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Association of SNPs/haplotypes in promoter of TNF A and IL-10 gene together with life style factors in prostate cancer progression in Indian population

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Abstract

Objective Levels of proinflammatory (*TNF A*) and anti-inflammatory (*IL-10*) cytokines play a key role in the progression of inflammation as well as cancer disease. We were investigating the potential association of single-nucleotide polymorphisms (SNPs)/haplotypes in proinflammatory (*TNF A*) and anti-inflammatory (*IL-10*) cytokines locus with the development of PCa in Indian population.

Materials and methods We had genotyped 235 BPH/PCa samples (130 BPH and 105 cancer) along with 115 control samples for proinflammatory (TNF A −238G/A and −308G/A) and anti-inflammatory (IL-10 −1082A/G, −819C/T and −592C/A) cytokines SNPs in the gene promoter region using ARMS-PCR method.

Results Allelic frequencies of *TNF A* and *IL-10* SNPs were found to be significantly associated with the risk of prostate cancer and BPH when compared to controls ($p = 0.05$). Further haplotypic analysis showed that two haplotypes of TNF A (AG and AA) and IL-10 gene (CCG and CTG) were serving as risk haplotypes for prostate cancer development. IL-10 risk haplotypes were found to be positively associated with aggressiveness of prostate cancer. We also noticed successively increasing percentage of *TNF A* and *IL-10* risk haplotypes with life style habits like smoking (10 and 26%) and alcohol consuming (9 and 27%).

Conclusions According to our data, TNF A −238G>A and IL-10 −1082A>G, −819C>T and −592C>A may be associated with the development of prostate cancer and BPH. We could also notice higher frequency of *TNF A* and *IL-10* risk haplotypes in smoker and alcohol user. Interestingly, IL-10 risk haplotype was positively associated with aggressiveness of tumor. This information can be used for the early diagnosis of disease and to improve tissue-specific treatment's efficacy which will be moving ultimately towards the discovery of personalized therapy.

Keywords Prostate cancer · TNF A · IL-10 · Haplotype

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Introduction

Presently, prostate cancer (PCa) is one of the most common malignancies of males not only in western countries but in Asian countries also like India. According to "The Global Burden of Cancer 2013", prostate cancer incidences were found 1.4 million and deaths 2.93 lac globally. Incidence of PC is ranked first in developed country and sixth in

developing counties including India [1]. It has been found as an emerging cancer with about 80% of newly diagnosed reproductive cancer in India [2]. The primary etiology of PCa is weakly understood with genetic affinity and environmental factors likely contributing to disease progression. It may not be possible to distinguish the effects of both factors.

The most familiar factors associated with PCa risk are ethnicity, age, and changes in life style (physical inactivity, obesity and high tobacco-smoke, and alcohol consumption) of an individual [3]. Various studies suggested that consumption of tobacco/smoking and alcohol is associated with the risk of prostate cancer progression [4].

TNF has the ability to activate the oncogenic transcription factors like NF-κB and AP-1 in cells. These factors further accelerate the cell proliferation, anti apoptotic activity, and inflammation reaction. Interleukin-10 (IL-10) is a key regulator of immune responses during inflammation reaction. The prolonged inflammation in prostate tissue and evasion of the immune response may increase the chances of initiation and progression of cancer. Epidemiologic and molecular evidences have been supported the key role of tumor necrosis factor (TNF-α and TNF-β) and interleukin (IL-10) cytokines in both inflammatory and cancer promoting pathway in various cancer like oral, cervical, breast, etc [5–9]. Genes, encoding TNF-α and IL-10 cytokines, contain various nucleotide variations like single-nucleotide polymorphisms (SNPs) which are coupled with different levels of gene translation and determines the inter-individual differences in TNF-α and IL-10 production [10, 11].

The TNF A gene is situated in the major histocompatibility complex III region on chromosome 6p21.3. Its plays an important role in the regulation of cell differentiation, proliferation, and death as well as in innate and adaptive immune responses. Impairment in DNA sequence of gene might interface variety of human diseases (infectious, cancer, autoimmune, etc). The previous data have been shown that expression of TNF alpha depends on its genetic variation in promoter region specially at position 308 and 238 [12, 13]. The TNF alpha protein induces the expression of adhesion molecules, facilitating the invasion of metastatic tumor cells [14].

The gene structure of IL-10 comprises five exons and is situated on chromosome 1q31–32 [15]. According to database, promoter region contains approx 40 polymorphic sites, but three functional SNPs (rs1800896, rs1800871, and rs1800872) are emerging more frequently in different cancers [8, 9, 16]. A few studies have also reported positive interaction of IL-10 SNPs with the risk of PCa. Unfortunately, results were not always consistent [17, 18].

The previous studies have also shown that modulated gene expression is due to linkage disequilibrium of their haplotype on the gene promoter region which may increase the probability of different cancer in a population [19, 20]. Discrepancies have been generated due to poor understanding and lack of data from different population. Therefore, need to explore more information with clinical parameters for the association of TNF A and IL-10 genes SNPs either individually or together with PCa is necessary. In addition, to best of our knowledge, no such studies have been performed in Indian population for the association of these SNPs with PCa risk. Here, we have studied the association of pro-inflammatory (TNF A) and anti-inflammatory (IL-10) gene polymorphisms with prostate cancer risk in the Indian population.

Materials and methods

Sample collection

In the present study, a total of 350 consecutive subjects consisting of 105 histologically confirmed prostate cancer case tissue biopsy samples, 130 BPH cases and 115 samples from control (persons coming for routine physical checkups or with other problems like fever and minor injury, etc.) of similar age and ethnicity were included. Categorization and grading of the Cancer were done according to Gleason grading system [21]. Information on demographic features and clinical details were collected through personal interview and written consent form from patients. The study was also approved by the ethics committee review board of Institute of Cytology and Preventive Oncology (Indian Council of Medical Research), Noida, India (Ref. No. ICPO-ICMR/IEC/2011/P-003).

DNA extraction

High-quality genomic DNA was extracted from freshly collected blood samples of prostate cancer, BPH patients, and healthy controls by standard method using proteinase K followed by phenol/chloroform/isopropanol treatment [22].

