like immune response (26). In other words, GGT release at low but constant levels has pro-oxidant and immuno-modulatory efficacy, while GGT increase may display an antioxidant function with the increased GSH cycle rate in situations with increased oxidative stress.

PCa has similar carcinogenesis mechanisms to many other adenocancers. Oxidative stress and antioxidants play important roles in these onco-genesis processes. Additionally, the effect of oxidative stress on carcinogenesis has still not been fully explained. Variable antioxidant mechanisms are among factors contributing to limiting carcinogenesis. Without being able to make a definite generalization, many primary tumors are observed to have high antioxidant enzyme activity (27). In light of this information, it appears there are differences in antioxidant-pro-oxidant mechanisms linked to carcinogenesis stages and even to early or advanced stages of the tumor. This situation may be associated with the identification of different GGT levels in different clinical stages of cancer.

When the literature is investigated, there are two different opinions in studies investigating the relationship between GGT levels and malignancy. Some researchers reported they identified statistically significant increases in GGT levels in malignant processes including PCa, while some researchers reported changes in GGT levels in malignant processes were not statistically significant (28). Some researchers stated that cancers metastasizing to the liver may cause an increase in serum GGT levels; for this reason, they stated that GGT levels may be misleading in malignancy patients (29). In the literature, there are studies reporting serum GGT levels are independent of

GGT expression in tumoral tissue (30). A large prospective cohort study in 2011 reported GGT was associated with tumor development, additionally, they identified that there was variability in glucose levels in the correlation of GGT and cancer. In conclusion, researchers did not find evidence of a direct relationship between GGT, modeled as categoric variable, with prostate cancer (28). After sequential studies reported a correlated increase in GGT levels with urinary system cancers, a prospective cohort study was published in 2017 investigating the PCa-GGT association. The researchers reported that long-term follow-up of GGT was positively and independently associated with prostate cancer risk in the future (18). Another study published in the same year reported that GGT activity in serum exosomes in PCa patients were significantly higher compared to BPO patients (17).

The conclusion reached from all this valuable literature information is that GGT levels may increase or reduce with different mechanisms in different stages of malignant transformation. Our study is the first assessment to include patients with PSA elevation and PCa diagnosis after TPB. In other words, this is not a patient population with previously known PCa, and detailed cancer staging or spread revealed. There is low probability of the PCa patients included in our study being in the advanced stage, we predict that there is high probability most of our patients are early-stage PCa. For this reason, we associated the presence of low GGT levels in PCa patients identified in our study with secondary increased pro-oxidant and reduced antioxidant levels in the malignant-inflammatory process.

In short, the relationship between GGT levels with a variety of tissue organ cancers in many previous studies, the relationship with PCa, in fact the relationship with secondary malignancies identified in PCa patients, was investigated (20). Some of these studies were based on serum GGT levels, some were based on tissue GGT levels and some on serum exosomal GGT levels. In our study, serum GGT levels were measured and contrary to the two main views in the literature, the serum GGT levels measured before biopsy in

our patients with PCa diagnosis after TPB were identified to be significantly low compared to the control group. When we consider the mechanisms above, the main reason may be the assessment of early-stage PCa patients in our study.

Conclusion

Serum and tissue GGT levels can be said to show variability between early and advanced stages of PCa. In this sense, serum GGT levels being evaluated only in the first assessment in our study, the patients not being grouped according to PCa stages with staging after the diagnosis of PCa, and the changes in GGT levels not being evaluated according to PCa stage are important limitations. Perhaps if we had performed these assessments, we would be able to say that low serum GGT levels before TPB will be a guide in predicting the diagnosis of early-stage PCa.

Acknowledgements

Conflict of Interest

The authors declare that there is no conflict of interest.

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