

RESEARCH ARTICLE

Pro-(IL-18) and Anti-(IL-10) Inflammatory Promoter Genetic Variants (Intrinsic Factors) with Tobacco Exposure (Extrinsic Factors) May Influence Susceptibility and Severity of Prostate Carcinoma: A Prospective Study

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Abstract

Background: It has been hypothesized that IL-18 (pro-) and IL-10 (anti-) inflammatory genetic variants at -607 C/A-137G/C and -819C/T,-592C/A, respectively, may generate susceptibility and severity risk with various modes of tobacco exposure in prostate carcinoma (PCa) patients. IL-18 is a pro-inflammatory cytokine expressed on various cells including prostate gland elements, and is a key mediator of immune responses with anti-cancerous properties. IL-10 is an anti-inflammatory cytokine that is associated with tumour malignancy which causes immune escape. **Materials and Methods:** The present study was conducted with 540 subjects, comprising 269 prostate carcinoma patients and 271 controls. Genotyping was performed by PCR-RFLP and confirmed by real time PCR probe-based methods. **Results:** The findings indicated that the mutant heterozygous and homozygous genotype CC and GC+CC showed significant negative associations ($p=0.01$, OR=0.21; 95% CI: 0.08-0.51 and $p=0.011$, OR=0.43; 95% CI: 0.22-0.81, respectively) thus, less chance to be diagnosed as cancer against GG genotype of tobacco smoking patients. In addition, a heterozygous GC genotype at the same locus of IL-18 pro-inflammatory cytokine may aggravate the severity (OR=2.82; 95% CI 1.09-7.29 : $p=0.001$) so that patients are more likely to be diagnosed in advanced stage than with the GG wild homozygous genotype. Our results also illustrated that anti-inflammatory cytokine (IL-10) genetic variants, although showing no significant association with susceptibility to cancer of the prostate, may gave profound effects on severity of the disease, as -819 TC (OR=4.60; 95% CI 1.35-15.73), and -592 AC (OR=5.04; 95% CI 1.08-25.43) of IL-10 in tobacco chewers and combined users (both chewers and smokers) respectively, are associated with diagnosis in more advanced stage than with other variants. **Conclusions:** We conclude that promoter genetic variants of IL-18 and IL-10 with various modes of tobacco exposure may affect not only susceptibility risk but also severity in prostate cancer.

Keywords: Prostate carcinoma - tobacco smokers/chewers - combined users - IL-18 - IL-10 - genetic polymorphisms

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Introduction

Prostate cancer is multi-factorial disease and relentless progress of past few decades in molecular biology has unravelled the complexity of cancer that it is regulated by intrinsic factor (gene) as well as by extrinsic factor (Environmental viz. tobacco exposure and infective agents). Inflammation has been deciphered as an important link with genetic makeup and environment which fosters the carcinogenesis (Colotta et al., 2009; Malakar et al., 2014). Its contribution in proliferation, malignancy, angiogenesis, metastasis, adaptive immunity modulation, and unresponsiveness to hormones and chemotherapeutic

agents has been reported in various cancers (Mantovani, 2005).

Net inflammatory response is determined by a delicate balance between pro- and anti-inflammatory cytokines levels which is influenced by accumulation of random genetic alterations or mutations. Genetic variations in promoter region of cytokine can alter the level of cytokines thus can affect immunity and susceptibility (Chen et al., 2013; Das et al 2014). In current study we have targeted, Interleukin-18 an important member of pro-inflammatory cytokine family, initially called interferon gamma inducing factor (IGIF), and a molecule responsible for interferon gamma production is well known for anti-

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tumor activity. IL-18 gene when transfected into tumor cells enhances both specific and nonspecific antitumor immune responses, as it has been shown that when IL-18 gene were transferred into dendritic cells, it induces highly effective antitumor immune responses (Xia et al., 2002). IL-18 has been reported as an anti-neoplastic agent with anti-angiogenesis and tumor suppressor (Tysome et al., 2013), and can be used in anti-tumor gene therapy (Tatsumi et al., 2002). Significant genotype differences in the IL-18 promoter polymorphisms have been reported in colorectal, nasopharyngeal, cervical (Nong et al., 2009; Sobti et al., 2008), and prostate cancer in Chinese population (Liu et al., 2007).