Genotyping by ARMS-PCR

Amplification refractory mutation-specific (ARMS) PCR methodology was used for genotyping of anti-inflammatory (IL-10) and pro-inflammatory (TNF A) gene polymorphisms by thermocycler (Applied biosystem). DNA was amplified in a 15 μl reaction. Final concentrations of reagents were 1× PCR buffer, 200 μM each dNTP, 0.25 units DNA polymerase (ABgene), Optimised $MgCl_2$ concentration, 5 μM-specific primer mix and 2.5 μM each internal control primer, and 50–100 ng DNA as per the following cycling conditions: 1 min at 96 °C, followed by 10 cycles of 96 °C for 15 s,

annealing temperature according to T_m for 50 s, 72 °C for 40 s; then 20 cycles of 96 °C for 10 s, 60 °C for 50 s, and 72 °C for 40 s. Allele-specific and internal control primer sequences synthesis by Sigma-Aldrich company. PCR products were loaded directly onto 2% agarose gels (containing 0.5 mg/ml ethidium bromide) and electrophoresis, and documented by gel doc detection system [23]. For verification of ARMS-PCR results, some of the samples were tested twice randomly.

Statistical analysis

The data analysis was performed using the computer software Graphpad Instat version 3.3. Chi-square test/Fisher's exact test (for smaller numbers on subgroup analysis) was used to compare the distributions of *IL-10-gene* polymorphisms between cancer patients, BPH, and controls. Haplotypes were constructed from genotypes of these three polymorphic markers and LD estimation was determined by Haploview (http://www.broad.mit.edu/mpg/haploview) [24].

Results

The demographic and clinico-pathological characteristics of the cases and controls group are listed in Table 1. The mean age of PCa patients, BPH patients, and controls has 64.47 ± 4.7, 67.18 ± 8.37, and 62.85 ± 9.93 years, respectively. Percentage of alcohol consumer and non-vegetarian was found higher in PCa (36, 42%) and BPH (24, 22%) as compared to controls (20, 14%). Interestingly, these habits were having significant association with PCa patients ($p = 0.01$; $p < 0.0001$) only. Smokers and tobacco chewers have not shown significant association with case and controls (Table 2). PSA level was observed greater than normal value (4 ng/ml) in PCa patients 94% (99/105) as compared to BPH 51% (66/130). Advance histological grade (MDCC and PDCC) (65%) and clinical stage (III and IV) (65%) were also found in higher percentage as compared to lower grade (WDCC) (35%) and early stage (I and II) (35%)in prostate cancer patient. Out of 105 PCa patients, 79 (75%) were having Gleason score ≤7 and 26 (25%) were having Gleason score >7 (Table 2).

The genotypic analysis of SNPs in IL-10 and TNF A locus

Three different types of genetic models (dominant, recessive, and co-dominant) were used for genotypic frequency analysis of IL-10 (−1082A/G, −819C/T, and −592C/A) and TNF A (−308G/A and 238G/A) locus in PCa and BPH in comparison with controls (Table 2).

Table 1 Demographic details of the studied population

	BPH cases [*n* (%)] *N* = 130	PCa cases [*n* (%)] *N* = 105	Controls [*n* (%)] *N* = 115
Age (mean ± SD)	67.18 ± 8.37	64.47 ± 4.7	62.85 ± 9.93
Alcohol habit			
User	31 (24)	38 (36)	23 (20)
Non-user	99 (76)	67 (64)	92 (80)
p value	0.57	**0.01**	Ref
OR (95% CI)	1.25 (0.68–2.30)	2.26 (1.24–4.16)	1
Smoking habit			
User	44 (34)	34 (31)	33 (29)
Non-user	86 (66)	71 (69)	82 (71)
p value	0.47	0.66	Ref
OR (95% CI)	1.27 (0.74–2.19)	1.19 (0.67–2.11)	1
Tobacco habit			
User	30 (23)	15 (14)	25 (22)
Non-user	100 (77)	90 (86)	90 (78)
p value	0.92	0.21	Ref
OR (95% CI)	1.08 (0.59–1.97)	0.60 (0.30–1.21)	1
Food habit			
Non-vegetarian	28 (22)	44 (42)	16 (14)
Vegetarian	102 (78)	61 (58)	99 (86)
p value	0.16	**<0.0001**	Ref
OR (95% CI)	1.6 (0.86–3.33)	4.46 (2.32–8.59)	1

Significant *p* values are shown in bold

Table 2 Clinico-pathological details of studied cases

	BPH cases [*n* (%)] *N* = 130	Cancer cases [*n* (%)] *N* = 105
PSA level		
≤4	64 (49)	6 (6)
>4	66 (51)	99 (94)
Clinical stage		
I + II	–	37 (35)
III + IV	–	68 (65)
Histological grade		
WDCC	–	37 (35)
MDCC	–	43 (41)
PDCC	–	25 (24)
Gleason's score		
≤7	–	79 (75)
>7	–	26 (25)

TNF A −308G/A polymorphisms and risk of prostate cancer

The genotypic distributions of GG, GA, and AA of −308 G/A locus were 77, 14, and 9%, for PCa; 96, 2, and 2% for BPH; and 90, 6, and 4% for controls, respectively. In dominant model, carrier genotype (GA + AA) was observed significant in PCa ($p = 0.01$) while non-significant in BPH ($p = 0.12$). Interestingly, in recessive model, variant AA genotype had shown non-significant association with both PCa and BPH. While in co-dominant model, only heterozygous GA had shown a significant association with PCa ($p = 0.05$). Allelic frequency of variant 'A' allele has also showed a significant association only with PCa ($p = 0.003$) (Table 3).

TNF A −238G/A polymorphism and risk of prostate cancer

The genotypic distributions of GG, GA, and AA of −238 G/A locus were 31, 57, and 12% for PCa; 52, 42, and 63% for BPH; and 8, 75, and 17% for controls, respectively. In dominant model, carrier genotype (GA + AA) was significantly associated with PCa ($p \leq 0.0001$) as well as BPH ($p \leq 0.0001$). Interestingly, in recessive model, variant AA genotype has shown higher risk for BPH ($p = 0.005$, OR = 3.69) as compared to PCa ($p = 0.28$). While in co-dominant model, heterozygous GA as well as variant AA were found significantly associated with PCa ($p \leq 0.0001$; $p = 0.0008$) and BPH ($p \leq 0.0001$; $p \leq 0.0001$). Allelic frequency of variant A allele has also shown a significant association with BPH ($p \leq 0.0001$) as well as PCa ($p = 0.002$) (Table 4).

