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Original Article

Expression of Genetic Polymorphism of TMPRSS2 Gene (Rs12329760) in Different Ethnicities of Prostate Cancer Patients: A Case Control Study

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ABSTRACT

Objective: To determine the frequency and clinical relevance of the TMPRSS2 gene polymorphism (rs12329760) in prostate cancer patients of different ethnicities.

Study Design: Case-control study.

Place and Duration of Study: Department of Biochemistry, Islamic International Medical College, Rawalpindi, Surgical unit of Railway General Hospital, Rawalpindi, and Urology Department of National Institute of Rehabilitative Medicine, Islamabad, Pakistan, from Oct 21 to Sep 22.

Methodology: There were 200 participants included in this study, with 100 cases of prostate cancer and 100 age-matched controls. Blood samples were collected from the outpatient department (OPD) and ward. The Chelex method was used for DNA extraction. Tetra-ARMS Polymerase chain reaction was carried out to determine the respective allelic frequencies of the TMPRSS2 gene polymorphism (rs12329760) using specific primers.

Results: CT and TT genotypes of TMPRSS2 gene polymorphism (rs12329760) showed a significant association in prostate cancer patients (p-value<0.001). T-allele of TMPRSS2 gene polymorphism (rs12329760) also showed a significant association in prostate cancer patients (p-value<0.001). Significant association was also found in Pathans with CT and TT genotype of TMPRSS2 gene polymorphism (rs12329760) with p-values of 0.01 and 0.004. Punjabis also showed a significant association with CT genotype of TMPRSS2 gene polymorphism (rs12329760) with a p-value of 0.004.

Conclusion: TMPRSS2 gene polymorphism (rs12329760) is strongly linked to a higher risk of developing prostate cancer in Pathans and Punjabis within the Pakistani population.

Keywords: Prostate Cancer, Single Nucleotide Polymorphism, TMPRSS2 Gene Polymorphism (rs12329760).

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INTRODUCTION

Prostate cancer is one of the most common neoplastic diseases among men.[1] The worldwide expected incidence is 1.1 million cases, influenced by ethnicity and geographic location.[2] With an estimated nearly 1.4 million new cases and 375,000 deaths globally, prostate cancer was the second most common malignancy and the fifth leading cause of cancer-related death among men in 2020.[3] According to WHO figures (2020), the occurrence of prostate cancer in Pakistan is 6.7%, ranking 13th among other carcinomas.[4] The death rate per 100,000 is 1.9%, placing it 16th among other cancers in Pakistan. The rate of prostate cancer varies expressively by race, ethnicity, and geography. These variances can be explained by differences in access to screening and treatment, differences in exposure to prostate cancer risk factors, and differences in the essential biology of prostate carcinogenesis (including genomic tendency of some groups to grow biologically aggressive disease).[5]

Among all main tumors, this cancer has the highest heritability rate.[6] Many genetic susceptibility markers have been identified through genome-wide association studies, family-based studies, and candidate gene association studies.[7] Other important risk factors are family history of prostate cancer, parental consanguinity, obesity, and smoking.[8] One of the major fusion genes recognized in solid human tumors is TMPRSS2-ERG, and it is present in about 50% of localized prostate carcinomas.[9] TMPRSS2 (Transmembrane serine protease-2) is expressed in prostate epithelial cells and is essential for normal prostate function. Samples from prostate cancer patients showed an upregulation of TMPRSS2 in

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response to androgens. Specifically, TMPRSS2 fuses with ERG (Endrogen response gene), an oncogenic (cancer-promoting) transcription factor. Usually, ERG is not controlled by androgens, but after this oncogenic fusion, ERG regulation is controlled by androgens fueling prostate cancer and progression to metastasis.[10]

Deviations in a genome's base pair in DNA organization is known as single nucleotide polymorphisms (SNPs), like rs12329760, and it occurs in almost 1 out of 800 base pairs. Likewise, for a single nucleotide deviation to be considered as polymorphism, it must occur in the DNA of at least 1% of the persons. SNPs cause disparities in genes that change the protein and enzymatic mechanism of the cell. SNPs strongly affect the inheritance of genes in families and rs12329760 polymorphism is strongly associated with the susceptibility to prostate cancer. Similarly, the incidence of prostate cancer is higher in some individuals than in others.

This study aims to investigate the prevalence and clinical relevance of TMPRSS2 gene polymorphism (rs12329760) among prostate cancer patients of different ethnic backgrounds. By examining ethnic trends and genotype–phenotype correlations, the study seeks to clarify the potential role of this polymorphism as a biomarker for early diagnosis, disease stratification, and personalized treatment. Understanding such genetic variability may ultimately contribute to more effective risk prediction and tailored therapeutic strategies in prostate cancer management.