Interleukin-10 (IL-10) an anti-inflammatory cytokine produced by Th2 cells, was originally termed as cytokine synthesis inhibitory factor (Fiorentino et al., 1989), and it has a role in inhibiting cytokine production of Th1 cells. Meanwhile studies have shown the actions of IL-10 on inhibition of pro-inflammatory cytokine production by both T and NK cells were indirect, acting via inhibition of accessory cell function (Asadullah et al., 2003). In vitro and in vivo studies revealed pleiotropic activities of IL-10 on B and T cells and that its critical function is to suppress multiple immune responses through individual actions on T cells, B cells, antigen presenting cells and other cell types and to skew the immune response from Th1 to Th2 (Moore et al., 2001). In malignancy, this might imply a priori that IL-10 might promote tumour development, acting to suppress anti-tumour immune responses.

Genetic variants cause genetic instability which fosters the carcinogenesis mechanism, although net effect of immunity modulation or severity of disease depends on many variables, including environmental factors. Recent study showed that the cause of chronic inflammation in cancer patients was chronic infection in 20%, tobacco exposure and inhaled pollutant in 30%, and dietary factors in 35% (Aggarwal et al., 2009). Inflammation has also been linked in various steps including tumorigenesis, cellular transformation, survival, proliferation, invasion, angiogenesis, and metastasis (Aggarwal et al., 2009; Dwivedi et al., 2013). Tobacco consumption has been correlated with various human cancers including lung, oral cavity, breast, esophagus, pharynx, larynx, and urinary bladder cancers (Pleasant et al., 2010; Ebadi et al., 2014; Malakar et al., 2012), but its association with prostate carcinoma is controversial (Islami et al., 2014; Bae JM et al., 2013). Among the identified environmental risk factors for cancers, tobacco exposure is the leading preventable risk factor. The habit of smoking and betel quid chewing is most frequent in many Asian countries including India (Moore and Tsuda, 2002).

Thus in current study our research group pin-pointed to explore the link of genetic variants of inflammatory cytokine (IL-10 and IL-18) with various modes of tobacco exposure in susceptibility and severity of the prostate carcinoma.

Materials and Methods

Patient and control selection

All newly diagnosed and previously untreated 269

men with prostate carcinoma attending urologic clinics of Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow and King George Medical University (Earlier CSMMU) Lucknow, India between 2007 and 2014 were included in the study. During the same period, age matched 271 independent (of patients) healthy subjects as controls were recruited for comparison. The subjects having pure alcoholic (only alcohol drinking) habit were excluded from the study. All controls were free from personal or family history of cancer or any other serious illnesses, enrolled by organizing various camps. The patients and controls suffering with diabetes, arthritis, cardiovascular disease, hepatitis, AIDS and other inflammatory diseases including prostatitis were excluded. All the subjects were above 40 years and below 80 years in age.

The ethical clearance was received from the Institutional Ethics committee. Following an informed consent, information was obtained from the subjects as age, gender, habitual attributes (recall basis) and family history for any cancer. All study subjects completed a questionnaire covering medical, residential and occupational history. All newly diagnosed biopsy approved (pathological test) men with carcinoma prostate were recruited and sampling were done before their treatment. To rule out the effect of hormone or medicine, no specific standard medicine/hormonal therapy was given before sampling and diagnosed men with carcinoma prostate were suggested treatment according to disease status. 2.5 ml blood samples of all subjects were taken at the time of admission (base line) and stored in EDTA for DNA isolation.

Exposure factors

The exposure factors were recorded retro-prospectively in prostate cancer and controls subjects who were tobacco users in any forms and modes (smoking and chewing tobacco), alcohol alone users were excluded from the study. Tobacco habit was categorized and classified by using Dwivedi et al. 2014 criteria into smokers, who were using cigarettes, bidis (hand-manufactured cigarettes consisting of tobacco wrapped in a tendu or temburini leaf) or any other smoked form as hookah (Indian water pipe), chillum and chewers (use of non-smoking tobacco as powder or in beetle leaf or areca nut, catechu), combined users (using both smokeless and smoked product) and non users as those who were not smoking, chewing and drinking (Dwivedi et al., 2012).

Genomic DNA extraction

Genomic DNA was extracted from peripheral blood leukocytes by invitrogen kit and by salting out procedure. Briefly, 2.5 ml of blood was mixed with triton lysis buffer (0.32 M sucrose, 1% Triton X-100, 5 mM MgCl₂, H₂O, 10 mM Tris-HCl, and pH 7.5). Leukocytes were spun down and washed with H₂O. The pellet was incubated with proteinase K at 56 °C and subsequently salted out at 4 °C using a concentrated NaCl solution. Precipitated proteins were removed by centrifugation. The DNA in supernatant fluid was precipitated with ethanol. The DNA pellet was dissolved in 50 µl of sterile distilled water.

