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Implications of risk conferred by 5p15.33 loci genetic variants; human telomerase reverse transcriptase rs2736098 and rs2736100 in predisposition of bladder cancer

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ABSTRACT

Background: The polymorphic variations of human telomerase reverse transcriptase (*hTERT*) gene play an important role in predisposition to carcinogenesis. The current study aimed to elucidate the genetic predisposition to bladder cancer in two important variants, rs2736098 and rs2736100 of *hTERT* gene.

Materials and methods: Confirmed 130 patients of bladder cancer and 200 healthy controls were genotyped by PCR-RFLP to determine different variants of *hTERT* rs2736098 and rs2736100.

Results: *hTERT* rs2736098 homozygous variant AA genotype frequency was observed to significantly differ 2-fold between cases and controls (26.15% *vs.* 13.5%) (p = 0.02). In addition, rare 'A' allele significantly differed among two groups (cases: 47% versus controls: 39%: p = 0.03). *hTERT* rs2736098 was observed to be presented significantly more in high stage tumors (p = 0.02). *hTERT* rs2736100 genotype AA or variant allele A showed no significant difference between cases and controls. Haplotype CA displayed significantly different pattern of frequency as 0.5 in cases as compared to 0.16 in controls (p < 0.0001). Combination of variant A/G haplotype frequency implicated more in cases than in controls (0.34 *vs.* 0.14, p = 0.001).

Conclusions: It is concluded that *hTERT* rs2736098 polymorphic variant has a vital role to confer a strong risk to bladder cancer in our population. Further, *hTERT* haplotypes CA and AG in*hTERT* could prove to be a promising tool to screen the risk for bladder cancer.

Key words: human telomerase reverse transcriptase; bladder cancer; homozygous variant; haplotype; allele

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Introduction

Bladder cancer (BC) is one of the most stressful prevalent cancers for men and women and, thus, requires significant expenditure on health care. BC is related to diverse risk factors that prominently include smoking, occupations, some drugs, and family history [1]. Although bladder cancers in

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numerous cases occur due to the exposure to various hazardous aspects, there are conditions where this tumor arises exclusive of these risk factors. This proposes that propensity of many genes may participate in the etiology of bladder cancer. Even though the frequency rate of bladder cancer has shown a constant rise over the last few decades, the rate has also declined recently in some geographic areas due to curtailment of exposure to risk factors [2]. It is the 6th most common cancer in men and the 9th most common cause of cancer related mortalities [3]. In a study by Arshad (2012) [4], overall urinary tract cancers here in Kashmiri population represent 9.1% of all common cancers wherein frequency of bladder cancer amounts to 5.9%.

Newly conducted genome-wide association studies have recognized germline variants as significant contributors in the pathogenesis of BC [5]. Genetic single nucleotide polymorphisms (SNPs) have been substantiated to confer a little but absolute risk to different cancers where their individual or combined variants can be a rationale for the disproportion of vital metabolism implicated in cancer predilection [6]. Although there is a range of polymorphic variations that cause predisposition to bladder cancer, currently, human telomerase reverse transcriptase (*hTERT*) is an important gene — a catalytic subunit of the telomerase [7] implicated in bladder cancer. Telomerase retains stability in the telomere regions to maintain the genetic information which consequently shorten with each replication cycle [8–10].

While in normal human tissues telomerase activity is repressed, in tumors, its activity is restored that implies telomerase involvement in malignant conversion of tumor [11]. Over expression of the *hTERT* gene can probably direct the cell to unlimited division that becomes a cause of tumor development in different forms [12]. The *hTERT* gene contains different genetic polymorphic variants that relate with risk to cancers [13]. The *hTERT*rs2736098(G>A) and rs2736100(C>A) polymorphisms are the most frequently studied SNPs and their relationship with the risk of cancer has been demonstrated in different malignancies [14–16]. *hTERT*rs2736098 SNP impacts the telomerase action and curtails telomere length owing to its propensity in the gene regulatory elements [17]. It has been substantiated recently from two meta-analyses that the association of variant rs2736098 with cancer risk is in coherence [18, 19]. The *hTERT* rs2736100 C allele has been seen to be related with long telomeres in white blood cells [20]. The relation of this SNP with cancer predisposition has been broadly investigated where the reports are questionable.

