

ORIGINAL RESEARCH

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Role of serum cathelicidin in diagnosis of patient with prostatitis and prostate carcinoma

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Abstract

Background: This study investigated the diagnostic role of 75 levels measured in serum prostatitis and prostate carcinoma and in the differentiation of these two conditions.

Methods: The study was conducted with 75 patients histopathologically diagnosed with prostate carcinoma or prostatitis and followed up at the Departments of Urology and Medical Oncology and 21 healthy male subjects. Serum cathelicidin levels were investigated using the ELISA method. Statistical analyses were performed using the SPSS for Windows 22.0 package software. Compliance of the variables to normal distribution was examined using visual and analytic methods. In the Kolmogorov–Smirnov test, cases with a p value of greater than 0.05 were accepted as normal distribution.

Results: A total of 75 patients including 45 diagnosed with prostate carcinoma and 30 diagnosed with prostatitis, as well as 21 healthy control subjects were included in the study. Prostate-specific antigen (PSA) was detected as 23 (4–1200) ng/mL in the patients with prostate carcinoma and as 9.85 (3.9–405 ng/mL) in the patients with prostatitis. The cathelicidin levels were diagnostically significant when assessed by ROC analysis in the prostate cancer, prostatitis and control groups ($p = 0.005$). The cutoff values derived from the ROC curve analysis were 3.5151 ng/mL for distinguishing prostate cancer from prostatitis, 2.2620 ng/mL for prostate cancer versus control group and 1.2340 ng/mL for prostatitis versus control group.

Conclusions: In this study we showed that the serum cathelicidin levels were significantly higher in the patients diagnosed with prostate carcinoma. Measurement of serum cathelicidin levels could be used as a diagnostic marker in prostate carcinoma as well as facilitating differential diagnosis to strengthen the diagnostic suspicion before prostate biopsy and distinguish the diagnosis from prostatitis cases.

Keywords: Prostate cancer, Prostatitis, Cathelicidin

1 Background

Prostate cancer is the most common type of cancer in men and the most common cause of death after lung cancer [1]. In Europe, an estimated 2.6 million new cases

of cancer are diagnosed each year. Prostate cancer constitutes about 11% of all male cancers in Europe and accounts for 9% of all cancer deaths among men within the European Union. While these men with clinically localized disease are faced with a number of different treatment options, the majority will choose either radical prostatectomy or some form of radiation therapy as definitive therapy [2]. Therefore, early diagnosis of these patients is important.

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Prostatitis describes a combination of infectious diseases (acute and chronic bacterial prostatitis), a chronic pelvic pain syndrome and asymptomatic inflammation [3]. Of note, African-American males suffer disproportionately from prostate disease. The incidence of prostate cancer, a potential etiology for prostatitis, is 274.3 per 100,000 African-American men, while white men have an incidence of 171.2 per 100,000. For African-American men under the age of 65, the incidence of prostate cancer is double that of whites [4]. The exact reasons are not known, but contributing factors may include genetics, morbidity from other disease states, socioeconomic status and access to health care.

Cathelicidin (LL-37) belongs to a group of antimicrobial peptides. LL-37 is chemotactic for leukocytes through interaction with formylpeptide receptors 2 (FPR2) [5]. LL-37 is released from various cells such as skin epithelial cells, leucocytes, keratinocytes, melanocytes and the bone marrow. Due to these properties, it plays a role in first-step defense against foreign pathogens [6]. In addition to its function of stopping the development of bacteria, LL-37 has the capacity to neutralize the lipopolysaccharides (LPS) released by gram-negative bacteria. LL-37 exhibited the potential of eliminating foreign pathogens through various processing pathways such as membrane-disrupting activity, antiseptic activity, apoptosis, angiogenesis, wound healing, chemotaxis and immune modulation (both humoral and cellular components) [7, 8]. Studies have shown that LL-37 levels increased in tumor cells of prostate, head and neck cancers and melanoma showing proliferation, invasion and migration [9–11].

Our study investigated the diagnostic role of LL-37 assay in prostatitis and prostate carcinoma.

