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Original Article

TMPRSS2–ERG gene fusion in Turkish patients with localized prostate cancer: results of radical prostatectomy specimens

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ABSTRACT

Objective: Our aim was to evaluate and determine the frequency of Transmembrane protease, serine 2 (TMPRSS2)-ERG fusion in Turkish patients with clinically localized prostate cancer by using immunohistochemistry and reveal its relationship with clinicopathologic variables.

Material and methods: Radical prostatectomy specimens of 99 patients, who underwent radical retropubic prostatectomy for localized cancer, between January 2002 and December 2011 were analyzed in the study. To detect ERG fusions, monoclonal ERG antibodyclone ID: EPR3864 (Epitomics, San Diego, CA, USA) and monoclonal anti-ERG antibody (9FY) (BiocareMedical, LLC, USA) were used. The immunistochemical expression of ERG protein was assessed as positive or negative regardless of stain intensity. Patients' age, total and primary Gleason scores, PSA levels, prostate volumes, tumor volumes, tumor stages and perineural invasion status were analysed retrospectively. Total fusion rate and correlation between the variables and fusion were evaluated.

Results: Mean age, prostate volume, tumor volume, PSA value of 99 patients were 62.02 years (±5.93), 50.02 cc (±20.67), 3.19 cc (±4.16), and 9.34 ng/mL (±3.37) respectively. TMPRSS2-ERG fusion was seen in 46 (46.5%) of 99 patients. When the variables analysed with independent samples t test to predict fusion (+) status, none of them was found to be statistically significant. When evaluated by logistic regression analysis for (+) or (-) status, only tumor stage was found to be statistically significantly correlated with fusion (p=0.049).

Conclusion: The incidence of TMPRSS-ERG fusion in patients with localised prostate cancer in our study with Turkish population was found as 46.5%. Only tumor stage correlated with TMPRSS2-ERG fusion.

Keywords: Prostate Ca, TMPRSS-ERG fusion gene, tumor stage.

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Introduction

Prostate cancer is the most common cancer in men in Western societies. It constitutes 29% of all types positive cancers in men and accounts for 9% of all deaths from cancer.[1] Biochemical screening tests as prostate spesific antigen (PSA), PSA derivatives, pro-prostate specific antigen (ProPSA) and prostate health index were used to detect the tumor at an early stage.[2] Studies are ongoing to predict the relationship between histopathological results of prostate biopsy material, prostatectomy specimens and the clinicopathological factors and progression of the tumor.[3,4]

Discovery of recurrent gene rearrangements, mostly between androgen-regulated 5' Transmembrane protease, serine 2 (TMPRSS2) and ETS-Related Gene (ERG) a member of E26 transformation-specific (ETS) family, in prostate cancer[5] suggests that translocations may occur more commonly than previously assumed in epithelial tumors.[6] The fusion of TMPRSS2 and ERG seems to be specific for prostate cancer.[7] The results of prevalence studies from different countries have shown some discrepancy[8-12] which might be attributed to genetic variations between nations.[13] Many studies have been conducted to evaluate the fusion of these genes in different subtypes of prostate cancer to detect their importance in predicting aggressive forms.[10,14-16] Some controversy also remains about the association of the fusion with clinical variables, such as Gleason score, pT stage, and prognosis.[17]

Our aim was to evaluate the frequency of TMPRSS2-ERG fusion in Turkish patients with clinically localized prostate cancer by using immunohistochemistry and determine the correlation between TMPRSS2-ERG fusion, and clinicopathologic variables.

Material and methods

The patients, diagnosed with localized prostate cancer and underwent radical retropubic prostatectomy in our institute between January 2002 and December 2011, were enrolled in the study. After getting local ethics committee's approval, radical prostatectomy specimens of 99 patients were prepared for detecting TMPRSS2-ERG gene fusion.

For detecting ERG fusions, we used monoclonal ERG antibody clone ID:EPR3864 (Epitomics, San Diego, CA, USA) and monoclonal anti-ERG antibody (9FY) (Biocare Medical, LLC, USA) which were shown to be correlated with TMPRSS2-ERG fusions in previous studies.[18] Immunohistochemical analysis was performed on sections cut from tissue macroarray blocks which were previously constructed from formalin-fixed paraffin-embedded tumor tissues, and composed of 3 mm tissue cores from each tumor specimen. Immunohistochemical staining was performed using an automated stainer (Ventana MedicalSystems, Tucson, AZ, USA).

Immunohistochemical expression of ERG protein was evaluated as positive or negative regardless of the staining intensity. Only nuclear staining was considered as positive, and vascular endothelial cells were used as internal positive controls.

Patients' age, total and primary Gleason scores of prostatectomy specimens, PSA levels, prostate volumes, tumor volumes, tumor stages and perineural invasion status were analysed retrospectively. Total fusion rate and correlation between the above- mentioned variables and fusion were evaluated.

Power analysis: We planned to study 59 experimental, and 40 control subjects. Prior data indicated that probability of exposure among controls was 0.41. If the true probability of exposure among cases was 0.55, we could be able to reject the null hypothesis that the exposure rates for cases and controls were equal with probability (power) 0.275. The type 1 error probability associated with this test of this null hypothesis was 0.05.

Statistical analysis

Statistical Package for the Social Sciences 16.0 (SPSS Inc; Chicago, IL, USA) was used for statistical analysis. TMPRSS2/ERG fusion status was categorised as (+) and (-). Age, prostate and tumor volume were described as continuous, total and primary Gleason score, stage and positive perineural invasion as categorical variables. Correlation between variables and fusion (+) and (-) status was analysed with independent samples t test. Logistic regression analysis was used to evaluate the factors which were related with fusion (+) status. Statistically significant level was accepted as $p<0.05$.

