Ahead of Print

Turk J Urol 2019 • DOI: 10.5152/tud.2019.46416

TURKISH JOURNAL OF UROLOGY

UROONCOLOGY

Original Article

Can the expression level of *PIWIL 2* gene be a serum marker for prostate cancer? A single-center prospective study

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Cite this article as: Tosun H, Demirtaş A, Sönmez G, Tombul ŞT, Akalın H, Özkul Y. Can the expression level of *PIWIL2* gene be a serum marker for prostate cancer? A single-center prospective study. Turk J Urol 2019; DOI: 10.5152/tud.2019.46416

ABSTRACT

Objective: The aim of the present study was to evaluate the predictive value of the serum expression level of Piwi-like 2 (*PIWIL2*), a stem cell protein, for prostate cancer (PCa).

Materials and method: This randomized and prospective study included a total of 60 volunteers between 50 and 75 years old. Cases were assigned to three groups according to prostate-specific antigen (PSA) elevations and pathology reports, with 20 participants in each group. The first group included patients with a PSA level of >4 ng/dL and with PCa, the second group included patients with a PSA level of >4 ng/dL and with benign prostate hyperplasia, and the third group included patients with a PSA level of ≤4 ng/dL and with benign prostate hyperplasia. The levels of serum PSA and *PIWIL2* expressions were compared between the groups.

Results: The median serum PSA levels were 28.5 (4.6–98.1) ng/mL, 8.89 (4.3–24.1) ng/mL, and 2.4 (0.3–3.8) ng/mL for groups 1, 2, and 3, respectively. The PSA levels were significantly different between the groups ($p<0.001$). The median *PIWIL2* gene expression levels were 2.54 (0.28–9.27), 2.27 (0.6–9.38), and 1.17 (0.26–3.07) for groups 1, 2, and 3, respectively. The *PIWIL2* gene expression level was found to be lower in patients with a PSA level of <4 ($p=0.02$). No significant difference was observed between patients with and without cancer among those with a PSA level of ≥4 ($p>0.05$). Patients diagnosed with cancer were grouped according to the criteria of the International Society of Urological Pathology (ISUP), and *PIWIL2* gene expression was observed to be significantly higher among patients with ISUP of >3 than among those with ISUP of ≤3 ($p=0.04$).

Conclusion: In our study, it was observed that the serum level of *PIWIL2* gene expression could not be a diagnostic indicator of PCa; however, it could be a beneficial prognostic indicator particularly for progressed disease.

Keywords: Cancer; marker; *PIWIL*; prostate.

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Submitted:
18.06.2018

Accepted:
22.11.2018

Available Online Date:
04.02.2019

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Available online at
www.turkishjournalofurology.com

Introduction

According to recent data, prostate cancer (PCa) is the second most frequent type of cancer among men and constitutes approximately 15% of all cancers worldwide.[1] The basic methods used in the diagnosis of PCa are digital rectal examination, measurement of the serum level of prostate-specific antigen (PSA), and needle biopsy from the prostate with the guidance of transrectal ultrasonography.[2] It has been observed with the introduction of PSA and PSA derivatives as indicators of diagnosis that cancer-related mortality is decreased, whereas the number of biopsies obtained is increased at an important rate[3], because PSA is an organ-specific indicator and a cancer-specific marker. Therefore, new markers are needed to reduce the number of unnecessary biopsies and to differentiate clinically insignificant cancers and cancers with aggressive prognosis. Thus, derivatives, such as PSA doubling time, PSA velocity, PSA density, and free/total PSA rate, have recently begun to be used.[4-6]

With advances in genetics, alterations have been observed in various gene regions and proteins, such as prostate antigen 3, androgen-dependent transmembrane serine 2, and glutathione S-transferase P1 in PCa, as in many other cancer types.[7-9]

Piwi genes belong to a class of genes that are needed for self-regeneration of stem cells in different organisms. The Piwi-like 2 (*PIWIL2*) region is a member of the Piwi family that includes *PIWIL1*, *PIWIL2*, *PIWIL3*, and *PIWIL4*. *PIWIL2* plays a key role in the signal transduction pathway of cancer stem cells that regulates proliferation and apoptosis.[10,11] Previous studies demonstrated that the overexpression of the *PIWIL2* gene blocked the normal functioning of the DNA repair mechanism and thereby led to the formation and progression of several cancer types, such as gastric, colorectal, papillary thyroid, cervical, and breast cancers.[12-16] Tissue studies have demonstrated that the *PIWIL2* gene increased epithelial–mesenchymal transformation and expression of matrix metalloproteinase and led to a related increase in invasion and metastasis in PCa. In addition, *PIWIL2* gene expression was observed to significantly increase in PCa and was related to a high Gleason score. The importance of *PIWIL2* gene as a prognostic biochemical indicator and a potential target in cancer treatment has been emphasized.[17,18]

The aim of the present study was to investigate in PCas whether serum PSA level correlates with detected *PIWIL2* gene expression that may be associated with PCa in animal experiments. In this respect, we aimed to determine whether the expression of *PIWIL2* gene is a valuable marker for predicting PCa.