IL-10 −1082 (A/G) polymorphism and risk of prostate cancer

The genotypic distributions of AA, GA, and GG of −1082 A/G locus were 34, 43, and 23% for cancer; 38, 49 and 13% for BPH; and 60, 30, and 10% for controls, respectively. Variant allele 'G' either in homozygous (GG) or in heterozygous (AG) condition had shown a significant association with PCa in all three models dominant ($p = 0.0002$), recessive ($p = 0.012$), and co-dominant ($p = 0.004$, $p = 0.0008$), while in BPH, carrier genotype (GA + GG) was observed significantly associated ($p = 0.0008$) in dominant model only. Allelic frequency of variant G allele was observed highly significant for PCa patient ($p = 0.0001$) as compared with BPH ($p = 0.003$) and controls. These findings illustrated the dominant effect of GG genotype in the development of PCa (Table 5).

Table 3 Distribution of TNF A −308 G/A genotypes among PCa, BPH, and healthy controls

−308 G/A (rs1800629)																	
Sample	Genotyping *n* (%)			Dominant model (GG vs. GA + AA)			Recessive model (GG + GA vs. AA)		Co-dominant model				Allelic association				
									(GG vs. GA)		(GG vs. AA)						
	GG	GA	AA	*p* value	OR (95% CI)		*p* value	OR (95% CI)	*p* value	OR (95% CI)	*p* value	OR (95% CI)	G	A	*p* value	OR (95% CI)	
Control (115)	104 (90)	7 (6)	4 (4)	Ref.	**1.0**		Ref.	**1.0**	Ref.	**1.0**	Ref.	**1.0**	**215 (93)**	**15 (7)**	Ref.	**1.0**	
BPH (130)	125 (96)	3 (2)	2 (2)	0.1213	2.64	(0.89–7.85)	0.5712	2.31	0.2294	2.80	0.5378	2.40	253 (97)	7 (3)	0.0681	2.52	(1.00–6.30)
								(0.41–12.84)		(0.71–11.12)		(0.43–13.39)					
Cancer (105)	81 (77)	15 (14)	9 (9)	**0.0121**	0.36	(0.17–0.77)	0.1888	0.38	**0.0520**	0.36	0.1354	0.35	177 (84)	33 (16)	**0.0033**	0.37	(0.20–0.71)
								(0.11–1.29)		(0.14–0.93)		(0.10–1.17)					

Significant *p* values are shown in bold

Table 4 Distribution of TNF A −238 G/A genotypes among PCa, BPH, and healthy controls

−238 G/A (rs361525)															
Sample	Genotyping *n* (%)			Dominant model (GG vs. GA + AA)		Recessive model (GG + GA vs. AA)		Co-dominant model				Allelic association			
								(GG vs. GA)		(GG vs. AA)					
	GG	GA	AA	*p* value	OR (95% CI)	*p* value	OR (95% CI)	*p* value	OR (95% CI)	*p* value	OR (95% CI)	G	A	*p* value	OR (95% CI)
Control (115)	9 (8)	86 (75)	20 (17)	Ref.	**1.0**	Ref.	**1.0**	Ref.	**1.0**	Ref.	**1.0**	**104 (45)**	**126 (55)**	Ref.	**1.0**
BPH (130)	68 (52)	55 (42)	7 (6)	**<0.0001**	12.92 (6.02–27.70)	**0.0053**	3.69 (1.50–9.11)	**<0.0001**	11.81 (5.45–25.60)	**<0.0001**	21.59 (7.13–65.29)	191 (73)	69 (27)	**<0.0001**	3.35 (2.29–4.89)
Cancer (105)	33 (31)	60 (57)	12 (12)	**<0.0001**	5.40 (2.44–11.92)	0.2884	1.63 (0.75–3.53)	**<0.0001**	5.25 (2.34–11.79)	**0.0008**	6.11 (2.19–17.08)	126 (60)	84 (40)	**0.0027**	1.81 (1.24–2.65)

Significant *p* values are shown in bold

Table 5 Distribution of IL-10 −1082 A/G genotypes among PCa, BPH, and healthy controls

−1082 A/G (rs1800896)															
Sample	Genotyping *n* (%)			Dominant model (AA vs. AG + GG)		Recessive model (AA + AG vs. GG)		Co-dominant model				Allelic association			
								(AA vs. AG)		(AA vs. GG)					
	AA	AG	GG	*p* value	OR (95% CI)	*p* value	OR (95% CI)	*p* value	OR (95% CI)	*p* value	OR (95% CI)	A	G	*p* value	OR (95% CI)
Control (115)	69 (60)	35 (30)	11 (10)	Ref.	**1.0**	Ref.	**1.0**	Ref.	**1.0**	Ref.	**1.0**	**173**	**57**	Ref.	**1.0**
BPH (130)	49 (38)	64 (49)	17 (13)	**0.0008**	2.48 (1.48–4.15)	0.5086	1.42 (0.63–3.17)	**0.0011**	**2.57 (1.48–4.46)**	0.1046	2.17 (0.93–5.05)	162	98	**0.0030**	**1.83 (1.24–2.71)**
Cancer (105)	36 (34)	45 (43)	24 (23)	**0.0002**	2.87 (1.66–4.98)	**0.0121**	**2.80 (1.29–6.05)**	**0.0046**	**2.46 (1.35–4.48)**	**0.0008**	**4.18 (1.84–9.49)**	117	93	**<0.0001**	**2.41 (1.60–3.61)**

Significant *p* values are shown in bold

IL-10 −819 (C/T) polymorphism and risk of prostate cancer

The frequency of CC, CT, and TT genotype of IL-10 −819 C/T locus was 26, 59, and 15% in PCa; 32, 51, and 17% in BPH; and 45, 35, and 20% in controls, respectively. Variant allele was found significantly associated with PCa in heterozygous condition in both dominant (CC vs. CT + TT) ($p = 0.004$) and co-dominant (CC vs. CT) model ($p = 0.0007$). The difference in the allelic frequencies of C and T allele was not observed significant in both PCa as well as BPH (Table 6).

IL-10 −592 (C/A) polymorphism and risk of prostate cancer

The genotypic distribution of −592 locus CC, CA, and AA was found 42, 48, and 10% in PCa; 44, 38, and 18% in BPH; and 40, 32, and 28% in controls, respectively. The significant difference was not observed in BPH as well as PCa in dominant model. The interesting observation illustrated that homozygous variant allele AA genotype was significantly associated with PCa risk in both recessive ($p = 0.0021$) as well as co-dominant ($p = 0.018$) model. Therefore, the frequency of A allele was found significant in PCa ($p = 0.04$), while in BPH, it was not significant ($p = 0.16$) (Table 7).