Table 1. Variables of the patients

	Mean±SD
Age	62.02±5.93
PSA (ng/mL)	9.34±3.37
Prostate volume (cc)	50.02±20.67
Tumor volume (cc)	3.19±4.16

PSA: prostate spesific antigen

Table 2. Independent samples t test for detecting the variables that predict fusion (+)

Variables	Fusion (+)	Fusion (-)	p
Age	61.86±6.02	62.15±5.91	0.815
Prostate volume (cc)	46.53±12.94	52.91±25.12	0.135
Tumor volume (cc)	3.66±5.44	2.77±2.53	0.309
Total Gleason score	6.60±0.65	6.67±0.75	0.622
Primary Gleason score	3.10±0.37	3.20±0.45	0.247
Perineural invasion	0.71±0.45	0.73±0.44	0.832

Significance level was $p<0.05$. None of the variables could reach a statistically significant level

Results

Mean age, prostate volume, tumor volume, PSA value of 99 patients were 62.02±5.93 years (45-74), 50.02±20.67 cc (20-150), 3.19±4.16 cc (0.1-35), 9.34±3.37 ng/mL (1.54-17.60), respectively (Table 1). Total Gleason score was 6 in 45 (45.5%) patients, 7 in 47 (47.5%), 8 in 4 (4%) and 9 in 3 (3%) patients. Primary Gleason scores of 3, 4 and 5 were detected in 85 (85.9%), 12 (12.1%) and 2 (2%) patients, respectively. Tumor was localized in one lobe (pT2a/b) in 59 (59.6%), and two lobes (pT2c) in 40 (40.4%) patients. Perineural invasion was seen in 70 (70.7%) patients.

TMPRSS2-ERG gene fusion was seen only in 46 (46.5%) of 99 patients in our study on Turkish population.

The variables of age, tumor stage, prostate volume, tumor volume, total Gleason score, primary Gleason score and perineural invasion were analysed with independent samples t test in the prediction of fusion (+) status, and none of them was found to be statistically significant (Table 2). Logistic regression analysis was used for evaluating the correlation between variables and fusion (+) or (-) status and only tumor stage was found to be statistically significant ($p=0.049$). The TMPRSS2-ERG gene fusion were found in 41.4%, and 55% of the patients with tumors located in one and two lobes, respectively.

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Discussion

The most frequent genomic alteration in prostate cancer, detected in 50-70% of the patients from Western countries, is TMPRSS2-ERG fusion.[19,20] Socioeconomic status and access to healthcare services may explain disparities in diagnosis, treatment and survival of prostate cancer patients of different ethnicities, with different genotypes.[21] Many studies performed on the frequency rates of *TMPRSS2-ERG* gene fusions detected by immunohistochemistry *per se* or in combination with fluorescence *in situ* hybridization (FISH) test in different populations have demonstrated variations in incidence rates worldwide. The patients from United States and Europe have displayed much higher fusion rates (50%-70%) relative to Asian countries (15.9-29.7%) such as China, Korea, Malaysia, Japan and India.[11,22-26]

To date, no study has evaluated the frequency of TMPRSS2-ERG gene fusion in patients with prostate cancer in Turkish population. As a first and unique study, we evaluated the patients with localized cancer who had undergone radical retropubic prostatectomy to determine the exact l status of TMPRSS2-ERG gene fusion. Overall frequency of the fusion in our study (45.5%) was higher than Asian part of the world and similar with Western countries. We also evaluated possible correlations between fusion and some clinichopathological variables such as age, Gleason score, stage, which have yielded variable results in different studies due to heterogeneity of patient cohorts. In some studies patient age and TMPRSS2-ERG fusion were found to be correlated.[26,27] But we didn't find any significant correlation between age and the fusion. More studies are needed to demonstrate the exact state of that relationship.

The other parameter evaluated in some studies is Gleason score. Furusato et al.[18] found that higher Gleason score was associated with higher ERG gene alterations, in contrast, Darnel et al.[16] showed that lower Gleason scores are related with higher number of fusion. We evaluated total and primary Gleason scores but we could not find any significant correlation with Gleason scores and TMPRSS-ERG fusion in our study population.

Mehra et al.[9] concluded that there was a significant relation between TMPRSS2-ERG fusion and higher pathological stage. In our study we also found that pathological stage (pT2a/b vs.pT2c) was the only variable associated with positive fusion sign. Patients with tumor that invaded two prostatic lobes had higher incidence of TMPRSS2-ERG fusion than patients with one lobe involvement (p=0.049). Genetic alteration may accelerate tumoral spread in fusion (+) patients. We also evaluated the association between perineural invasion status and fusion. Our study is the first one that handles this subject. We could not find any significant correlation between perineural invasion and fusion.

The limitation of our study may be that we used only immunohistochemical analyses for detecting the fusion status. Confirmation of fusion with FISH method might have emphasized our results. One of the limitation may be the scarce number of patients with high Gleason sum.

In conclusion, the incidence of TMPRSS-ERG fusion in patients with localized prostate cancer in our study with Turkish population was found as 46.5% which is compatible with the data reported from European countries. Clinicopathological variables such as age, Gleason score, perineural invasion, prostate volume and tumor volume were not associated with fusion. In our study only tumor stage was correlated with fusion in our original study performed with the first sampling Turkish population.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Gülhane Military Medical Academy Haydarpaşa Training and Research Hospital.

Informed Consent: Written informed consent was obtained from patients who participated in this study.

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