2 Methods

2.1 Study population

The study was conducted with 75 patients histopathologically diagnosed with prostate carcinoma or prostatitis and followed up at the Departments of Urology and Medical Oncology and 21 healthy subjects admitting to the same clinic with no urological disease detected upon examination. All patients with prostate cancer were non-metastatic early-stage prostate cancer patients. Informed consent forms were obtained from the patients for the study.

2.2 Procedures

Approximately 5 mL venous blood was taken under suitable conditions from all subjects participating in the study. Pre-treatment blood samples were taken from patients with prostate cancer and prostatitis. These blood samples were centrifuged at 4000 rpm for 10 min

and then separated into Eppendorf tubes and stored at -80°C . These samples were kept at 4°C in a refrigerator one night before the measurements and were brought to room temperature 2 h before the study using the ELISA method. Then the samples were mixed using vortex and repeating twice for each sample for performing the measurement procedures.

Human Antibacterial Peptide LL-37 levels were measured quantitatively in accordance with the manufacturer instructions of the Cusabio brand commercial kits with the Catalog Number CSB-E14948h (Wuhan Hi-tech Medical Devices, Hubei/China). The analysis was performed using the sandwich enzyme immunoassay technique. All concentration/absorption graphic curves of the Human Antibacterial Peptide LL-37 test and calculations regarding the results were performed on the software of the Biotek_ELx808 (Winooski, Vermont, USA) device. The test was determined to have a sensitivity of 0.039 ng/mL and detection range of 0.156–10 ng/mL. The intra-assay and inter-precision assay variation coefficients were found as 6.7% and 5.3%, respectively.

2.3 Statistical analyses

Statistical analyses were performed using the SPSS for Windows 22.0 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–Smirnov test, cases with a p value of greater than 0.05 were accepted as normal distribution. Since the LL-37, age and PSA variable were not found to have a normal distribution, the differences between the prostatitis, prostate cancer and the control group were examined using Kruskal–Wallis test. In cases which normal distribution was not detected, pairwise comparisons were made with Mann–Whitney U test and evaluated using Bonferroni correction ($p < 0.017$). The total Type I error level was used as 5% for statistical significance. The capacity of serum LL-37 values in predicting prostate cancer was analyzed by ROC (receiver operating characteristics) curve analysis. The cutoff values were determined using the Youden index to maximize the sum of sensitivity and specificity using the sensitivity + specificity - 1 equation. A p value less than 0.05 was considered statistically significant.

3 Results

A total of 75 patients including 45 diagnosed with prostate carcinoma and 30 diagnosed with prostatitis, as well as 21 healthy subjects were included in the study. As the LL-37, age and PSA did not show a normal distribution, the difference among the groups was investigated using Kruskal–Wallis test. The patients diagnosed with

prostatitis had a median age of 62 (31–83). The patients diagnosed with prostate carcinoma had a median age of 70 (58–82). The median age of the control group was 64 (36–80). There was a statistically significant difference between the three groups in terms of age ($p=0.001$) (Table 1). PSA mean values were detected as 23 (4–1200) ng/mL in patients with prostate cancer, 9.85 (3.9–405 ng/mL) in patients with prostatitis and 1.23 (0.1–3.5) in control group (Table 1).

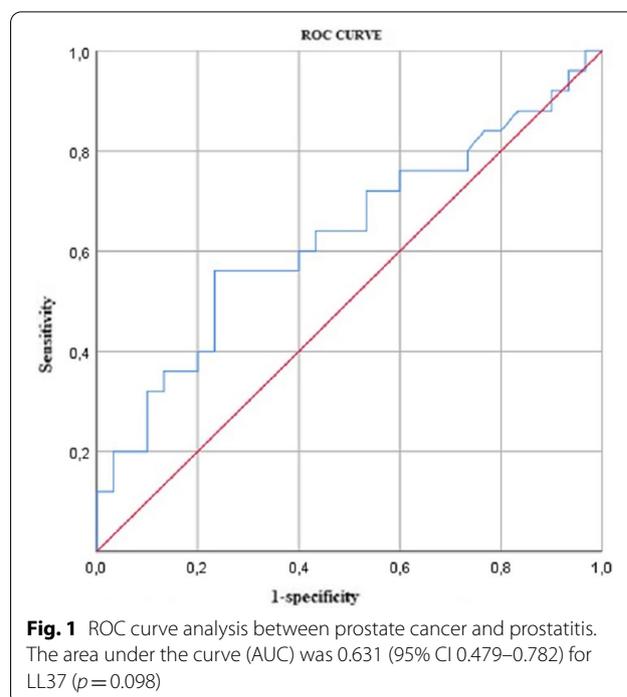
Serum LL-37 mean values were detected as 3.73 (1.32–26.65) ng/mL in patients with prostate cancer, 2.74 (1.24–7) ng/mL in the prostatitis group and 2.16 (0.8–5.04) ng/mL in the control group. There was also a significant difference between the prostate carcinoma patients, the prostatitis group and the control group ($p=0.005$) (Table 1).