Effects of lifestyle habits on risk of prostate cancer

Further analysis was performed to determine the correlation between the SNPs and life style habits (tobacco, alcohol smoking, and food habit) of the patients. The data revealed that carrier genotypic frequencies of IL-10 −1082A/G were significant and more susceptive to tobacco chewers and alcohol user making them more prone in the development of the risk of prostate cancer and BPH ($p < 0.05$). Smoker had also showed significant data with *IL-10 −592C/A* for PCa ($p = 0.03$). As concluded, these life style habits were associated with IL-10 and *TNF A* polymorphisms and were contributing in the development of risk for prostate cancer (Supplementary tables 1a–e).

Role of SNPs in the progression of prostate cancer

Further analysis was performed to determine correlation between SNPs and clinical characteristic of PCa in Fig. 1.

TNF A

We had observed increased carrier genotypic frequency of TNF A −238G/A SNP only with the advancement of prostate cancer. The carrier (GA + AA) genotypic

Table 6 Distribution of IL-10 −819 C/T genotypes among PCa, BPH, and healthy controls

−819 C/T (rs1800871)																	
Sample	Genotyping *n* (%)			Dominant model (CC vs. CT + TT)		Recessive model (CC + CT vs. TT)		Co-dominant model				Allelic association					
								(CC vs. CT)		(CC vs. TT)							
	CC	CT	TT	*p* value	OR (95% CI)	*p* value	OR (95% CI)	*p* value	OR (95% CI)	*p* value	OR (95% CI)	C	T	*p* value	OR (95% CI)		
Control (115)	52 (45)	40 (35)	23 (20)	Ref.	**1.0**	Ref.	**1.0**	Ref.	**1.0**	Ref.	**1.0**	**144**	**86**	Ref.	**1.0**		
BPH (130)	41 (32)	67 (51)	22 (17)	**0.0385**	1.79 (1.06–3.02)	0.6488	**0.81 (0.43–1.56)**	**0.0131**	**2.12 (1.20–3.74)**	0.7273	1.213 (0.5942–2.477)	149	111	0.2704	1.247 (0.8676–1.793)		
Cancer (105)	27 (26)	62 (59)	16 (15)	**0.0041**	2.38 (1.35–4.22)	0.4550	**0.72 (0.36–1.45)**	**0.0007**	**2.98 (1.62–5.50)**	0.6004	1.34 (0.61–2.95)	116	94	0.1406	1.36 (0.93–1.98)		

Significant *p* values are shown in bold

Table 7 Distribution of IL-10 −592 G/A genotypes among PCa, BPH, and healthy controls

−592 C/A (rs1800872)																	
Sample	Genotyping *n* (%)			Dominant model (CC vs. CA + AA)			Recessive model (CC + CA vs. AA)		Co-dominant model				Allelic association				
									(CC vs. CA)			(CC vs. AA)					
	CC	CA	AA	*p* value	OR (95% CI)		*p* value	OR (95% CI)	*p* value	OR (95% CI)		*p* value	OR (95% CI)	C	A	*p* value	OR (95% CI)
Control (115)	46 (40)	37 (32)	32 (28)	Ref.	**1.0**		Ref.	**1.0**	Ref.	**1.0**		Ref.	**1.0**	**129**	**101**	Ref.	**1.0**
BPH (130)	57 (44)	49 (38)	24 (18)	0.6320	0.85 (0.51–1.42)		0.1119	0.58 (0.32–1.07)	0.9373	1.06 (0.60–1.90)		0.1809	0.60 (0.31–1.16)	163	97	0.1631	0.76 (0.52–1.09)
Cancer (105)	44 (42)	50 (48)	11 (10)	0.8810	0.92 (0.53–1.58)		**0.0021**	**0.30 (0.14–0.64)**	0.3206	1.41 (0.78–2.55)		**0.0180**	**0.35 (0.16–0.80)**	128	72	**0.0491**	**0.66 (0.45–0.98)**

Significant *p* values are shown in bold

frequency of TNF A 238 was higher and significant in advanced clinical stage III + IV 71% ($p = 0.0003$) and histological grade MDCC 74% ($p = 0.006$) and PDCC 64% ($p = 0.0005$) as compared to lower clinical stage (I + II) 65% ($p = 0.0001$) and histological grade WDCC 65% ($p = 0.0001$). Interestingly, Gleason's score was not showing the same trend in comparison between GS >7, 62% ($p = 0.0001$) and GS ≤7, 71% ($p = 0.0002$) while non-significant in TNF A −308 SNP (Fig. 1).

IL-10

We had observed increased carrier genotypic frequency of IL-10 1082, −819, and −592 SNPs with the advancement of prostate cancer. The carrier (AG + GG) genotypic frequency of IL-10 1082 was higher and significant in advanced clinical stages III + IV 69% ($p = 0.0003$) and histological grade MDCC 70% ($p = 0.0003$) and PDCC 68% ($p = 0.02$) as compared to the early clinical stages (I + II) 59% ($p = 0.06$) and lower histological grade WDCC 59% ($p = 0.06$). Interestingly, Gleason's score was also showing same trend with more positive association in comparison between GS >7, 73% ($p = 0.004$, OR = 4.07) and GS ≤7, 63% ($p = 0.002$, OR = 2.59). In IL-10 −819 loci, the percentage of carrier (CT + TT) genotypic frequency was higher in advanced histological grade MDCC 65% ($p = 0.32$) and PDCC 84% ($p = 0.01$) and Gleason's score >7 85% ($p = 0.009$) both as compared to lower histological grade WDCC 78% ($p = 0.01$) and lesser Gleason's score ≤7, 71% ($p = 0.03$). The statistically significant association was not found in IL-10 −592 carrier (CA + AA) genotypic frequencies of both advanced as well as lower clinical site of prostate cancer (Fig. 1).

What haplotype does in the succession of prostate cancer?

For haplotypic analysis, we had used Haploview and PLINK statistical software. All the haplotypes were present in >1% of population.

In TNF alpha, four haplotypes were constructed for this study. All four haplotypes GG (49%), GA (34%), AG (11%), and AA (6%) were found statistically significant in cancer ($p = 0.02$; $p \leq 0.0001$; $p \leq 0.04$ and $p = 0.04$), but in BPH, only three GG (71%), GA (26%), and AG (1.5%) were found statistically significant ($p \leq 0.0001$; $p \leq 0.0001$; $p = 0.04$), respectively, in comparison with controls. The haplotype AA with minor alleles of TNF A −238A and TNF A −308A was significantly associated with prostate cancer only (Table 8).

