ORIGINAL ARTICLE

ASSOCIATION OF SMOKING WITH PROSTATE CANCER IN OLD AGE PAKISTANI MALES WITH *TMPRSS2* GENE POLYMORPHISM (rs12329760)

Saadia Sadiq, Sarwat Abbasi*, Bushra Rehman, Sahar Ikram***, Anwar Shahzad†, Tehseen Ashraf Kohistani††**

Department of Biochemistry, Abbottabad International Medical College, *Ayub Medical College, Abbottabad, **Foundation University Medical College, ***Federal Medical and Dental College, Islamabad, †Department of Community Medicine, Abbottabad International Medical College, Abbottabad, ††Department of Medicine, CMH, Bahawalpur, Pakistan

Background: Second most common cause of cancer related deaths in men is prostate cancer. Obesity, old age and smoking are the common modifiable risk factors for this cancer. Smoking and prostate cancer together can lead to higher mortality rate, especially in heavy smokers. *TMPRSS2* gene polymorphism (rs12329760) is also associated with this cancer in smokers. This study was conducted to find out the association of smoking with prostate cancer patients with *TMPRSS2* gene polymorphism (rs12329760) in old age Pakistani men. **Methods:** It was a case control study conducted from Oct 2021 to Sep 2022 in Railway Hospital, Rawalpindi, and Urology Department, National Institute of Rehabilitative Medicine, Islamabad. Sample size was 200 with 100 prostate cancer cases and 100 age-matched controls. Chelax method was used for DNA extraction from blood samples. Polymerase Chain Reaction (PCR) was carried out to find out the relationship of smoking with prostate cancer development with *TMPRSS2* gene polymorphism (rs12329760). **Results:** Significant association was found in smokers in age group 76–100 years with CT-genotype of *TMPRSS2* gene polymorphism (rs12329760) in cases (p<0.001). **Conclusion:** Smoking is a modifiable risk factor for development of prostate cancer with *TMPRSS2* gene polymorphism (rs12329760) in old age Pakistani men.

Keywords: Prostate cancer, Polymerase chain reaction, Smoking, *TMPRSS2* Gene

Pak J Physiol 2024;20(2):54–7, **DOI:** https://doi.org/10.69656/pjp.v20i2.1656

INTRODUCTION

The second most common tumour in men is prostate cancer. It is also one of the causative factor for cancer related deaths in men in developing countries.[1] The incidence of prostate cancer is more in western compared to Asian countries.[2] The intake of fat, red meat and dairy products are associated with progressive risk for prostate cancer.[3] Old age, obesity and smoking are also important risk factors for this cancer.[4] More than 70% of all the prostate cancer patients in the United States were typically more than 65 years and very rare cases were noticed among men less than 50 years. After the age of 50 years, the incidence and death rates present a loud increase with death rate as 1.9% and it is graded at 16th position as compared with other cancers rendering to death rate in Pakistan.[5] Genetic factors like chromosomal changes and gene mutations like single nucleotide polymorphism (*TMPRSS2* gene polymorphism, SNP rs12329760) play major role in pathogenesis of prostate cancer.[6,7] Highly expressed-androgen responsive gene in prostate epithelial cells and prostate carcinoma is *TMPRSS2*. This gene is expressed in normal and neoplastic prostate tissue.[8,9] A main landmark in prostate cancer investigation was the identification of repeated fusions between *TMPRSS2* and Oestrogen Responsive Gene (ERG).[10]

Cigarette smoking is a modifiable risk factor for the development of prostate cancer. Several proposed mechanisms have been explained through which smoking can develop risk of prostate cancer. Circulating levels of steroid hormones are disturbed in smokers. Higher levels of bio available testosterone and lower levels of estradiol are found in smoker men.[11] Significantly positive correlations (p<0.001) were found between cigarettes smoked per day and serum total rostenedione as well as total and free testosterone levels in men.[12] This is noteworthy because its more active metabolite Dihydrotestosterone (DHT) are not only essential for normal growth and development of prostate gland but are also found involved in malignant changes, i.e., increased cell proliferation in prostate gland. When œstrogens are considered, they basically act on the hypothalamus and pituitary to reduce the production of testicular androgen.[13] Proficiently, cigarette smoking may produce a hormonal atmosphere that is favourable for growth and development of prostate cancer. Cigarettes comprise notable levels of cadmium, which is associated with prostate carcinogenesis.[14,15] One or both of these mechanisms might support a link between smoking and prostate cancer. When the *TPMRSS2* gene polymorphism (rs12329760) was considered in smoker prostate cancer patient, highly significant results were obtained.