In the pairwise comparisons made among these three groups in terms of their LL-37 levels, a significant difference was observed between the prostate carcinoma patients and the control group ($p=0.004$; Bonferroni correction $p<0.017$). There was no statistically significant difference between the prostatitis group and the control group ($p=0.02$; Bonferroni correction $p<0.017$). Moreover, there was no statistically significant difference between the prostatitis and the prostate carcinoma patients ($p=0.1$; Bonferroni correction $p<0.017$). The cutoff values derived from the ROC curve analysis were 3.5151 ng/mL for distinguishing prostate cancer from prostatitis ($p=0.098$) (Fig. 1), 2.2620 ng/mL for prostate cancer versus control group ($p=0.004$) (Fig. 2) and 1.2340 ng/mL for prostatitis versus control group ($p=0.025$) (Fig. 3).

The LL-37 levels were diagnostically significant when assessed by ROC analysis in the prostate cancer, prostatitis and control groups ($p=0.011$) (Fig. 1). When the LL-37 cutoff point was accepted as 2 ng/mL, sensitivity was 88%, and specificity was 70%.

The correlation between PSA and LL-37 was investigated using Spearman test. There was no statistically significant difference between PSA and LL-37 ($p=0.121$).

When the patients with prostate carcinoma were evaluated based on their Gleason score and PSA, 2 (8.4%) had low risk, 8 (33.3%) had moderate risk, and 14 (58.3%) had high risk.



The relationship between the LL-37 and prostate cancer risk groups was investigated. There was no significant difference among the three groups ($p=0.714$).

4 Discussion

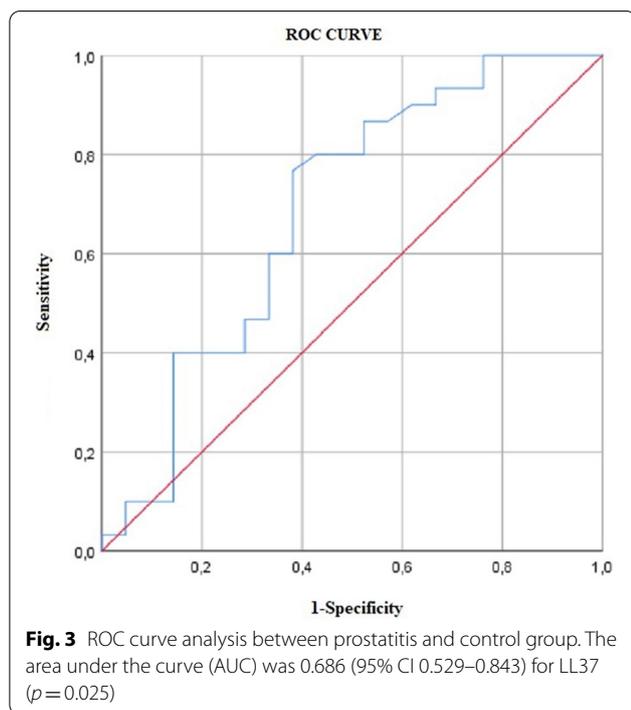
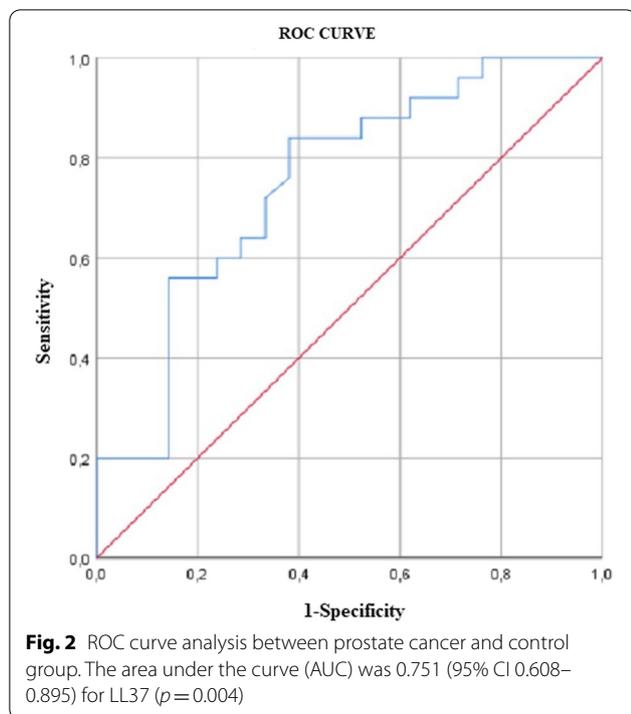
Some of the main purposes of cancer research are to facilitate diagnosis methods, increase predictiveness and develop non-invasive screening methods. Serum PSA levels may be detected on highly varying levels in bacterial prostatitis. Many studies have reported that an elevated PSA level is related to prostatitis. Screening for prostate cancer with serum prostate-specific antigen (PSA) aims to detect prostate cancer at an early, intervenable stage amenable to curative treatment and reduction in overall and disease-specific mortality. However, the evidence has so far not demonstrated that screening for prostate cancer saves lives [12, 13].