In IL-10, eight haplotypes were constructed for this study (Table 4). Three haplotypes CTG (6.7%), CCG (17.2%), and ACA (9.3%) were found statistically

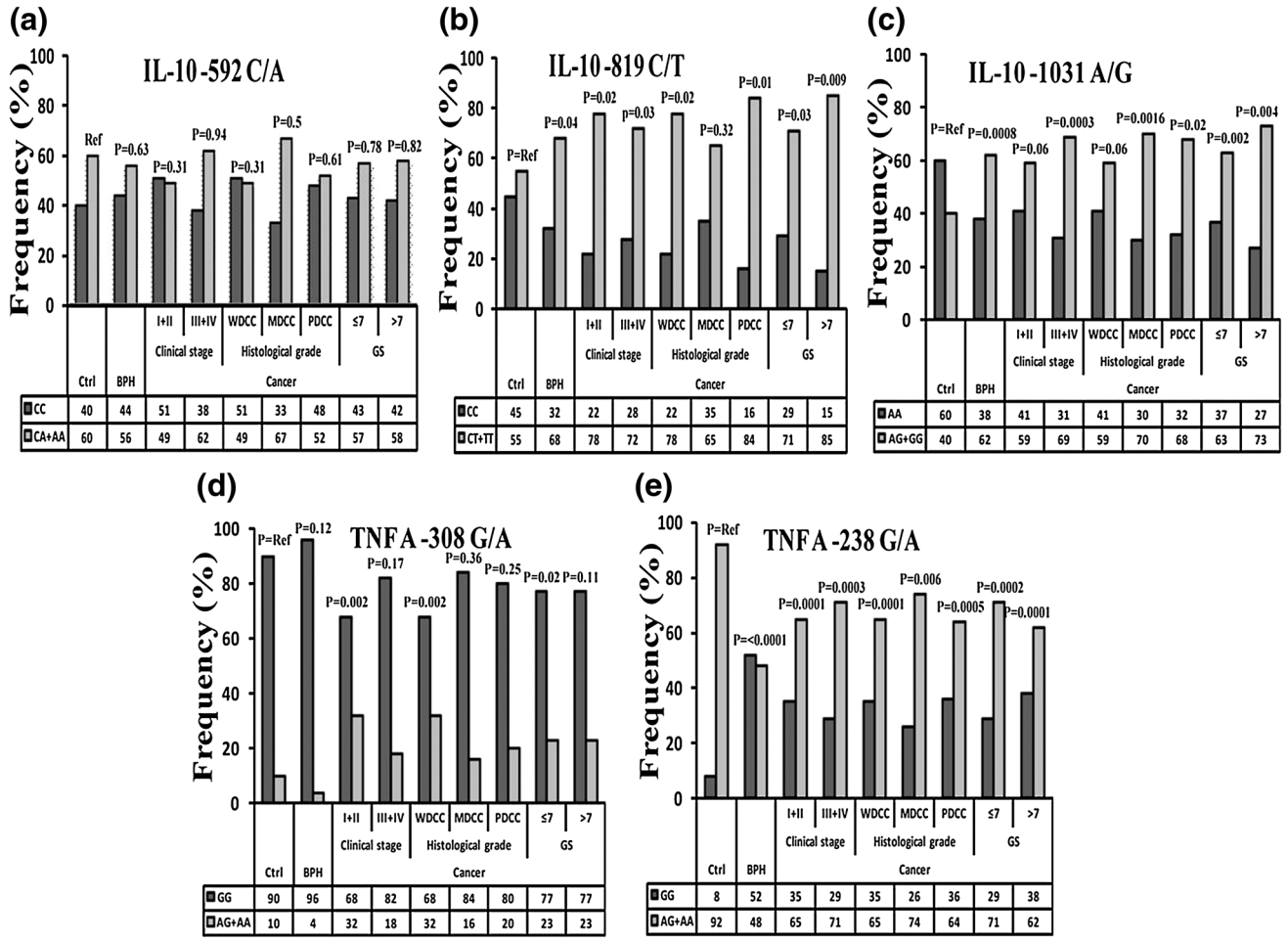

Fig. 1 Genotypic distributions of SNPs in *IL-10* and *TNF A* in prostate cancer, BPH and controls. **a** *IL-10* -592C/A, **b** *IL-10* −819C/T, and **c** *IL-10* −1082A/G, **d** *TNF A* −308G/A, **e***TNF A* −238G/A (Chi-square test significant $p \leq 0.05$)

Table 8 Distribution frequencies of TNF A gene (−308 G/A and −238 G/A) haplotypes among PCa, BPH, and healthy control subjects

S. no.	Haplotype (TNF −308, −238) (frequency) $n = 350$ (%)	BPH $n = 130$ (%)	Cancer $n = 105$ (%)	Controls $n = 115$ (%)
1	GG (191; 0.545)	92 (71)	52 (49)	47 (41)
	p value	**<0.0001**	**0.0214**	Reference
	OR	8.83	1.74	1
2	GA (130; 0.371)	34 (26)	36 (34)	60 (52)
	p value	**<0.0001**	**<0.0001**	Reference
	OR	0.161	0.299	1
3	AG (18; 0.051)	2 (1.5)	11 (11)	5 (4)
	p value	0.047	**0.049**	Reference
	OR	0.233	2.05	1
4	AA (11; 0.031)	2 (1.5)	6 (6)	3 (3)
	p value	0.607	0.047	Reference
	OR	0.708	3.03	1

Significant p values are shown in bold

significant in both BPH ($p \leq 0.0001$; $p = 0.0014$; $p = 0.0007$) as well as cancer groups ($p \leq 0.0001$; $p = 0.0017$; $p = 0.0016$) when compared with controls. The association of minor alleles of SNPs in haplotypes in BPH and cancer groups were found more interesting. ATG haplotypes were containing all three minor alleles of

Table 9 Distribution frequencies of IL-10 gene (−592 C/A, −819 C/T, and −1082 A/G) haplotypes among PCa, BPH, and healthy control subjects