Therefore, *hTERT* gene polymorphic variations that show dissimilarities among different individuals or ethnic-racial groups' confer risk and severity to cancer and may be perceived as an important candidate gene for bladder cancer. Although *hTERT* gene polymorphic nucleotide variations seem biologically connected for their possible impact on bladder tumors but evidence to support it is very scarce, especially in the Indian subcontinent. Therefore, the current case-control study was initiated to demonstrate the frequency and association between *hTERT* gene polymorphic variants (rs2736100 and rs2736098) and bladder cancer in a highly ethnic Kashmiri population (North India).

Materials and methods

Study population

The current study was taken up at the Advanced Centre for Human Genetics and Department of Urology in Sheri-I-Kashmir Institute of Medical Sciences (SKIMS) Srinagar, India, between 2018 and 2020.The present study enrolled consecutively all the prospective cases of 130 bladder cancer patients that were frequency matched to age and gender with 200 healthy controls (144 males and 56 females) free from any kind of malignancy, in particular the urinary tract. BC patients included 103 (79%) males and 27 (21%) females with a ratio of 4:1, respectively. The controls were almost frequency matched to cases and no gender, age or smoking related differences were observed among both groups ($p > 0.05$). These subjects (case-control) were studied prospectively and were randomly recruited from the Department of Urology, SK Institute of Medical Sciences (SKIMS), J&K (India). All the cases were chosen and only their confirmation was ascertained as transitional cell carcinoma (TCC) by histopathological examination. The study was approved by the Ethics Committee of SK Institute of Medical Sciences (SKIMS Study ref: IEC-SKIMS Protocol #RP 25/2019), and all participating patients' approvals were obtained through a native written information consent form. Peripheral blood

sample (5 mL) and corresponding tumor tissue samples were collected from the Department of Urology (SKIMS) and were preserved at –20°C for analysis.

PCR amplification and SNP detection by PCR-RFLP

DNA was extracted from blood samples using the phenol chloroform method and also by using DNA Extraction kit (Zymo Research Corporation, USA). PCR-RFLP genotyping procedure was used to detect possible different genotypes of *hTERT* SNPs. For *hTERT* rs2736100, primers used were F: 5'-GGTGCCTCCAGAAAAGCAG-3' R: 5-GACACGGATCCAGGACCTC-3'. The following PCR protocol was used: 94°C for 5min; 35 cycles of 94°C for 30 s, 56°C for 30 s, 72°C for 30 s; and final extension cycle 72°C for 7 min. The PCR product was directly digested with *SfcI* restriction enzyme for 3 hours at 60°C. Digestion with *SfcI* produced an uncut 161-bp fragment from the mutant allele (A) and 106-bp and 55-bp fragments from the wild allele (C) [21]. For *hTERT* rs2736098, primers were F: 5'-GCCAGACCCGCCGAAGAAG-3' R: 5´-GCGCGTGGTCCCAAGCAG-3´. The PCR protocol was: 94°C for 30s, 65°C for 30s, 72°C for 30s; and final extension cycle 72°C for 7min. The PCR product was directly digested with *PspOmI* restriction enzyme at 37°C overnight and yielded an uncut 379-bp fragment from the mutant allele (A) and 289-bp and 90-bp fragments from the wild-type allele (G) [22]. PCR digested amplicons of both SNPs were put to electrophoresis on 3% agarose gel and visualized with ethidium bromide in a gel documentation system (Protein simple, Alpha Imager). The representative pictures of RFLP for both SNPs of *hTERT* are given in Supplementary Figures 1 and 2. To ensure quality control, distilled water was used instead of DNA as a negative control. We chose 10% of the samples randomly from both groups for RFLP to confirm reproducibility of the results and the experiments were conducted by researchers who were blinded with previous genotype findings to avoid bias.