Tobacco Exposure and Genetic Variability in IL-18/IL-10 May Affect Susceptibility and Severity of Prostate Cancer Genotyping (IL-18 -607 C/A, -137 G/C and IL-10 -819 T/C, 592 A/C)

IL-18 polymorphisms were genotyped using the restriction fragment length polymorphism of polymerase chain reaction (RFLP-PCR) analysis. All PCR reactions were performed by thermal cycler in 25 μ l reaction volume containing 50-100 ng of genomic DNA, 200 μ M dNTP (Bangalore Genei, India), 2 mM MgCl₂, 1xTaq polymerase buffer, 0.5 unit of Taq DNA polymerase (Amersham, Paris, France) and 0.5 μ M of primers (Bangalore Genei, India). Then thermal cycler amplified DNA was digested by using restriction enzymes RsaI, MboII (Fermentas Inc., USA), MaeIII, MseI (New England Biolabs and Fermentas Inc., USA). All digested products were run on a 2% (w/v) agarose gel and RFLP bands were detected by ethidium bromide staining. To improve the genotyping quality and validation, all samples were re-genotyped in duplicate and

further if any discrepancy was noted then it was confirmed by customized probe based amplification on Light Cycler 480 (Roche, Real time thermal cycler).

Statistical analysis

Genotype and allele frequencies of IL-18 were compared between prostate cancer and controls using the χ^2 -test and Fisher's exact test. χ^2 -test was used to compare the observed no. of each genotype with those expected for a population in Hardy-Weinberg equilibrium. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated to assess the relative risk conferred by a particular allele and genotype. The linkage disequilibrium (LD) between the polymorphisms was quantified using Shi's standardized coefficient D₀ (ID₀) (Shi and He, 2005). A two-tailed p<0.05 was considered statistically significant. All statistical analyses were done using

Table 1. Association of Promoter Genetic Variants of Pro-Inflammatory (IL-18) and Anti-Inflammatory (IL-10) with Various Strata of Tobacco Exposed Prostate Carcinoma Patients

Loci and Genotypes		Smokeless tobacco (Chewers group) (N=143)			Tobacco smokers (Smokers group) (N=180)		
	Genetic Variants	Control (n=71)	Prostate Ca (n=72)	Control Vs Prostate Ca OR(95%CI) p-value	Control (n=88)	Prostate Ca (n=92)	Control Vs Prostate Ca OR(95%CI) p-value
IL-18 -137G/C	GG	21	35	1.00 (Ref.)	21	39	1.00 (Ref.)
	GC	35	31	1.8 (0.26-1.09) 0.102	41	43	0.57(0.29-1.12) 0.125
	CC	15	6	0.24(0.08-0.71) 0.011*	26	10	0.21(0.08-0.51) 0.001**
	GC+CC	50	37	0.44(0.22-0.88) 0.026*	67	53	0.43(0.22-0.81) 0.011*
	CA	28	31	1.45(0.60-3.51) 0.503	35	38	1.45(0.67-3.11) 0.439
IL-18 -607 C/A	CC	17	13	1.00 (Ref.)	24	18	1.00 (Ref.)
	CA	28	31	1.45(0.60-3.51) 0.503	35	38	1.45(0.67-3.11) 0.439
	AA	26	28	1.41(0.57-3.46) 0.501	29	36	1.65(0.75-3.62) 0.238
	CA+AA	54	59	1.43(0.63-3.21) 0.418	64	74	1.54(0.76-3.09) 0.29
IL-10 -819 T/C	TT	13	22	1.00 (Ref.)	16	28	1.00 (Ref.)
	TC	28	29	0.61(0.26-1.44) 0.288	43	38	0.51(0.24-1.07) 0.092
	CC	30	21	0.41(0.17-1.00) 0.078	29	26	0.512(0.23-1.15) 0.155
	TC+CC	58	50	0.50(0.23-1.12) 0.119	72	64	0.508(0.25-1.02) 0.059
IL-10 -592 A/C	AA	13	11	1.00 (Ref.)	20	19	1.00 (Ref.)
	AC	32	23	0.85(0.32-2.23) 0.807	42	41	1.03(0.48-2.20) 1
	CC	26	38	1.72(0.67-4.45) 0.336	26	32	1.73(0.67-4.45) 0.336
	AC+CC	58	61	1.24(0.52-2.99) 0.661	68	73	1.24(0.52-2.99) 0.66