This study was conducted to identify the association of smoking in old age Pakistani men with *TMPRSS2* gene polymorphism (rs12329760).

METHODOLOGY

It was a case control study carried out at the Biochemistry Department of Islamic International Medical College, Rawalpindi in alliance with the Surgical Unit of Railway General Hospital, Rawalpindi and Urology Department of National Institute of Rehabilitative Medicine, Islamabad from Oct 2021 to Sep 2022.

Samples were collected after permission from the Ethical Review Committee under letter No. Riphah/IIMC/IRC/21/69. Non-probability convenience sampling technique was used and the sample size was adjusted by the formula, $n= z^2 (pq)/e^2$ where disease prevalence (p) of prostate cancer was 6.7%[16], 1.96 was considered as confidence interval (z) and margin of error (e) at 5%. Aged above 50 year diagnosed cases of prostate cancer willing to participate, and healthy age matched controls were selected. Benign prostatic hypertrophy, bladder and renal carcinoma patients were excluded from this study.

Informed written consent was taken before blood sampling in the Outpatient Department (OPD). Blood samples were preserved at 4–8 °C in EDTA containing 1.5 ml tubes. Chelex method was used for DNA extraction. Extracted DNA was then stored in Eppendorf tubes at 80 °C until further analysis. Tetra-ARMS Polymerase Chain Reaction (PCR) was performed and respective allelic frequencies were recorded.[17] Primer3Plus software was used.[18] Forward and reverse primers designed for *TMPRSS2* gene were used, and 5 to 3′ end was sequenced. Primers used were the following:

rs 760- RI-T (GACCAAACTTCATCCTTCCGA),
rs 760- FO (AGGAGTCTATAGAGGCCAAGGAGGA),
rs 760-RO (GGTGAAACCCCATCTCTAATAAAACAG) and
rs 760-FI-T (CAGGACTTCCTCTGAGATGAGTAGAT)

Twenty-five µL was the final volume for each PCR reaction, which contain 8.5 µL water by Invitrogen™, 12.5 µL 2× Thermoscientific™ Master mix containing 0.05 U/µL Taq DNA polymerase, dNTPs and reaction buffer, 1 µL of primer mixture from four designed primers, and 3 µL of extracted DNA sample for genotyping.

For polymerase chain reactions, initial denaturation of DNA was carried at 95 °C for 3 minutes followed by 35 amplification cycles, each consisting of denaturation at 95 °C for 30 seconds, annealing at 59 °C for 40 seconds, extention at 72 °C for 30 seconds. Final extension was carried out at 72 °C for 5 minutes. After completing 35 cycles, the amplification was completed to hold at 4 °C. Then Agarose gel electrophoresis was run using current of 700 mA and 100 v for 50 minutes. Then the gel was visualized to view the DNA fragments as white band against dark field on a UV transilluminator by Gene System. UV camera was used to store the images from gel document system.[19]

Data were analysed on SPSS-22. Independent *t*-test and Chi-square test were applied to find out the possible association between smoking and *TMPRSS2* gene polymorphism (rs12329760) in aged men. Frequencies and percentages were calculated for descriptive statistics, and $p \leq 0.05$ was considered statistically significant.

RESULTS

In 200 total subjects, 100 were cases of already diagnosed prostate cancer and 100 were healthy age matched who served as controls. Forty-one percent of the cases were in age group 50–75 years and the rest were in age group 76–100 years. In cases the mean age was 77±8.4 (range=59–93) years. In controls the mean age was 77±9.1 (range 54–94) years. Thirty percent of controls and 64% of cases were smokers.

There were 41% cases and 43% controls included in 50–75 years age group. The CC genotype was present in 40 (97.60%) cases and 43 (100%) controls, CT genotype was present in 1 (2.40%) cases and 0 (0.0%) controls and TT genotype was present in 0 (0.0%) cases and controls in age group 50–75 years. In age group 76–100 years there were 59 (59%) cases and 57 (57%) controls. The CC genotype was present in 1 (1.70%) case and 29 (50.90%) controls. CT genotype was present in 46 (78.0%) cases and 25 (43.90%) controls. TT genotype was present in 12 (20.30%) cases and 3 (5.30%) controls. Significant association was found in age group 76–100 years with CT-genotype of TMPRSS2 gene polymorphism (rs12329760) in cases with $p<0.001$. (Table-1).