Statistical analysis

Statistical evaluation was done by using IBM Statistics SPSS software (Version-23). The cases and controls were compared using the chi square test for categorical variables like sex and age of the demographic variables. A goodness-of-fit chi-square test was employed to evaluate whether the polymorphisms were in Hardy-Weinberg equilibrium between cases and controls. Odds ratios (OR) were used as estimates of the relative risk, and 95% confidence intervals (CI) were calculated to estimate the association between certain genotypes or other related risk factors of bladder cancer. The patients were followed up to determine the overall survival (OS) from the date of the diagnosis and were deduced at the time patient developed a new lesion. Different tests for homogeneity of proportions including Chi square and Kaplan Meier (KM) analysis to evaluate survival outcome probabilities were used to determine significance of the distribution patterns with respect to different clinico-analytical parameters. The haplotype association analysis was performed by using SPSS software (Version-23). Statistical significance was set at the level of $p < 0.05$.

Results

The current study enrolled 130 bladder cancer patients and 200 healthy controls free from any malignancy where no gender status, age or smoking related differences were observed between two groups ($p > 0.05$). Overall 75 (57.69%) cases belonged to lower stages pTa/pT1 and the cases that were non-smokers numbered as 42 (32.3%) *vs.* 88 (67.7%) smokers. The details of other clinic-pathological characteristics, like smoking status, grade and gender are given in Supplementary File — Table S1.

The details about cases and controls with overall genotypic/allelic frequencies of *hTERT* rs2736100/rs2736098 are shown in Table 1. The *hTERT* SNPs in controls were in Hardy-Weinberg Equilibrium (HWE).

In BC cases, the observed frequencies of *hTERT*rs2736098 G/A genotypes GG, GA and AA were 30.76%, 43.07% and 26.15% as compared to 35%, 51.5% and 13.5%, respectively, ($p < 0.05$) in controls. Homozygous AA genotype frequency was observed to significantly differ between cases and controls as 26.15% *vs.* 13.5% respectively [odds ratio (OR) = 2.20, confidence interval (CI) = 1.16–4.16), $p = 0.02$]. In addition, rare 'A' allele significantly differed between the two groups (cases: 47% *vs.* controls: 39%) (OR = 1.5, CI = 1.02–1.93), $p = 0.03$) (Tab. 1). Distribution

Table 1. Distribution of genotypes/alleles of *hTERT* rs2736098/ rs2736100 among bladder cancer cases and controls

***hTERT* rs2736098**	**Cases (n = 130)**	**Controls (n = 200)**	**OR (95% CI)**	**p-value**
Homozygous wild (GG)	40 (30.76%)	70 (35%)	Reference	Reference
Homozygous mutant (AA)	34 (26.15%)	27 (13.5%)	2.20 (1.16–4.16)	**0.02**
Heterozygous (GA)	56 (43.07%)	103 (51.5%)	0.95 (0.57–1.57)	0.89
G allele	136 (52.38%)	243 (60.75%)	Reference	Reference
A allele	124 (47.69%)	157 (39.25%)	1.5 (1.02–1.93)	**0.03**
***hTERT* rs2736100**	**Cases (n = 100)**	**Controls (n = 200)**	**OR (95% CI)**	**p-value**
Homozygous wild (CC)	35 (35%)	71 (35.5%)	Reference	Reference
Homozygous mutant (AA)	16 (16%)	21 (10.5%)	1.54 (0.71–3.32)	0.31
Heterozygous (CA)	49 (49%)	108 (54%)	0.92 (0.54–1.55)	0.78
C allele	119 (59.5%)	250 (62.5%)	Reference	Reference
A allele	81 (40.5%)	150 (37.5%)	1.13(0.80–1.60)	0.53

Bold represent significant value; the odds ratio (OR) for genotypes is adjusted with respect to other covariates like age, sex, smoking status; CI — confidence interval

of *hTERT* rs2736098 genotype variation based on sex, gender or any other characteristic showed no association. On classification of various features of bladder cancer, as shown in Table 2, *hTERT*rs2736098 was observed to be presented significantly more in high stage tumors (OR = 2.4,

Table 2. Frequency of different genotypes of *hTERT* rs2736098 in various clinic-pathological parameters of bladder tumor cases and healthy controls