Material and methods

This was a randomized and prospective study conducted between May 2014 and May 2015. The study was approved by the Clinical Researches Ethical Committee of Erciyes University (no.: 2013-171). All participants were informed verbally and in written form. Written informed consent was obtained from all participants.

Patient selection

A total of 60 volunteers between 50 and 75 years old were included in the study. Owing to the risk of interference for serum level of *PIWIL*2, patients with another known malignancy or risk of malignancy and those receiving dialysis treatment due to chronic renal failure were excluded from the study. Cases were assigned to three groups according to PSA elevations and pathology reports, with 20 participants in each group. The first group (n=20) included patients with a PSA level of >4 ng/dL and histopathologically diagnosed with PCa following transrectal prostate biopsy or transurethral resection of the prostate (TUR-P). The second group (n=20) included patients with a PSA level of >4 ng/dL and histopathologically diagnosed with benign prostate hyperplasia following transrectal prostate biopsy or TURP. The third group (n=20) included patients with a PSA level of ≤4 ng/dL who underwent TURP due to obstructive complaints and histopathologically diagnosed with benign prostate hyperplasia. Patients in the third group were accepted as controls. Blood and tissue samples were collected from all participants for genetic analysis. The levels of serum PSA and *PIWIL2* expressions were compared between the groups.

Sample collection and analysis

Peripheral venous blood samples of 7–10 cc were collected from volunteers assumed to participate in the study prior to the surgical procedure (TURP or biopsy). The samples were obtained into EDTA tubes and centrifuged at 3000 g for 5 min. The sera obtained were kept at –20°C. The samples of the cases that fulfilled the inclusion criteria into groups were analyzed in a proper manner.

Genetic analysis

Leukocyte and total RNA isolations were performed from the peripheral blood samples obtained from volunteers according to the TRIzol reagent method. cDNA synthesis was performed using Transcriptor High Fidelity cDNA Synthesis Kit (Sigma-Aldrich, Taufkirchen, Germany). Gene expression analysis was performed using the LightCycler 480 II instrument (Roche Diagnostics Ltd., Rotkreuz, Switzerland), and analyses were organized using the LightCycler 480 Software (version 1.5.0. SP4).

Statistical analysis

The Student's t, the one-way ANOVA, the Mann–Whitney U, and the Kruskal–Wallis tests were used for comparison of the groups. Compatibility of the quantitative data to normal distribution was evaluated using the Shapiro–Wilk test. Data were expressed as mean (±SD) and median (min–max). A p value of <0.05 was accepted as statistically significant.

Results

The mean ages among the groups were 68.4 (±10.1), 68.5 (±7.8), and 66.5 (±10.4) years, respectively. No significant difference was observed (p=0.763). The median serum PSA levels were 28.5 (4.6–98.1) ng/mL, 8.89 (4.3–24.1) ng/mL, and 2.4 (0.3–3.8) ng/mL for the groups, respectively. A significant difference was observed between the groups with regard to the PSA level (p<0.001). The median levels of *PIWIL2* gene expression were 2.54 (0.28–9.27), 2.27 (0.6–9.38), and 1.17 (0.26–3.07), respectively. The *PIWIL2* gene expression level was lower in patients with a PSA level of <4 than in others (p=0.02). No significant difference was observed between the groups among those with a PSA level of ≥4, with and without cancer (p>0.05). Data have been presented in Table 1.

The patients were grouped as those with malignant and benign pathologies according to histopathological examination, which revealed a significantly higher level of PSA in those with adenocarcinoma (n=20) than in those with benign histopathology (n=40) (p<0.001). However, no difference was observed in the same groups with regard to the level of *PIWIL2* gene expression (Table 2).

Table 1. Comparison of age, serum PSA, and serum level of PIWIL2 gene expression between the groups

	Group 1 (n=20)	Group 2 (n=20)	Group 3 (n=20)	p
Age (years)	68.4 (±10.1)	68.5 (±7.8)	66.5 (±10.4)	0.763
PSA (ng/dL)	28.5 (4.6–98.1)	8.89 (4.3–24.1)	2.4 (0.3–3.8)	<0.001
PIWIL2	2.54 (0.28–9.27)	2.27 (0.6–9.38)*	1.17 (0.26–3.07)*	0.029*

*Shows the statistical analysis between the two groups. PSA: prostate-specific antigen; PIWIL2; Piwi-like 2

Table 2. Comparison of the levels of serum *PIWIL2* gene expression and PSA according to pathology outcomes

	Adenocarcinoma (n=20)	Benign (n=40)	p
PSA (ng/dL)	28.5 (4.6–98.1)	6.42 (0.3–24.1)	<0.001
PIWIL2	2.54 (0.28–9.27)	2.01 (0.26–9.38)	0.103

PSA: prostate-specific antigen; PIWIL2: Piwi-like 2

Table 3. Comparison of the levels of *PIWIL2* gene expression in patients diagnosed with cancer according to ISUP scores

	ISUP ≤3 (n=9)	ISUP >3 (n=11)	p
PIWIL2	1.91 (0.28–4.20)	2.72 (1.42–9.27)	0.04

PIWIL2: Piwi-like 2

Patients diagnosed with cancer were grouped according to the criteria of the International Society of Urological Pathology (ISUP) (Table 3). The *PIWIL2* level was found to be significantly higher in ISUP >3 cases than in ISUP ≤3 cases (p=0.04).