S. no.	Haplotype (IL-10 592 C/A, 819 C/T and −1082 A/G) (frequency) $n = 350$(%)	BPH $n = 130$ (%)	Cancer $n = 105$ (%)	Controls $n = 115$ (%)
1	CCA (84; 0.241)	30 (23)	21 (20)	33 (29)
	p value	0.13	**0.02**	Reference
	OR	0.728	0.602	1
2	ATG (13; 0.038)	6 (5)	4 (4)	3 (3)
	p value	0.184	0.552	Reference
	OR	2.33	1.57	1
3	CTG (24; 0.067)	7 (5)	13 (12)	4 (4)
	p value	**<0.0001**	**<0.0001**	Reference
	OR	2.27	8.4	1
4	ACG (29; 0.078)	9 (7)	10 (10)	10 (9)
	p value	0.521	0.755	Reference
	OR	0.775	1.13	1
5	CCG (59; 0.172)	27 (21)	20 (19)	12 (10)
	p value	**0.001**	**0.001**	Reference
	OR	2.68	2.97	1
6	ATA (61;0.175)	24 (18)	16 (15)	21 (18)
	p value	0.811	0.349	Reference
	OR	1.06	0.777	1
7	CTA (47; 0.136)	18 (14)	14 (14)	15 (13)
	p value	0.948	0.72	Reference
	OR	1.02	1.11	1
8	ACA (33; 0.093)	9 (7)	7 (7)	17 (15)
	p value	**0.0007**	**0.001**	Reference
	OR	0.27	0.268	1

Significant p values are shown in bold

−595A, −891T, and −1082G SNPs. Minor allele of −891T and −1082G was significantly present in CTG haplotype with positive association for the risk of BPH ($p \leq 0.0001$; OR 2.27) and prostate cancer ($p \leq 0.0001$; OR 8.4) (Table 9).

How demographic and clinical parameters affect the risk of prostate cancer incidence in population?

For a better understanding of prostate cancer etiology, we had examined the association between haplotype findings, lifestyle habits (alcohol drinking, smoking, tobacco chewing, and food), and clinical characteristic. All haplotypes were divided in three groups (wild, risk, and other) on the basis of haplotypic frequency (greater frequency in cases than controls) and statistical value in studied population for analysis.

In TNF A, the percentage of risk haplotypes (AG and AA) was found higher in smoker and alcohol user (10, 9%) as compared to non-user (7, 8%) (Table 5). Analysis between risk haplotype and wild haplotype with aggressiveness of clinical parameter had not shown significant value. Interestingly, risk haplotype percentage was more than twofold higher in prostate cancer as compared to controls (Figs. 2, 3).

For IL10, risk haplotypes (CTG and CCG) were compared with wild haplotype (CCA). After analysis, percentage of risk haplotypes was found higher in smoker and alcohol user (26, 27%) as compared to non-user (23, 23%) (Table 5). During further analysis, we had found significant ($p = 0.05$) association of risk haplotypes with clinical parameter (CS, HG, and GS) of PCa as well as BPH when compared to wild haplotype. Interestingly, percentage of risk haplotype was twofold higher in prostate cancer as well as BPH when compared to controls but almost equal with increasing aggressiveness of prostate cancer (Figs. 4, 5).

Discussion

The development of PCa is multifactorial and can be distinguished by the type of cellular infiltrates, cytokines, and growth factors. Association studies have assumed an

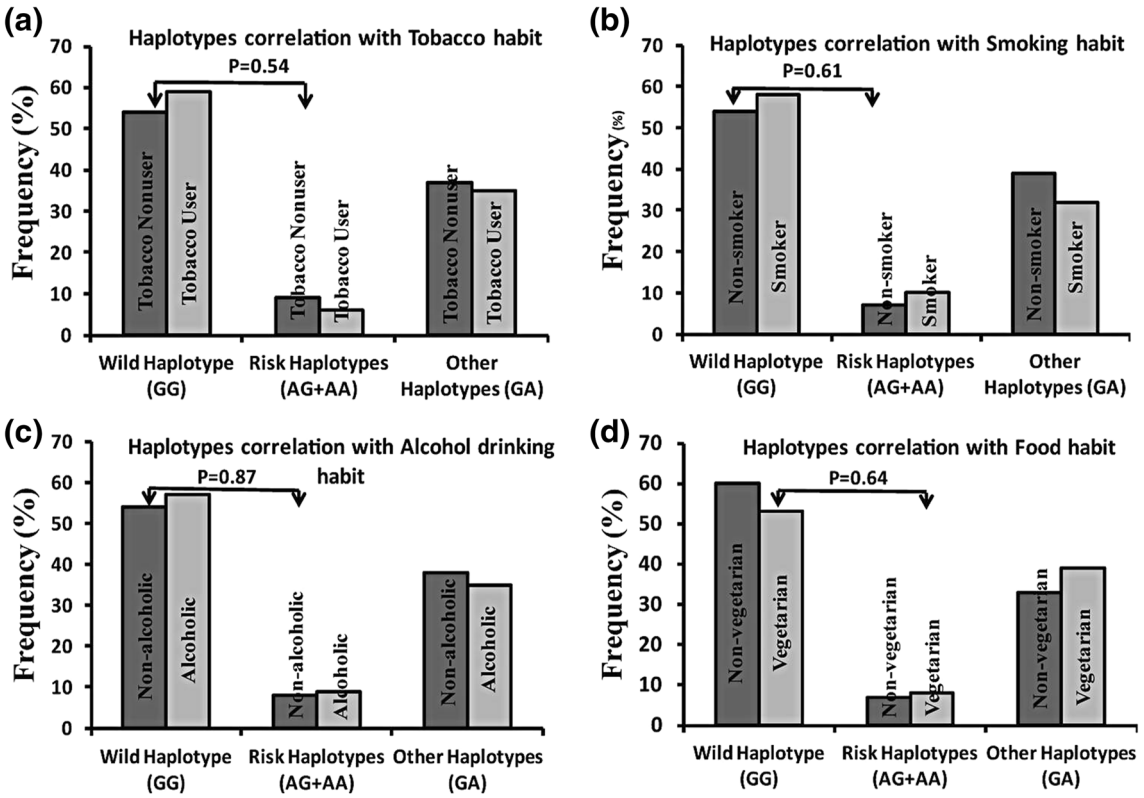

Fig. 2 Correlation of *TNF A* (−308, −238) haplotypes with various lifestyle related risk factors. Comparison between wild haplotype and risk haplotypes. **a** Tobacco user and tobacco non-user. **b** Non-smokers and smokers, **c** non-alcoholic and alcoholic, and **d** non-vegetarian and vegetarian (Chi-square test significant $p \leq 0.05$)