Parameter	**Cases (%)**	**GG (%)**	**GA + AA (%)**	**Controls**	**GG (%)**	**GA + AA (%)**	**Adjusted OR (95% CI)**	**p-value**
Overall genotype	n = 130	40 (30.76)	90 (69.23)	n = *200*	70 (35)	130 (65)	1.21 (0.751.94)	0.47
Age								
< 50	46 (35.38) 84 (64.61)	13 (32.5)	33 (36.66)	75 (37.5)	32 (45.71)	43 (33.07)	1.88 (0.854.15)	0.16
≥ 50		27 (67.5)	57 (63.33)	125 (62.5)	38 (54.28)	87 (66.92)	0.92 (0.50–1.67)	0.87
Sex								
Male	103 (79.2)	32 (80)	71 (78.88)	144 (72)	42 (60)	102 (78.46)	0.91 (0.52–1.58)	0.77
Female	27 (20.76)	8 (20)	19 (21.11)	56 (28)	28 (40)	28 (21.53)	2.37 (0.89–6.31)	0.10
Smoking status								
Never	42 (32.30)	16 (40)	26 (28.88)	86 (43)	32 (45.71)	54 (41.53)	0.96 (0.44–2.06)	1
Ever	88 (67.69)	24 (60)	64 (71.11)	114 (57)	38 (54.28)	76 (58.46)	1.33 (0.72–2.45)	0.36
Dwelling								
Rural	99 (76.15)	31 (77.5)	68 (75.55)	135 (67.5)	46 (65.71)	89 (68.46)	1.13 (0.65–1.97)	0.67
Urban	31 (23.84)	9 (22.5)	22 (24.44)	65 (32.5)	24 (34.28)	41 (31.53)	1.43 (0.56–3.60)	0.49
Histological type								
GI/GII	72 (55.38)	26 (65)	46 (51.11)				0.9 (0.54–1.67)	0.8
GIII/GIV	58 (44.61)	14 (35)	44 (48.89)				0.56 (0.26–1.21)	0.18
Tumor stage								
pTa/pT1	75 (57.69)	30 (75)	45 (50)				0.8 (0.46–1.39)	0.4
pT2/higher	55 (42.30)	10 (25)	45 (50)				2.4 (1.17–5.20)	**0.02**
Procedure								
TURBT	116	35 (87.5)	81 (90.0)				1.5 (0.7–3.7)	0.3
Cystectomy	14	5 (12.5)	9 (10.0)				0.3 (0.09–1.1)	0.9

Bold represent significant value; OR — odds ratio; CI — confidence interval; TURBT — transurethral bladder resection

Table 3. Frequency of different genotypes of *hTERT* rs2736100 in various clinico-pathological parameters of bladder tumor cases and healthy controls

Parameter	Cases (%)	CC (%)	CA + AA (%)	Controls	CC (%)	CA + AA (%)	Adjusted OR (95% CI)	p-value
Overall genotype	n = 100	35(35)	65 (65)	n = 200	71 (35.5)	129 (64.5)	1.02 (0.61–1.68)	1
Age								
< 50	35 (35) 65 (65)	17 (48.57)	18 (27.69)	75 (37.5)	32 (45.07)	43 (33.33)	0.78 (0.35–1.76)	0.68
≥ 50		18 (51.42)	47 (72.30)	125 (62.5)	39 (54.92)	86 (66.66)	1.18 (0.61–2.29)	0.73
Sex								
Male	79 (79) 21 (21)	30 (85.71)	49 (75.38)	144 (72)	49 (69.01)	95 (73.64)	0.84 (0.47–1.49)	0.56
Female		5 (14.28)	16 (24.61)	56 (28)	22 (30.98)	34 (26.35)	2.07 (0.66–6.46)	0.28
Smoking status								
Never	33 (33)	11 (31.42)	22 (33.84)	86 (43)	32 (45.07)	54 (41.86)	1.18 (0.50–2.76)	0.83
Ever	67 (67)	24 (68.57)	43 (66.15)	114 (57)	39 (54.92)	75 (58.13)	0.93 (0.49–1.75)	0.87
Dwelling								
Rural	76 (76) 24 (24)	28 (80)	48 (73.84)	135 (67.5)	48 (67.60)	87 (67.44)	0.94 (0.52–1.69)	0.88
Urban		7 (20)	17 (26.15)	65 (32.5)	23 (32.39)	42 (32.55)	1.32 (0.48–3.67)	0.62
Histological type								
GI/GII	51 (51)	17 (48.57)	34 (52.30)				1.16 (0.51–2.64)	0.83
GIII/GIV	49 (49)	18 (51.42)	31 (47.69)					
Tumor stage								
pTa/pT1	59 (59)	22 (62.85)	37 (59.92)				0.78 (0.33–1.81)	0.67
pT2/higher	41 (41)	13 (37.14)	28 (43.07)					
Procedure								
TURBT	116	37 (92.5)	80 (88.9)				1.1 (0.7–1.9)	0.5
Cystectomy	14	3 (7.5)	8 (11.1)				1.4 (0.3–3.5)	0.7