Loci and Genotypes		Combined Users (Smokers and chewers with alcohols) (N=118)			Non Users (N=101)		
	Genetic Variants	Control (n=53)	Prostate Ca (n=63)	Control Vs Prostate Ca OR(95%CI) p-value	Control (n=59)	Prostate Ca (n=42)	Control Vs Prostate Ca OR(95%CI) p-value
IL-18 -137G/C	GG	20	32	1.00 (Ref.)	22	20	1.00 (Ref.)
	GC	23	26	0.71(0.32-1.56)	24	17	0.71(0.29-1.70)
				0.426			0.506
	CC	10	5	0.31(0.09-1.05)	13	5	0.38(0.12-1.28)
				0.077			0.155
	GC+CC	33	31	0.58(0.28-1.23)	37	22	0.59(0.26-1.34)
0.191				0.222			
CC	16	12	1.00 (Ref.)	16	6	1.00 (Ref.)	
IL-18 -607 C/A	CA	26	29	1.49(0.59-3.72)	25	22	2.34(0.78-7.04)
				0.488			0.188
	AA	11	22	2.67(0.94-7.55)	18	14	2.07(0.64-6.68)
				0.075			0.262
	CA+AA	37	51	1.84(0.78-4.34)	43	36	1.61(0.56-4.64)
				0.194			0.448
TT	12	17	1.00 (Ref.)	17	12	1.00 (Ref.)	
IL-10 -819 T/C	TC	27	34	0.89(0.36-2.18)	26	21	1.14(0.45-2.91)
				0.824			0.816
	CC	14	12	0.61(0.21-1.76)	16	9	0.79(0.26-2.39)
				0.423			0.783
TC+CC	41	46	0.79(0.34-1.85)	42	30	1.01(0.42-2.42)	
			0.669			1	
IL-10 -592 A/C	AA	11	11	1.00 (Ref.)	8	12	1.00 (Ref.)
	AC	22	26	1.18(0.43-3.24)	32	18	0.37(0.12-1.08)
				0.8			0.108
	CC	20	26	1.30(0.47-3.60)	19	12	0.42(0.13-1.33)
				0.795			0.161
	AC+CC	42	52	1.24(0.48-3.14)	51	30	0.39(0.14-1.07)
0.813				0.078			

*OR-Odds ratio, CI-Confidence Interval, *Significant *p<0.05, **p<0.005

SPSS 13.0 (SPSS, Chicago, IL) and Graph Pad Prism 5.0 (Graph-Pad Software).

Results

The genotype and allele frequencies of IL-18 and IL-10 promoters

The distribution of genotypes of IL-18 and IL-10 polymorphisms among controls and cases for -137 G/C in both control and cases (p=0.255 and p=0.608) and at -607C/A (p=0.264 and p=.064) respectively were consistent with expectation under Hardy Weinberg equilibrium. Similarly the genotype frequencies of IL-10 among controls and cases for -819 T/C (p=0.463 and p=0.114) and at -592 A/C (p=0.605 and p=0.600) respectively did not depart significantly from Hardy Weinberg equilibrium.

Association of inflammatory polymorphism in tobacco

exposure in PCa and Controls

The promoter genotypes and allele frequencies of -137 G/C and -607 C/A of the Interleukin-18 (pro-inflammatory) and -819 T/C and -592 A/C of Interleukin-10 promoter's polymorphism among the various attributes of tobacco exposed subjects are summarized in Table 1.

Interleukin-18 promoter genetic variation and tobacco exposure

Smokeless tobacco and IL-18 Polymorphisms: When comparison was made among combined mutant heterozygous and homozygous CC and GC+CC genotypes against GG, it showed a significant (p=0.011, OR=0.24; 95% CI: 0.08-0.71 and p=0.026, OR=0.44; 95% CI: 0.22-0.88) protective association and less chance to be diagnosed as a prostate cancer in smokeless tobacco users. Further when genotype association was explored for the -607 of IL-18, it showed higher risk for prostate

carcinoma with CA, AA and CA+AA (OR=1.45; 95% CI: 0.60-3.51; OR= 1.41 95%CI: 0.57-3.46 OR=1.43; 95 % CI: 0.63-3.21) than CC wild homozygous, although difference were not statistically significant.