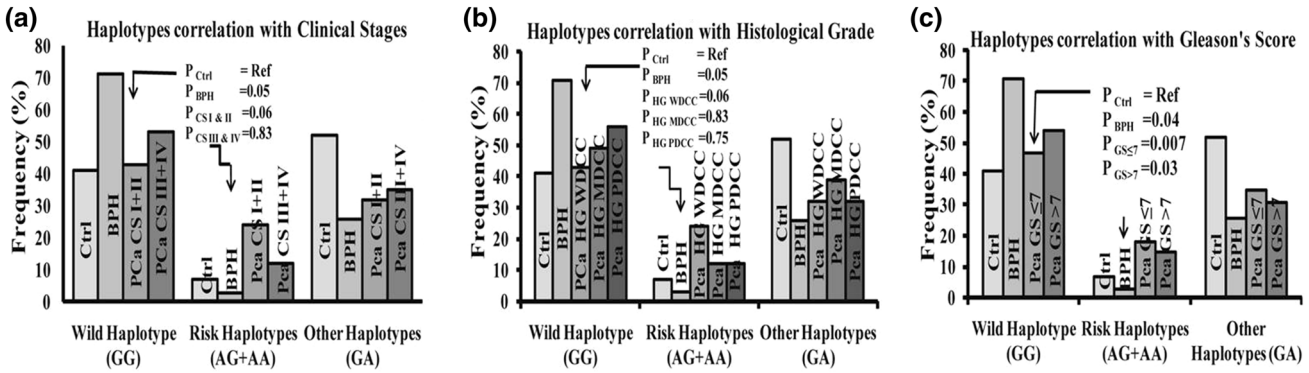

Fig. 3 Effect of *TNF A* (−308, −238) haplotypes on clinical parameters of PCa. Comparison between wild haplotype and risk haplotypes. **a** With clinical stage, **b** with histological grade, and **c** with Gleason's score (Chi-square test significant $p \leq 0.05$)

important role of both genetic and environmental factors in the development and progression of PCa [25]. The etiology of PCa is still indistinct; it has been postulated that genetic polymorphisms in the regulatory regions of proinflammatory and anti-inflammatory cytokine genes influence directly to its production [26]. Therefore, it is required to understand the role of different biomarkers susceptibility in prostate cancer.

In the present study, we had analyzed the association of pro-inflammatory (TNF A −238 G/A and −308 G/A) and anti-inflammatory (IL-10 −1082A/G, −819 T/C, and −592 A/C) cytokines SNPs with prostate cancer progression in Indian population. Results had revealed a significant association between TNF A −238 G/A and IL-10 −1082G/A, −819 T/C, −592 A/C polymorphism, and prostate cancer progression.

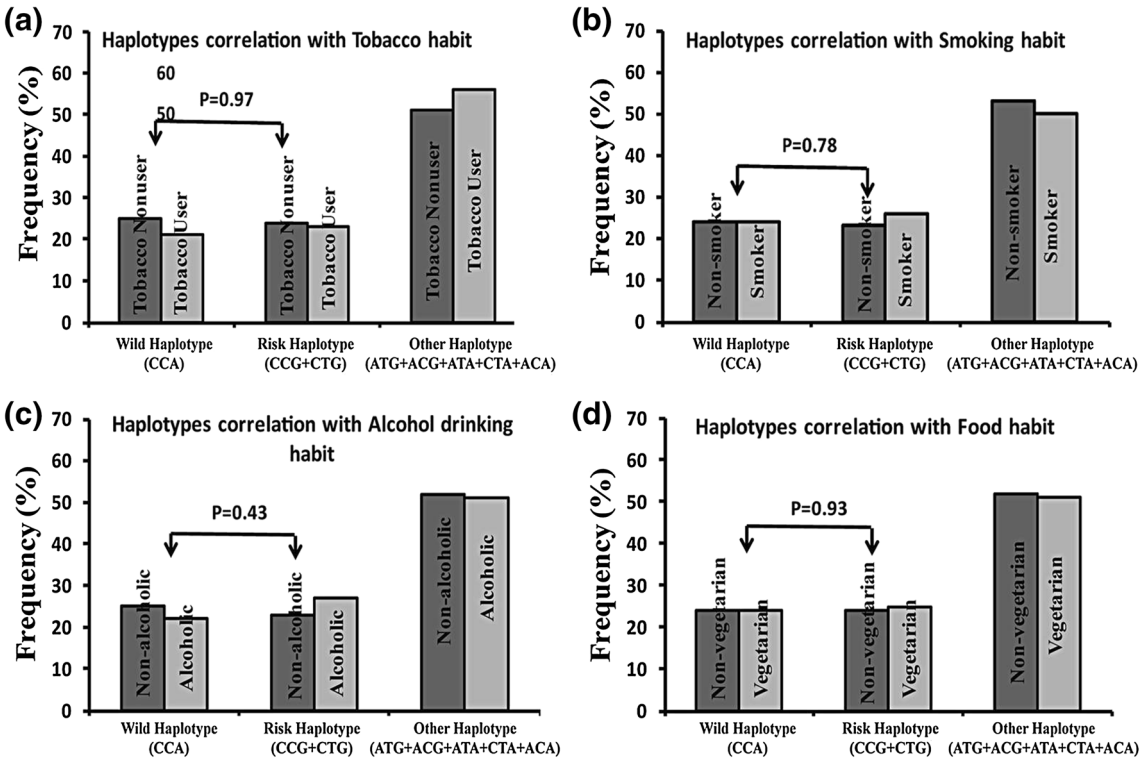

Fig. 4 Correlation of *IL-10* (−592, −819, and −1082) haplotypes with various lifestyle related risk factors. Comparison between wild haplotype and risk haplotypes. **a** Tobacco user and tobacco non-user. **b** Non-smokers and smokers, **c** non-alcoholic and alcoholic, and **d** non-vegetarian and vegetarian (Chi-square test significant $p \leq 0.05$)