OR — odds ratio; CI — confidence interval; TURBT — transurethral bladder resection

CI = 1.17–5.20, p = 0.02). In case of related *hTERT* rs2736100 SNP, CC, CA and AA genotypes in BC cases were 35%, 49% and 16% compared, respectively, to 35.5%, 54.0% and 10.5% in controls (Tab. 1). Homozygous 'AA' genotype showed no significant difference between cases and controls, with variant genotype frequency of 16.0% *vs*. 10.5%, respectively (p > 0.05) with OR = 1.54 (CI = 0.71–3.32). Also the distribution of rare allele A did not differ significantly between the two groups (cases: 40% *vs*. controls: 37% (p > 0.05) with OR = 1.13 (CI = 0.80–1.60). On stratification of various clinico-pathological characteristics of bladder cancer (Tab. 3), *hTERT*rs2736100 genotype distribution was observed to be comparable among all parameters of BC (p > 0.05). Kaplan Meier survival analysis was performed to determine the OS of all 130 patients and the disease-free survival (DFS) of 34 patients (in terms of recurrence). A marked difference in both OS and DFS was observed in histological types of bladder cancer wherein low stage and grade of the disease accounted for significantly higher OS (log rank p < 0.05) as depicted in Supplementary File — Figure 3A–D.

Multivariate analysis showed the smoking status and stage of the disease to have an independent significance in conferring a potential risk to the DFS with HR of 1.81 (95%CI = 0.48–2.84; log rank p = 0.03) and 2.60 (95%CI = 1.08–5.77; log rank p = 0.01), respectively (Table 4). Other independent variables like the gender, age, *hTERT* SNPs did not show any significant impact on the OS and recurrence (DFS) in multivariate models with respect to bladder cancer patients (Table 4). Further, the SNPs *hTERT*rs2736100 C/A and rs2736098 G/A did not show any association with any of the treatment modalities that were most suitable to offer for the patients with bladder tumors (p > 0.050. Patients with muscle invasive tumors were mostly suitable for organ preservation treatment (TMT), an organ preservation strategy based on transurethral bladder resection (TURBT) procedure,

Table 4. Multivariate (Cox regression model) analysis of clinic-pathological characters and *hTERT* gene with respect to overall survival (OS) and disease-free survival (DFS) of bladder cancer patients

Parameter	OS			DFS		
	HR	95% CI	p-value	HR	95% CI	p-value
Age	1.83	1.43–2.33	0.41	1.17	0.39–2.20	0.65
Sex	1.52	0.64–2.65	0.56	1.04	0.53–2.008	0.92
Smoking status	1.87	1.23–2.29	0.13	1.81	0.48–2.84	**0.03**
Dwelling	1.46	0.62–2.56	0.45	0.22	0.08–.60	0.64
Pesticide exposure	1.71	1.22–3.34	0.55	0.40	0.54–1.56	0.45
Grade	1.39	1.23–3.10	0.74	1.04	1.75–2.63	0.10
Stage	1.13	1.19–2.90	0.34	2.60	1.08–5.77	**0.01**
***hTERT* rs2736098**						
GG	Ref	–		Ref	–	
AA	1.08	1.24–2.75	0.38	1.07	0.59–3.28	0.07
GA	1.75	1.20–2.13	0.14	1.58	0.43–2.79	0.18
***hTERT* rs2736100**						
CC	Ref			Ref	–	
AA	1.08	1.15–3.57	0.44	1.28	0.61–1.91	0.33
CA	1.44	0.60–2.53	0.46	1.05	0.43–2.01	0.89