A member of the cathelicidins family, LL-37, has a peptide structure and is one of the elements of the immune system in addition to its anti-tumoral and anti-microbial

Table 1 Comparison of age, serum PSA levels and serum LL-37 levels in prostatitis, prostate cancer and control group

	Prostatitis (n:30)	Prostate cancer (n:45)	Control (n:21)	p
Age median (min–max) years	62 (31–83)	70 (58–82)	64 (36–80)	0.001
PSA median (min–max) ng/mL	9.85 (3.9–405)	23 (4–1200)	1.23 (0.1–3.5)	0.02
LL-37 median (min–max) ng/mL	2.74 (1.24–7)	3.73 (1.32–26.65)	2.16 (0.8–5.04)	0.005

n = number of individuals. Kruskal–Wallis test was used for statistical analysis



effects. Previous studies have shown that LL-37 was expressed in tumor tissue in prostate carcinoma as in many cancer types and asserted that it could be related to the progression of the disease [9]. Many studies reported

that serum LL-37 levels were detected to be higher than normal in the progression of diseases with chronic inflammation or in autoimmune diseases [14].

In the histopathological examinations on the prostate carcinoma patients, multifocal acute and chronic inflammation areas were observed especially in the regions not infiltrated by the tumor and were histologically benign. Acute inflammation of the prostate gland is characterized by infiltration of neutrophil leukocytes, and epithelial damage chronic inflammation is manifested with lymphocyte and macrophage reaction surrounding atrophic prostatic glands [15]. The etiology of this typical prostatic inflammation is not yet known. However, similar findings are observed also in the *Helicobacter pylori* infection of the stomach, and this situation has a relationship with inflammation, gastric atrophy and then gastric carcinoma. It was asserted that there could be such an infectious etiology also for prostate carcinoma. Although there is no microbiological agent clearly shown to cause prostate carcinoma development, there are scientific data which suggest that the inflammatory process has an important role in prostatic carcinogenesis [16]. Numerous inflammatory mediators have been implicated in cancer metastasis such as interleukin-6 (IL-6), IL-10 and tumor necrosis factor-alpha (TNF- α) [9].

No previous study was found about measurement of serum LL-37 levels in patients with prostate carcinoma or prostatitis. In the study conducted by Persson et al. about chronic obstructive pulmonary disease, an evident example of a similar chronic inflammation, it was found that the LL-37 levels were related to inflammation severity and bacterial colonization [16]. In their study on chronic viral hepatitis patients, Iacob et al. similarly detected LL-37 levels to be significantly higher in the active form of the disease [17]. In their study, Majewski et al. compared patients with acute pneumonia and pulmonary tuberculosis and showed that serum LL-37 levels were significantly higher in the pulmonary tuberculosis patients [18].