METHODOLOGY

This case-control study was conducted at the Department of Biochemistry, Islamic International Medical College, Rawalpindi, in collaboration with the Surgical unit of Railway General Hospital, Rawalpindi, and the Urology Department of National Institute of Rehabilitative Medicine, Islamabad, Pakistan, from October 2021 to September 2022 after receiving permission from the Ethical Review Committee via letter number Riphah/IIMC/IRC/21/69.

Sample size was calculated using WHO sample size calculator taking confidence level 95%, margin of error 5%, reported prevalence of prostate cancer was 6.7%4. The estimated sample size came out to be 200 patients, and a non-probability convenience sampling technique was carried out.

Inclusion Criteria: Exclusively Diagnosed cases of prostate cancer above 50 years of age belonging to any ethnicity. Controls were healthy, age and ethnicity-matched males.

Exclusion Criteria: Patients with benign prostatic hypertrophy, bladder and renal carcinoma, prostatitis, and cystitis were excluded from this study.

Written informed consent was taken from patients before taking blood samples. The outpatient department (OPD) and ward area were used for sample collection. Blood was then transported to the laboratory in Sodium-Ethylenediaminetetraacetic Acid (Na-EDTA) containing vacutainers and was preserved at 4-8 ℃. Chelex method was used for DNA extraction. Extracted DNA was then stored in Eppendorf tubes at 80℃ until further analysis. Tetra-ARMS PCR was performed, and respective allelic frequencies were recorded. Primer3Plus software was used.[11] (Table-I)

Separate PCR tubes were used for Polymerase Chain Reaction (PCR). Forward and reverse primers specific for TMPRSS2 gene were used. The final volume was 25µl for each PCR reaction, which contain 8.5µl water by Invitrogen™,12.5µl 2x Thermoscientific™ Master mix containing 0.05 U/µl Taq DNA polymerase, dNTPs and reaction buffer, 1µl of primer mixture from four designed primers (Table-I) and 3µl of extracted DNA sample to be genotyped.

PCR reaction procedure began with initial denaturation of DNA at 95℃ for 3 minutes followed by 35 amplification cycles, each comprising of denaturation at 95℃ for 30 seconds, annealing at 59℃ for 40 seconds, extension at 72℃ for 30 seconds. The final extension was carried out at 72℃ for 5 minutes. After completing 35 cycles, the amplification was finished to hold at 4℃.

The reaction products were then subjected to Agarose gel electrophoresis. The gel was prepared using 2g agarose powder mixed with 10 µg/ml of 1% Ethidium Bromide in 1x TBE buffer.

Current used was 700 mA and voltage was 100V for 50 minutes. The gel was placed on a UV transilluminator (Gene Box) by Gene Sys. The enlarged DNA fragments were seen as white bands against a dark field. A complete record of the gel was kept by capturing an image in secure SGD format with a UV camera and stored in a gel document system. (Figure)

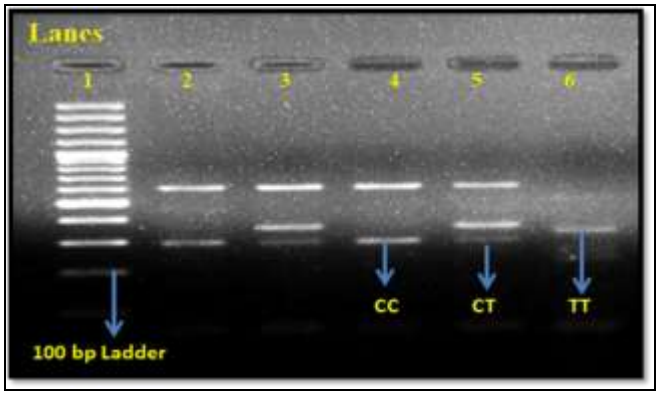

Figure: Electrophoretogram on 2% agarose gel showing amplified PCR products of TMPRSS2 gene polymorphism (rs12329760) showing CC, CT & TT genotype with DNA marker of 100 bp. Control band is at 600bp, CC genotype at 293bp and TT genotype at 353bp.Lane 1 represents Gene Ruler TM 100 bp ladder.Lanes 2 & 4 represent CC genotype. Lanes 3 & 5 represent CT genotype. Lane 6 represents TT genotype

The statistical analysis was executed via Statistical Package for Social Sciences (SPSS) version 22. Possible association between TMPRSS2 gene polymorphism in prostate cancer patients in different ethnicities was determined by using chi-square test and Independent t-test. Frequencies and percentages were determined for descriptive statistics. The statistically significant difference was indicated by *p*-value <0.05.