HR — hazard ratio; CI — confidence interval

Table 5. Haplotypic distribution of*hTERT*rs2736100 C/A and rs2736098 G/A between bladder tumor cases and healthy controls

Haplotype ***hTERT rs2736100 C/A*** hTERT rs2736098 G/A		Cases (n = 100)	Controls (n = 200)	Adjusted OR (95% CI)	p-value
C	G	0.16	0.65	Ref. (1.00)	
C	A	0.5	0.16	12.3 (3.5–42.9)	**< 0.0001**
A	G	0.34	0.16	8.2 (2.2–30.09)	**0.001**
A	A	0	0.035	–	–

and 14 cases underwent radical cystectomy. There was only a single case of invasive bladder cancer patient suitable for trimodality therapy. Further, haplotypic analysis was done to evaluate the model of linkage disequilibrium for *hTERT*rs2736100 C/A and rs2736098 G/A for their combined impact in conferring risk to patients with bladder cancer (Tab. 5). Haplotypes were seen with distribution of frequencies >5% among cases and > 4% in controls. Two haplotypes of *hTERT*rs2736100/rs2736098 were identified to confer much more risk to the patients with bladder cancer. Haplotype CA displayed significantly different pattern of frequency as 0.5 in cases as compared to 0.16 in controls with ($p < 0.0001$). Combination of variant A/G haplotype frequency implicated more in cases than in controls (0.34 *vs*. 0.16, $p = 0.001$).

Discussion

Many reports have confirmed a close relationship between the polymorphic variants of the *hTERT* gene and susceptibility to cancer and numerous additional pathological ailments [23–33] but their association with bladder cancer has been least explored. Of the two important *hTERT* variants studied (rs2736098 and rs2736100), the current case-control study found the risk conferred by *hTERT* polymorphic variant rs2736098 at 5p15.33 loci and haplotypic variants to bladder cancer cases, wherein significant differences were found among cases and controls ($p < 0.05$) and prominently homozygous variant genotype AA displayed more than 2-fold risk for cases ($p = 0.02$). The sequence variation of *hTERT* rs2736098 has been implicat-

ed to cause susceptibility to different malignancies [34] and our results also found this variant to be associated with bladder cancer ($p < 0.05$). Likewise, further validation of *hTERT* rs2736098 variant has come across various corners of the world where it has been associated previously with multiple tumors like basal cell carcinoma [35], lung cancer [36], bladder cancer [14] and prostate cancer [14]. In consistence with our findings, a recent meta-analysis conducted by Ru Wang (2019) [37] on six studies comprising of 1974 cases and 2887 controls found significant association between the *hTERT* gene polymorphic variant rs2736098 and bladder cancer. This meta-analysis found almost matched result with our report as A *vs*. G: OR = 1.22 *vs*. 1.5 (our study) and AA *vs*. GG: OR = 1.53 *vs*. 2.20 (our study). The studies from Asia as reported by Ru Wang (2019) [37] meta-analysis imply and confirm that *hTERT* gene rs2736098 variation confer susceptibility to bladder cancer in Asians [38–41] but not in Caucasians [38–41]. Bladder cancer is intricate due to multi-factorial etio-pathogenesis where numerous risk elements are known to cause its growth and development [42]. The *hTERT* rs2736098 (G>A) sequence variation is currently most reported SNP in the *hTERT* gene not only in bladder but in many other malignancies [43]. The *hTERT* rs2736098 connection apart from many reports, showed association with bladder cancer in our report where individuals with the variant A allele showed significant association and thus a higher risk for the disease than G homozygote carriers which may be due to its impact on the activity of telomerase to shorten telomere length that may prop up the initiation and development of bladder cancer [5, 44]. Similarly, substantiation of *hTERT* rs2736098 SNP for its association has been earlier reported from different ethnic regions of the world with several malignancies such as lung cancer [36], prostate cancer [14], basal cell carcinoma [35], hepatocellular cancer [45] and glioma [34]. However, there are a few reports that refute the association between *hTERT* rs2736098 and bladder cancer as reported by Jaworowska et al. 2011 [46] in the Polish Population and Ma et al. 2013[40] in the Chinese population. Interestingly study by Savage et al. (2007) [47] found association of *hTERT* rs2736098 with lower risk of familial breast cancer. Further, studies suggest that *hTERT* rs2736098 confer no risk in some other cancers, like breast, or with non-Hodgkin lymphoma [48, 49]. These discrepant results can be attributed to different genetic surroundings in the investigation of ethnic inhabitants and further involvement of a diverse set of pathways implicated in varied cancers or interactive effect of heritable and environmental elements [50]. Apart from high stage bladder tumors that showed significant association, no other clinical confounding factor was seen to have any relation with variant *hTERT* rs2736098 genotypes. Such a scenario has also been reported by Singh et al. (2014) [38] where tumor stage of bladder cancer cases suggests association with *hTERT* rs2736098 genotypic data.