Although it is known that prostatitis etiology is not always bacterial or viral, it was observed that LL-37 levels are higher with respect to acute diseases as the inflammation becomes more chronic. Nielsen et al. studied urine LL-37 levels regarding urinary infections on female patients, and urinary system infections were found to correlate with urine LL-37 levels [19]. Sensitivity against LL-37 was found to be lower in fecal *Escherichia coli* isolates observed in the patient group. In this study, it was stated that LL-37 concentration in the urinary tract and low sensitivity to LL-37 may increase the possibility of urinary tract infections [19]. Hensel et al. investigated LL-37 expression in cell culture series in human and animal prostate tumor tissues using both the

immunohistochemical method and polymerase chain reaction. They found that LL-37 expression was related to tumor angiogenesis, proliferation and invasion [9]. They asserted that LL-37 could be a potential new treatment goal for prostate carcinoma.

Cha et al. showed that in prostate cancer progression LL-37 mediated the differentiation of myeloid progenitors in the microvicinity to M2 macrophages and thus promoted tumor development. In their study, they also determined that tumor progression was inhibited when LL-37 expression was downregulated from prostate tumors [20]. In this study, it was shown that serum LL-37 levels could be both diagnostic and a guide in disease progression.

In our study, there was no statistically significant difference in the LL-37 serum levels between the prostatitis patient group and the healthy group ($p=0.02$). Examining the other results of the study, the serum LL-37 level was higher in the prostate carcinoma patients with respect to the control group ($p=0.004$). The fact that, in our study, the serum LL-37 levels of the prostate carcinoma patients were higher than the healthy group and there was no difference with the prostatitis group, could be explained with the inflammation, invasion or metastasis status of the tumors. We found that the cutoff values for LL37 serum level were 3.5151 ng/mL to differentiate prostate cancer from prostatitis, 2.2620 ng/mL to differentiate prostate cancer from control group (suggesting that a LL37 value above this threshold might indicate greater odds of inflammation and hence prostate cancer) and 1.2340 ng /mL to differentiate prostatitis from control group (meaning that a LL37 value above this threshold can indicate an increased likelihood of having a prostatitis).

Our study has a number of limitations. Firstly, the sample size was small. Secondly, our study did not involve a comparison of urinary LL37 levels in a different cancer.

Our study has a number of limitations. In particular, small sample size is an important limitation and may have precluded demonstration of less clear associations between studied variables.

Our study was the first study where serum LL-37 levels in prostate carcinoma and prostatitis patients were compared to a healthy group. In the future studies, we might plan to make comparisons among different stages of prostate cancer in a larger patient sample.

5 Conclusions

In this study we showed that the serum LL-37 levels were significantly higher in the patients diagnosed with prostate carcinoma. There was no statistically significant difference in the LL-37 serum levels between the prostatitis patient group and the healthy group. We consider that

measurement of LL-37 protein serum levels could facilitate differential diagnosis to strengthen diagnostic suspicion before prostate biopsy and distinguish the condition from prostatitis cases.

Abbreviations

PSA: Prostate-specific antigen; LL-37: Cathelicidin; FPR2: Formylpeptide receptors 2; LPS: Lipopolysaccharides; ROC: Receiver operating characteristics.

Acknowledgements

Not applicable.

Author contributions

NB, MS, MY helped in the idea for research or article/hypothesis generation; NB, MS, MY planned the methods to generate hypothesis; MS, MY, ÖNS, NO were involved in responsibility for conducting experiments, management of patients, organizing and reporting data; NB, MS, HÇ, MY contributed to responsibility for presentation and logical explanation of results; NB, MS, MY, ZY helped in responsibility for conducting literature search; NB, MS, MY helped in responsibility for creation of an entire or the substantial part of the manuscript. All authors read and approved the final manuscript.

Funding

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

Availability of data and materials

Not applicable.

Declarations

Ethics approval and consent to participate

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study. Approval for the study was obtained from the Ethics Committee for Clinical Trials of SANKO University (Reference numbers. 04-(03/2018). Written consent was obtained from all volunteers participating in the study.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Received: 10 July 2020 Accepted: 17 October 2022

Published online: 03 December 2022

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