In cases, CC genotype was present in 9 (14.10%) smokers, CT genotype in 44 (68.80%) smokers and TT genotype was present in 11 (17.20%) smokers. In controls, CC genotype was present in 5 (16.70%) smokers, CT genotype in 23 (76.70%) smokers and TT genotype was present in 2 (6.70%) smokers. Heterozygous CT genotype of TMPRSS2 gene polymorphism (rs12329760) had shown significant association ($p<0.001$) in smoker prostate cancer patients. (Table-2)

Table-1: Association of different age groups with *TMPRSS2* gene polymorphism (rs12329760) [n (%)]

Variable	Cases (n=100)	Controls (n=100)	OR (95%CI)	*p*
Age 50–75 Yr.	**41 (41)**	**43 (43)**		
CC	40 (97.60)	43 (100)	Ref 1	
CT	1 (2.40)	0 (0.0)	-	0.11
TT	0 (0.0)	0 (0.0)	.-	0.10
Age 76–100 Yr.	**59 (59)**	**57 (57)**		
CC	1 (1.70)	29 (50.9)	Ref 1	
CT	46(78.0)	25 (43.9)	53.36 (6.85–415.37)	**<0.001**
TT	12 (20.30)	3 (5.3)	116 (10.94–1229.88)	1.884

Ref 1: Odds ratio is equal to 1, $p \leq 0.05$ clinically significant, CI: Confidence interval

Table-2: Association of Smoking with *TMPRSS2* gene polymorphism (rs12329760) (n=200) [n (%)]

Variable	Cases n=100	Controls n=100	OR (95%CI)	*p*
Smokers	**64 (64)**	**30 (30)**		
CC	9 (14.10)	5 (16.70)	Ref 1	
CT	44 (68.80)	23(76.70)	1.06 (0.31–3.54)	**<0.001**
TT	11 (17.20)	2(6.70)	3.05 (0.47–19.65)	0.23
Non-Smokers	**36 (36)**	**70 (70)**		
CC	32 (88.90)	67 (95.70)	Ref 1	
CT	3 (8.30)	2 (2.90)	3.14 (0.49–19.73)	0.33
TT	1 (2.80)	1 (1.40)	1.00	

Ref 1: Odds ratio is 1, $p \leq 0.05$ is clinically significant

Electrophoretogram showed amplified PCR products of *TMPRSS2* gene polymorphism (rs12329760) with CC and CT genotype in cases with DNA marker of 100 bp. Lanes 1, 3, 4, 6, 7, 9, 11, 13, 14, 16, and 17 represent CC genotypes and Lanes 2, 5, 8, 12, 15, and 18 represent CT genotypes. (Figure-1).

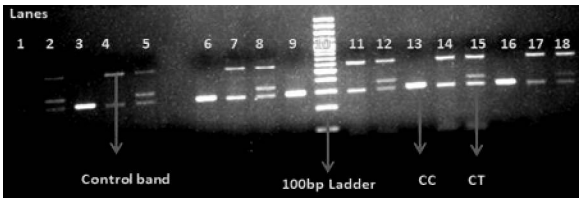

Figure-1: Electrophoretogram

DISCUSSION

Prostate carcinoma is the second most common male malignancy. It is a disease of old age and age is the chief risk factor for this cancer.[20] The average age of diagnosis for males with prostate cancer is more than 75 years and only 10% of males with prostate cancer are younger than 55 years at the time of diagnosis.[21] However, the ill-defined cancers of prostate are increasing in young men. The prostate cancer associated death rate between young men with high grade tumour is more compared to old men.[22] This recommends a different mechanism of prostate cancer development and the possible roles of sole oncogenic process among young and old men.[22]

This study was conducted to see the possible role of *TMPRSS2* gene polymorphism (rs12329760) in aetiology and pathogenesis of prostate cancer in old age smokers. We found significant association between *TMPRSS2* gene polymorphism (rs12329760) and age group 76–100 years with CT-genotype of *TMPRSS2* gene polymorphism (rs12329760). Significant association was also found between smoking and CT-genotype of *TMPRSS2* gene polymorphism (rs12329760).