Discussion

Prostate-specific antigen is the most common serum marker used in the diagnosis of PCa.[2] However, the organ-specific nature of PSA rather than the cancer-specific nature complicates its diagnosis, which has led the researchers to identify a PCa marker that is more effective than PSA. A total of 70 patients undergoing prostate biopsy were included in a recent study, 35 of whom had adenocarcinoma and 35 had benign pathologies.[17] It was reported that the *PIWIL2* levels in tissues with carcinomas were significantly higher than those in tissues without carcinomas. In the previous study, the level of *PIWIL2* gene expression was studied in biopsied tissues following paraffin blocking. In our study, an easier and less invasive sampling of tissues was targeted. Therefore, different to the study mentioned above, the level of *PIWIL2* gene expression was investigated from serum samples. However, the outcomes of our study revealed no significant difference in the level of *PIWIL2* gene expressions between the groups with and without cancer.

Yang et al.[18] showed a correlation between the level of *PIWIL2* gene expression and metastatic prostate cancer. A similar relationship between *PIWIL2* gene and metastasis in prostate and colon cancers was also demonstrated in other recent studies.[16,17] In our study, only three patients with PCa were observed to be metastatic on diagnosis. Thus, a sample size sufficient to evaluate the correlation between metastasis and the level of *PIWIL2* gene expression could not be reached. However, when the patients were divided into two groups according to the ISUP scores, *PIWIL2* gene expression was found to be significantly higher in patients with high ISUP than in those with low ISUP (Table 3). Therefore, related to the higher level of *PIWIL2* gene expression in cancers with higher ISUP, which are accepted to be more invasive and aggressive, it may be concluded that partially compliant outcomes were obtained with those of the above-mentioned studies.

The relationship between cancer invasiveness and the level of *PIWIL2* gene expression has been investigated in cancer tissues in the literature as well. However, the sample was serum instead of cancer tissues. Furthermore, the serum level of *PIWIL2* gene expression was detected to be high not only in patients with cancer but also in patients with a PSA level of >4 ng/dL and benign histopathology as well. It has been known that the level of *PIWIL2* expression is higher in colorectal and gastric cancers, certain thyroid cancers, and several soft tissue malignancies.[15,19-21] In our study, no other malignancy was present in patients with a ≥3 *PIWIL2* level. We have no data that would explain the increase in the level of *PIWIL2* gene expression in both cases with cancer and those with benign pathologies.

On the other hand, there are studies in the literature reporting no relationship between the level of *PIWIL2* gene and urological cancers. Nickpour et al.[22] demonstrated a lower level of *PIWIL2* expression in cells with bladder carcinoma than that with normal testicular tissue. Researchers have advocated that no relationship could be present between the level of *PIWIL2* expression and bladder carcinoma.

The limitations of our study include an insufficient sample size and genetic analysis conducted from serum samples only and not from prostate tissues.

In conclusion, data obtained in our study revealed no importance of serum *PIWIL2* gene expression level for diagnosis in patients with PCa. However, it may be used as a beneficial predictor of

advanced stage PCas, compatible with previous studies in the literature conducted on cancer tissues. Further prospective studies conducted from both the serum samples and tissues are needed.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Erciyes University School of Medicine (2013/171).

Informed Consent: In this study, the verbal and written consent was obtained from all volunteers.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – A.D.; Design – A.D., H.T.; Supervision – Y.Ö.; Resources – G.S., Ş.T.T., H.A.; Materials – G.S., H.A.; Data Collection and/or Processing – G.S., Ş.T.T.; Analysis and/or Interpretation – G.S., A.D.; Literature Search – G.S., H.T.; Writing Manuscript – G.S., H.T.; Critical Review – Y.Ö., A.D.

Acknowledgements: We thank Erciyes University Scientific Research Projects (SRP) Coordination Unit which provides financial support to our project, Erciyes University Medical Genetics Department who contributed to the conduction of the study and Prof. Dr. Atila Tatlışen who shared his vast knowledge with us throughout the study.

Conflict of Interest: The authors have no conflicts of interest to declare.

Financial Disclosure: This study with the project number TTU-2014-5212 was accomplished with the financial support of Erciyes University Scientific Research Projects Coordination Unit.

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