In yet another SNP*hTERT* rs2736100, both heterozygous CA and homozygous AA variant genotypes showed no significant differences between two groups (CA; 49% cases *vs*. 54% controls and AA; 16% cases *vs*. 10.5% controls). The *hTERT* rs2736100 has been documented among the main variants of the *hTERT* gene to be associated with a predisposition to cancer risk [51–54]. *hTERT* rs2736100 has been conjectured to be associated with the risk of cancer initiation by many reports, but the findings are not only contradictory but heterogeneous. In the meta-analysis conducted by Peng Zou et al. (2012) [55] on 25 case-control studies, *hTERT* rs2736100 variation was observed to be associated with a significantly enhanced risk of cancer. In contrast to our report, studies conducted on *hTERT* rs2736098 and bladder cancer from two different populations showed an association to confer risk for the disease [39, 40]. Further, the current study found a marked difference in survival wherein low stage of the disease accounted for significantly better OS and DFS (log rank $p < 0.05$). The scenario is a universally accepted norm. Both *hTERT* SNPs did not show any significant difference in OS and recurrences of the disease. We could not find any significant association of any SNPs with respect to treatment modalities as most of the cases were fit for TURBT with less cases for radical cystectomy. Trimodality therapy (TMT) was given to only a single patient and; therefore, TMT as an alternative to radical cystectomy [56] was not a possible option due to negligible patients deemed fit for surgery.

Further, *hTERT rs*2736100 C/A and *hTERT* 2736098 G/A haplotypic assessment were carried out to analyze the pattern of linkage disequilibri-

um for its collective effect in patients with bladder cancer risk. Interestingly, two haplotypes of *hTERT* rs2736100/2736098 were observed to confer greater risk: CA and AG haplotype as compared to normal CG haplotype in patients with bladder cancer ($p < 0.05$). This finding is in contrast with the only study that has conducted such analysis to demonstrate the joint effect of SNPs within the 5p15.33 region [39]. In agreement with Chen et al. (2011) [34], the haplotypes in *hTERT* were significantly associated with an increased risk of glioma. From haplotypic analysis of *hTERT* SNPs on bladder cancer, it is suggested that haplotypes may well prove to be a vital tool to monitor the risk of bladder cancer. Since there are almost negligible reports from the subcontinent, the data obtained needs additional reports to supplement the results. Besides, large cohort samples are further augmented for the future studies with respect to risk factors of bladder cancer and its other types like adenocarcinoma and squamous cell type which are seldom found in our region utilizing more robust techniques.

Conclusion

The study concludes that of the two SNPs studied, *hTERT* rs2736098 polymorphic variant has a vital role of presenting a strong risk of bladder cancer in our population. Further, haplotypes CA and AG in *hTERT* could prove as a promising tool to screen the risk for bladder cancer.

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Conflict of interest

Authors have no conflicts of interests to declare.

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Author contributions

A.A.P. — design of the study, conception and drafting the manuscript, I.A. — data interpretation, Experimentation, Statistical analysis, M.S.W. — provided sample, M.G., U.M., Z.A., H.M., I.A., S.M., S.M.B., A.M.K. — assisted in conducting experiments

Ethical approval

All procedures done involving human participants were done in compliance 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. The ethical sanction was attained from the Institutional Ethical Committee (SKIMS Study ref: IEC-SKIMS Protocol #RP 25/2019).

Data availability

All the data that support the results and conclusion of this manuscript will be made accessible to any eligible researcher.

Conflict of interest

None declared.

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