RESULTS

There were a total of 200 subjects with 100 cases and 100 age-matched controls. When we considered genotype frequencies, CC genotype was present in 41% cases, CT genotype in 47% cases, and TT-genotype was present in 12% cases. In controls CC genotype was present in 72% controls, CT genotype in 25% controls, and TT genotype in only 3% controls. When we considered allele frequencies of the C and T-allele, we found that C-allele was present in 88% cases and 97% controls. Whereas T-allele was present in 60% cases and 28% controls, as shown in Table-II. A highly significant association was found with CT and TT genotypes in cases with prostate cancer and TMPRSS2 gene polymorphism (rs12329760) with *p*-value<0.001. The T-allele had also shown a significant association with *p*-value <0.001 in prostate cancer patients.

The three ethnic groups were Pathans, Punjabis, and Kashmiris, as shown in Table-III. There were 51% Pathans in cases and 50% Pathans in controls. In Pathans CC genotype was present in 48.8% cases and 48.6% controls, CT genotype in 48.9% cases and 50% controls. Whereas in Pathans TT genotype was present in 66.7% cases and 33.3% controls. There were 33% Punjabis in cases and controls. In Punjabis CC genotype was present in 26.8% cases and 33.3% controls, CT genotype was present in 40.4% cases and 32.0% controls, and TT genotype in 25% cases and 33.3% controls. There were 16% Kashmiris in cases and 17% in controls. Kashmiris were having CC genotype in 24.4% cases and 18.1% controls, CT genotype in 10.6% cases and 12 % controls. The TT genotype was present in 8.3% cases and 33.3% controls. Strong association was found in Pathans with CT genotype (OR: 2.87 (95% CI: 1.21-6.80,*p*=0.01) and TT genotype (OR:14,95%CI:1.6-120.2, *p*=0.004) of TMPRSS2 gene polymorphism (rs12329760) in prostate cancer patients. Punjabis also showed a significant association with CT genotype of TMPRSS2 gene polymorphism (rs12329760) with *p*-value=0.004 (OR:5.18, 95% CI:1.73-15.43).

Table-I: Primer's SequencesOf TMPRSS2 Gene

Primer Name	Primer Sequence 5′ to 3′
rs760-FI-C	CAGGACTTCCTCTGAGATGAGTAAAC
rs760-RI-T	GACCAAACTTCATCCTTCCGA
rs760-FO	AGGAGTCTATAGAGGCCAAGGAGGA
rs760-RO	GGTGAAACCCCATCTCTAATAAAACAG

Table-II: Genotype and Allele Characteristics of Cases and Controls (n=200)

Genotype	Cases (n=100) n (%)	Control (n=100) n (%)	OR (95 % CI) *p*-value
CCCCCC	41(41)	72(72)	Ref 1
CT	47(47)	25(25)	3.30(1.77-6.12) < 0.001
TT	12(12)	03(3)	7.02(1.87-26.34) < 0.001
Allele			
C	88(88)	97(97)	1.59(0.98-2.57) 0.056
T	60(60)	28(28)	3.76(2.08-6.78) < 0.001

**OR = Odds Ratio, CI = Confidence Interval*

Table-III: Association of TMPRSS2 Gene Polymorphism (rs12329760) with Ethnicities (n=200)

Total (n=200)	Case (n=100)	Controls (n=100)	OR (95% CI) *p*-value
Ethnicities			
Pathan	51(51)	50(50)	
CC	20(48.8)	35(48.6)	Ref 1
CT	23(48.9)	14(50.0)	2.87(1.21-6.80) 0.01
TT	8(66.7)	1(33.3)	14(1.6-120.2) 0.004
Punjabi	33(33)	33(33)	
CC	11(26.8)	24(33.3)	Ref 1
CT	19(40.4)	08(32.0)	5.18(1.73-15.43) 0.004
TT	3(25.0)	1(33.3)	6.54(0.6170.23) 0.122
Kashmiri	16(16)	17(17)	
CC	10(24.4)	13(18.1)	Ref 1
CT	5(10.6)	3(12.0)	2.16(0.41-1.30) 0.43
TT	1(8.3)	1(33.3)	1.3(0.07-23.43) 1.00

**OR = Odds Ratio, CI = Confidence Interval*
Ref 1: It means that the odds ratio is 1.

DISCUSSION

This study aimed to investigate the potential role of the TMPRSS2 gene polymorphism (rs12329760) in the etiology and pathogenesis of prostate cancer across

different ethnic groups in Pakistan. The findings revealed that the single-nucleotide polymorphism rs12329760 of the TMPRSS2 gene may serve as a genetic risk factor for prostate cancer, particularly among the Pathan and Punjabi ethnic populations in Pakistan.