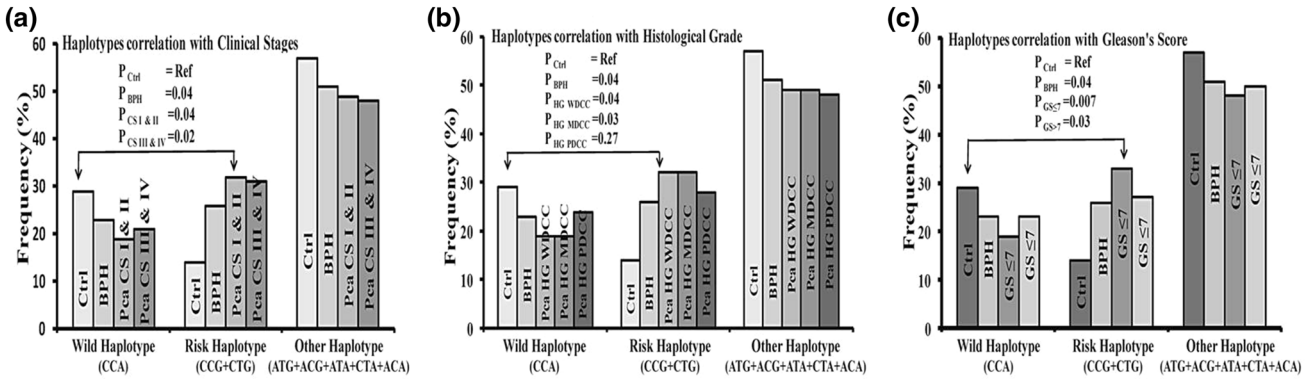

Fig. 5 Effect of *IL-10* (−592, −819, −1082) haplotypes on clinical parameters of PCa. Comparison between wild haplotype and risk haplotypes. **a** With clinical stage, **b** with histological grade, and **c** with Gleason's score (Chi-square test significant $p \leq 0.05$)

TNF-α has been linked directly and indirectly both to pathological processes such as inflammation, autoimmunity, and malignant disease [12]. The G-to-A substitution at position −308 and −238 in the TNF-a promoter increases its transcription activity and serum level [27]. Our results demonstrated that the TNF-α −238G/A polymorphism was significantly associated with PCa risk, while TNF-α −308G/A polymorphism was not associated significantly with PCa risk. Our data were also supported by the previous studies in reference to TNF-α −308G/A polymorphism and PCa risk [28, 29], but TNF-α −238G/A was not studied extensively in the previous studies in prostate.

The previous studies had showed that IL10 production has been associated with the presence of the IL10 −1082G/−819T/−592A haplotype on the IL-10 gene promoter [30] as well as with increased risk of different cancers such as cervical cancer, urothelial bladder cancer, prostate cancer, and so on [9, 31, 32]. Our data also supported the above findings in prostate cancer which revealed that carrier genotype of IL10 −819 (CT + TT) and −1082 (AG + GG) was observed significantly associated with

PCa and BPH ($p \leq 0.05$) both. Interestingly, homozygous variant of −592 (AA) and −1082 (GG) was associated with progression of PCa only. Haplotypic analysis also showed linkage between minor alleles of multiple SNPs of IL-10 gene.

Individual haplotype-pairs including *IL-10* −1082G and −819T were consistently associated with a significantly increased risk of PCa. CTG (with minor alleles *IL-10* −1082G and −819T) and CCG (with minor alleles −1082G) were found as a risk haplotype-pairs for PCa and BPH when compared to controls. Our consistent findings from the different statistical methodologies are quite meaningful and were also supported by other studies in prostate cancers in different population [33, 34]. Almost similar finding were observed in other cancer patient study, in which haplotype is formed by minor alleles of −1082G, −819T, and −592A of *IL-10* showing significant association [9, 16, 35].

Hence, overall IL-10 polymorphism may associate with the development of prostate cancer. In the Indian population, one of the previous study has shown association between the above one SNP (−1082) and risk of PCa, while in other SNPs, −819 was not associated and −592 was not studied [17]; this difference may be due to different lifestyle and habitat. Like Indian population, other population has also shown almost similar findings [33], while some other showed contradictory [18, 36, 37].

The strength of the present study was to find out the synergistic correlation between polymorphic gene (*TNF A* and *IL-10*) with life style habits (smoking, tobacco, alcohol, and food) which personalize the risk of prostate cancer.

Smoking, alcohol, and tobacco may play important roles in the etiology of many cancers including PCa [38, 39]. In our study, alcohol and smoking habit are more prone to develop PCa as compared to tobacco or non-vegetarian habits. Risk haplotype frequency was found with higher percentage in smoker and alcoholic PCa patient in both IL-10 and TNF A gene. Some other studies have reported that cigarette smoking and alcohol consumption suppresses the production of IL-10 and *TNF A*, adversely affecting the function of immune response in inflammatory and infectious disease and contributes to a higher incidence of cancer [40–42].

We had observed higher carrier genotypic frequencies of IL-10 −1082, −819, −592, and TNF A −238 locus in histologically more prognostic grade (MDCC and PDCC) as compared to lower grade (WDCC). The same trade was also found in risk haplotype of both genes. Advanced clinical stages (III + IV) were found with significantly higher carrier genotypic frequencies of IL-10 −1082 and TNF A −238 as compared to early stage (I + II) in PCa patients. Interestingly, only IL-10 −1082, −819, and −592 carrier genotypic and risk haplotype frequencies were found more in higher GS patient of PCa. One of the studies in Chinese population shows that IL-10 −1082 A allele and haplotype ATA are associated with increased risk of prostate cancer and GCC haplotype was higher in early stage [18], but in Austrian population was found no association of ATA haplotype with prostate cancer risk [33]. To best of our knowledge, no such kinds of study on association of haplotype along with lifestyle factor have been conducted previously in prostate cancer patient. In this study, we have some limitations due to small sample size which must be considered ethically.

Conclusions

Our study had shown that SNPs of TNF A −238 G>A and IL-10, i.e., −1082 A>G, −819 C>T, and −592 C>A to be associated with the development of prostate cancer and BPH. In combination, AG and AA in TNF A gene and CTG and CCG haplotypes in IL-10 gene have emerged as a major risk haplotype for prostate cancer progression. We could also notice the positive association of risk haplotypes of IL-10 gene with aggressiveness of tumor. Besides, we had also found higher frequency of risk haplotypes of IL-10 and TNF A SNPs in smoker and alcohol user. Hence, taking together all the above findings, we can demonstrate that both anti-inflammatory (IL-10) and proinflammatory (TNF A) genes may contribute to progression of PCa. This information can be used for the early diagnosis of disease, improving tissue-specific treatment's efficacy, which will ultimately move towards the discovery of personalized therapy.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

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