Tobacco smokers and IL-18 Polymorphisms: The mutant heterozygous and homozygous genotype CC and GC+CC showed a significant ($p=0.01$, OR=0.21; 95% CI: 0.08-0.51 and $p=0.011$, OR=0.43; 95% CI: 0.22-0.81 respectively) association and thus odd ratio have shown very less chance to be diagnosed as cancer against GG genotype of prostate cancer in tobacco smokers.

Further when the association at -607 of IL-18 was evaluated with its various genetic variants then it showed a higher risk statistically with mutant CA, AA and CA+AA (OR=1.45; 95% CI: 0.67-3.11; OR= 1.65 95%CI: 0.57-3.46 OR=1.43;95 % CI: 0.75-3.62) than

wild CC genotype.

Combined Users (Smokers and chewers with alcohols) and IL-18 Polymorphisms: Genotype analysis of combined users at -137 of IL-18 showed less susceptible to be diagnosed as cancer, although it was not statistically significant for CC and CC+GC against GG (OR=0.31; 95%CI 0.09-1.05 and OR=0.58; 95%CI 0.28-1.23). However at -607,when comparison was made with AA genotype to the wild CC genotype, it showed higher risk of susceptibility to be diagnosed as cancer prostate (OR=2.67; 95%CI 0.94-7.55).

Non Users and IL-18 Polymorphisms: Genetic variants of non-users at -137 showed minimal risk to be diagnosed for CC and CC+GC against GG (OR=0.38; 95%CI 0.12-1.28 and OR=0.59; 95%CI 0.26-1.34). However at-607 the AA genotype, when compared with wild CC genotype it

Table 2. Association of Promoter Genetic Variants (Y-axis) of Pro-Inflammatory (IL-18) and Anti-Inflammatory (IL-10) with Various Clinical Stages(X1-axis) in Stratified Tobacco Exposed(X2-axis) Prostate Carcinoma Patients

Clinical Stage (X1-axis) Genotypes (Y-axis)	Clinical Stage			Clinical Stage		
	I - II	III-IV	OR(95%CI)	I - II	III-IV	OR(95%CI)
			p-value			p-value
IL-18-137GG	18	21	1.00 (Ref.)	16	19	1.00 (Ref.)
IL-18-137GC	10	33	2.82(1.09-7.29) 0.037*	11	20	1.53(0.57-4.12) 0.458
IL-18-137CC	4	6	1.29(0.31-5.29) 1	4	2	0.42(0.06-2.60) 0.409
IL-18 -137GC+CC	14	39	2.39(0.99-5.74) 0.075	15	22	1.24(0.49-3.14) 0.812
IL-18-607CC	5	13	1.00 (Ref.)	5	8	1.00 (Ref.)
IL-18-607CA	13	25	0.74(0.22-2.53) 0.763	7	24	2.14(0.53-8.68) 0.295
IL-18-607AA	12	24	0.76(0.22-2.66) 0.763	11	17	0.97(0.25-3.73) 1
IL-18 -607CA+AA	25	49	0.75(0.24-2.35) 0.781	18	41	1.42(0.41-4.96) 0.743
IL-10-819TT	12	14	1.00 (Ref.)	12	10	1.00 (Ref.)
IL-10-819TC	14	24	1.47(0.53-4.05) 0.605	6	23	4.60(1.35-15.73) 0.018*
IL-10-819CC	8	18	1.92(0.62-6.00) 0.392	9	12	1.60(0.48-5.34) 0.546
IL-10 -819TC+CC	22	42	1.63(0.65-4.14) 0.342	15	35	2.80(0.99-7.88) 0.065
IL-10-592AA	9	10	1.00 (Ref.)	5	6	1.00 (Ref.)
IL-10-592AC	12	29	2.18(0.71-6.69) 0.245	11	12	1.67(0.42-6.69) 0.492
IL-10-592CC	10	22	1.98(0.61-6.38) 0.369	14	24	1.43(0.37-5.55) 0.73
IL-10 -592CC+AC	22	51	2.08(0.74-5.85) 0.179	25	36	0.8 1.20(3.29-4.37) 1