Nearly all prostate tumors are adenocarcinomas. Prostate cancer is the leading non-cutaneous cancer in men in many parts of the world, although incidence and mortality rates vary widely by region. The incidence and death rate of prostate cancer are higher in Black men than in White men. There is increasing evidence that prostate cancer risk, aggressiveness, and prognosis vary significantly by race, ethnicity, and geography.[12]

At the end of this study, we established a significant association of the CT-genotype (OR: 3.30, 95% CI: 1.77-6.12, p-value<0.001), TT-genotype (OR: 7.02, 95% CI: 1.87-26.3, p<0.001), and T-allele (OR: 3.76, 95% CI: 2.08-6.78, p<0.001) of the TMPRSS2 gene polymorphism (rs12329760) with increased risk of prostate cancer. This aligns with the study conducted by Bhanushali *et al*., among the Indian population, which concluded that this gene polymorphism (rs12329760) is strongly associated with a heightened risk of prostate cancer.[13]

Another study, conducted by Perdomo *et al*., in a Southwestern Colombian population free of prostatic carcinoma, showed that the gene polymorphism (rs12329760) was not common in the prostate cancer-free population, indicating a strong association between the TMPRSS2 gene polymorphism (rs12329760) and prostate cancer.[14] A Japanese study was conducted by Maekawa *et al*., to investigate the association between the TMPRSS2 gene polymorphism (rs12329760) and the risk of prostate cancer in Japanese men. This study showed that such a polymorphism was meaningfully related to the incidence of sporadic prostate cancer in Japanese men.[15]

In our study, two ethnic groups Pathans and Punjabis had shown a significant association with TMPRSS2 gene polymorphism (rs12329760). Pathans had shown significant association with the CT genotype with OR: 2.87 (95% CI: 1.21-6.80) and p=0.01, and TT genotype with OR: 14 (95% CI: 1.6- 120.2) and p=0.004 in prostate cancer patients. Whereas Punjabis had also shown a significant association with CT genotype (OR: 5.18,95% CI:1.73-15.43, p=0.004) of TMPRSS2 gene polymorphism (rs12329760) in prostate cancer patients. This was in accordance with the study done by Kimuru *et al*., which showed that TMPRSS2 gene polymorphism was strongly linked with the incidence of prostate cancer in Asian men.[16]

Multiple genes play a role in the development of this disease.[17] Achard *et al*., highlighted that patients with prostate cancer diagnosed with distant metastasis at initial presentation require early intervention. Close monitoring of PSA and serum testosterone changes is necessary during the process of endocrine therapy. After entering the CRPC stage, the etiological classification precision treatment can improve the therapeutic effect and improve the prognosis of patients. Therefore, there is a need to identify new genomic markers that can serve as indicators to predict the genes involved in this carcinoma as reported by Brawley *et al*.[18] He has also signified that racial disparities in prostate cancer survival are well documented, the relative importance of contributing factors remains unclear. Timely and efficient diagnostic markers can reduce the debilitating influence of various factors affecting variety of races and ethnicities.

The current study highlights the potential of identifying patients who visit urology OPDs with high PSA levels to undergo genetic testing for TMPRSS2 gene polymorphism (rs12329760) (CT and TT genotypes and T-allele) and be carefully monitored for early detection and treatment. The results of this study can be integrated with other diagnostic methods already available to diagnose and manage prostate cancer patients in our population.[19,20]

LIMITATIONS OF STUDY

In the current study, the authors have genotyped a single nucleotide polymorphism of TMPRSS2 gene and three ethnicities in this study. Future studies are required to see the association of other single-nucleotide polymorphisms of this gene in prostate cancer patients in other ethnicities as well.

RECOMMENDATIONS

Non-modifiable risk factors of prostate cancer, such as family history of prostate cancer and cousin marriage, can also be studied in the future with this gene polymorphism in prostate cancer patients.

CONCLUSION

Single-nucleotide polymorphism (rs12329760) of TMPRSS2 gene is found to be a genetic risk factor for prostate cancer in Pathans and Punjabi ethnic groups in the Pakistani population. The identification of this association highlights the importance of genetic diversity and ethnic-specific risk profiling in understanding the molecular

mechanisms underlying prostate cancer. These findings also emphasize the need for further functional and large-scale population studies to confirm this association and to explore its potential role in personalized risk assessment, early diagnosis, and targeted therapy for prostate cancer in ethnically diverse populations.

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Authors' Contribution

Following authors have made substantial contributions to the manuscript as under:

SS & AR: Data acquisition, data analysis, critical review, approval of the final version to be published.

MA & SI: Study design, data interpretation, drafting the manuscript, critical review, approval of the final version to be published.

TAK & FM: Conception, data acquisition, drafting the manuscript, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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