Very few studies are found in literature regarding prostate cancer genetics. A study by Bhanushali A *et al*[23] on a large cohort of Indian prostate cancer cases found the association of single nucleotide polymorphism rs12329760 with *TMPRSS2* ERG fusion. Our study is in accordance with that study. A study by Sharad S *et al*[24] found that more than 65% of all the diagnosed prostate cancer cases were above the age of 65 years. Our study has also found the association of prostate cancer in old age group (76–100 years). Our findings for association of the modifiable risk factor (smoking) with prostate cancer is in accordance with the review study of Ha Chung B *et al*[25] who explained that smoking may increase the risk of prostate cancer by affecting the steroid levels and contain many carcinogens. Bae JM *et al*[26] found no association between smoking and risk of prostate cancer. Studies done in Pakistan and North India by Bashir MN *et al*[27] and Thakur H *et al*[28] had shown that smoking increased the risk of prostate cancer. Our study is unique in that it not only considered the association of *TMPRSS2* gene polymorphism (rs12329760) with prostate cancer but it also considered smoking in prostate cancer patients with this gene polymorphism.

CONCLUSION

CT-genotype of *TMPRSS2* gene polymorphism (rs12329760) is a genetic risk factor for development and progression of prostate cancer in old age smokers. Smoking is a modifiable risk factor linked with prostate cancer in old age men with *TMPRSS2* gene polymorphism (rs12329760).

RECOMMENDATIONS

Other modifiable risk factors like diet (fats, meat, fish, eggs and dairy products), physical exercise, and obesity may also be studied on large scale with this gene polymorphism in prostate cancer patients.

REFERENCES

1. Ammar KA, Altaee MF. Recurrent of *TMPRSS2* genetic polymorphism and its role in Iraqi patients with prostate cancer. Iraqi J Agric Sci 2022;53(2):272–7.
2. Idrees R, Fatima S, Abdul-Ghafar J, Raheem A, Ahmad Z. Cancer prevalence in Pakistan: meta-analysis of various published studies to determine variation in cancer figures resulting from marked population heterogeneity in different parts of the country. World J Surg Oncol 2018;16(1):129.
3. Thunders M, Delahunt B. Gene of the month: *TMPRSS2* (transmembrane serine protease 2). J Clin Pathol 2020;73(12):773–6.
4. Rebbeck TR. Prostate cancer genetics: variation by race, ethnicity, and geography. Semin Radiat Oncol 2016;27(1):3–10. DOI: 10.1016/j.semradonc.2016.08.002.
5. Porzycki P, Ciszkowicz E. Modern biomarkers in prostate cancer diagnosis. Cent European J Urol 2020;73(3):300–6.
6. Bressi B, Iotti C, Cagliari M, Fugazzaro S, Cavuto S, Bergamaschi FAM, Moscato A, Costi S. Physical exercise habits, lifestyle behaviors, and motivation to change among men with prostate cancer: a cross-sectional study. Support Care Cancer 2022;30(6):5017–26.
7. Nanda S, Dash DK, Sahu AK, Aboulthana WM, Tripathi V, Chanda R, *et al*. Prostate Cancer : A short discussion. J Hosp Pharm 2022;17(1):12–9.
8. Zheng G, Sundquist J, Sundquist K, Ji J. Prostate cancer incidence and survival in relation to prostate cancer as second cancer in relatives. Cancer Med 2022;11(10)2117–24.
9. Fang L, Li D, Yin J, Pan H, Ye H, Bowman J, *et al*. *TMPRSS2-ERG* promotes the initiation of prostate cancer by suppressing

oncogene-induced senescence. Cancer Gene Ther 2022;29(10):1463–76.
10. Hussain M, Jabeen N, Amanullah A, Baig AA, Aziz B, Shabbir S, *et al*. Molecular docking between human *TMPRSS2* and SARS-CoV-2 spike protein: conformation and intermolecular interactions. AIMS Microbiol 2020;6(3):350–60.
11. Talluri R, Domgue JF, Gritz ER, Shete S. Assessment of trends in cigarette smoking cessation after cancer diagnosis among US adults, 2000 to 2017. JAMA Network Open 2020;3(8):e2012164.
12. Jiménez-Mendoza E,Vázquez-Salas RA, Barrientos-Gutierrez T, Reynales-Shigematsu LM, Labra-Salgado IR, Manzanilla-García HA, *et al*. Smoking and prostate cancer: A life course analysis. BMC Cancer 2018;18(1):160.
13. Jassem J. Tobacco smoking after diagnosis of cancer: clinical aspects. Transl Lung Cancer Res 2019;8(Suppl 1):S50–8.
14. Nuru F,Osei E, Kassim R. A review of the effects of tobacco smoking on the treatment of prostate cancer. J Radiother Pract 2022;21(1):109–16.
15. Miller EA, Pinsky PF, Pierre-Victor D. The relationship between diabetes, prostate-specific antigen screening tests, and prostate cancer. Cancer Causes Control 2018;29:907–14.
16. UICC. GLOBOCAN 2020: New Global Cancer Data. UICC 2020. URL: https://www.uicc.org/news/globocan-2020-global-cancer-data.
17. Applied Biosystems Veriti™ Thermal Cycler User Guide. Available from: https://www.yumpu.com/en/document/view/8844059/applied-biosystems-veritiitm-thermal-cycler-user-guide-pn-4375799
18. Primer3Plus-Pick Primers. URL: https://www.primer3plus.com/
19. Quick Guide Agarose Electrophoresis. Available from: https://www.edvotek.com/quick-guide-agarose-electrophoresis
20. Yang X, Chen H, Zhang S, Chen X, Sheng Y, Pang J. Association of cigarette smoking habits with the risk of prostate cancer: a systematic review and meta-analysis. BMC Public Health 2023;23(1):1150.
21. Giona S. The Epidemiology of Prostate Cancer. In: Bott SRJ, Ng KL, (Eds). Prostate Cancer. Chapter-1. Brisbane (AU): Exon Publications; 2021. p.1–16.
22. Abida W, Cyrta J, Heller G, Prandi D, Armenia J, Coleman I, *et al*. Genomic correlates of clinical outcome in advanced prostate cancer. Proc Natl Acad Sci USA 2019;116(23):11428–36.
23. Bhanushali A, Rao P, Raman V, Kokate P. Status of *TMPRSS2-ERG* fusion in prostate cancer patients from India: correlation with clinico-pathological details and *TMPRSS2* Met160Val polymorphism. Prostate Int 2018;6(4):145–50.
24. Sharad S, Allemang TC, Li H, Nousome D, Ku AT, Whitlock NC, *et al*. Age and tumor differentiation-associated gene expression based analysis of non-familial prostate cancers. Front Oncol 2021;10:584280.
25. Ha Chung B, Horie S, Chiong E. The incidence, mortality and risk factors of prostate cancer in Asian men. Prostate Int 2019;7(1):1–8.
26. Bae JM, Li ZM, Shin MH, Kim DH, Lee MS, Ahn YO. Cigarette smoking and prostate cancer risk: negative results of the Seoul Male Cancer Cohort Study. Asian Pac J Cancer Prev 2013;14:4667–9.
27. Bashir MN, Ahmad MR, Malik A. Risk factors of prostate cancer:a case-control study in Faisalabad, Pakistan. Asian Pac J Cancer Prev 2014;15:10237–40.
28. Thakur H, Gupta L, Sobti RC, Janmeja AK, Seth A, Singh SK. Association of *GSTM1T1* genes with COPD and prostate cancer in North Indian population. Mol Biol Rep 2011;38:1733–9.

Address for Correspondence:

Dr Saadia Sadiq, Assistant Professor, Department of Biochemistry, Abbottabad International Medical College, Abbottabad, Pakistan, **Cell:** +92-333-5057440
Email: saadiasadiq@gmail.com

Received: 24 Mar 2024 **Reviewed:** 26 May 2024 **Accepted:** 3 Jun 2024

Contribution of Authors:

SS: Substantial, data collection, data analysis and interpretation
SA: Data collection, data analysis, manuscript writing and interpretation
BR: Data collection and data analysis
SI: Data collection and data analysis
AS: Critical review
TAK: Statistical analysis

Conflict of Interest: None
Funding: None