Exposure (X2-axis)	Tobacco smokers (Smokers group) (N=92)			Tobacco chewers (Smokers group) (N=72)		
Clinical Stage (X1-axis) Genotypes	Clinical Stage			Clinical Stage		
(Y-axis)	I - II	III-IV	OR(95%CI) p-value	I - II	III-IV	OR(95%CI) p-value
IL-18-137GG	10	22	1.00 (Ref.)	9	11	1.00 (Ref.)
IL-18-137GC	11	15	0.62(0.21-1.82) 0.422	5	12	1.96(0.50-7.69) 0.497
IL-18-137CC	3	2	0.30(0.04-2.10) 0.321	3	2	0.55(0.07-4.01) 0.545
IL-18-137GC+CC	14	17	0.55(0.19-1.54) 0.306	8	14	1.43(0.42-4.93) 0.754
IL-18-607CC	5	7	1.00 (Ref.)	2	4	1.00 (Ref.)
IL-18-607CA	8	21	1.88(0.46-7.66) 0.468	6	16	1.33(0.19-9.27) 1
IL-18-607AA	7	15	1.53(0.36-6.57) 0.711	8	6	0.38(0.05-2.77) 0.629
IL-18-607CA+AA	15	36	1.71(0.46-6.26) 0.496	14	22	0.79(0.13-4.88) 1
IL-10-819TT	8	9	1.00 (Ref.)	5	7	1.00 (Ref.)
IL-10-819TC	10	24	2.13(0.63-7.11) 0.231	7	14	1.43(0.33-6.17) 0.716
IL-10-819CC	5	7	1.24(0.28-5.53) 1	4	5	0.89(0.16-5.12) 1
IL-10-819TC+CC	15	31	1.84(0.59-5.71) 0.379	11	19	1.23(0.31-4.84) 1
IL-10-592AA	6	5	1.00 (Ref.)	6	6	1.00 (Ref.)
IL-10-592AC	5	21	5.04(1.08-25.43) 0.050*	7	11	1.57(0.36-6.87) 0.711
IL-10-592CC	5	19	4.56(0.97-21.33) 0.062	5	7	1.40(0.28-7.02) 1
IL-10-592CC+AC	10	40	4.80(1.21-18.98)0.027*	12	18	1.50(0.39-5.77) 0.732
Exposure (X2-axis)	Combined Users (Smokers and chewers with alcohols) (N=63)			Non Users (N=42)		

*OR-Odds ratio, CI-Confidence Interval, *Significant *p<0.05

showed risk to be diagnosed as cancer (OR=2.67; 95%CI 0.94-7.55), although it was statistically non-significant.

Further at -607 all mutant heterozygous and homozygous CA, AA and CA+AA were associated with increased risk (OR=2.34; 95%CI 0.78-7.04, OR=2.07; 95%CI 0.64-6.68 and OR=1.61; 95%CI 0.56-4.64) respectively, but in none of these cases was it statistically significant.

Tobacco exposure and IL-10 Polymorphisms: Interleukin-10 is a novel biomarker of anti-inflammatory status, so its genetic variants at two loci of promoter's were also explored with various tobacco attributes of smokeless tobacco, tobacco smokers, combined users (smokeless,

smoked form with alcohol) and non-users groups.

Smokeless tobacco users and IL-10 Polymorphisms: Genotype analysis of Smokeless tobacco users at -819 of IL-10 showed protective correlation for TC and CC+TC, making individual less susceptible to be diagnosed for cancer prostate (OR=0.41; 95%CI 0.17-1.00 and OR=0.50; 95%CI 0.23-1.12) against TT. However, at-592 the CC and CC+AC genotypes when compared with wild AA genotype, showed higher risk to be diagnosed as prostate carcinoma (OR=1.72; 95%CI 0.67-4.45 and OR=1.24; 95%CI 0.52-2.99 respectively).

Tobacco smokers and IL-10 Polymorphisms: Genotype analysis of tobacco smokers at -819 of IL-10 showed no risk as these genotypes have almost equal odd ratio for TC, CC and CC+TC against TT (OR=0.51; 95%CI 0.24-1.07, OR=0.51; 95%CI 0.23-1.15 and OR=0.51; 95%CI 0.25-1.02). However, at -592 the CC and CC+AC genotype when compared against AA genotype showed higher risk (OR=1.73; 95%CI 0.67-4.45 and OR=1.24; 95%CI 0.52-2.99 respectively) to be diagnosed as prostate carcinoma.

Combined Users (Smokers and chewers with alcohols) and IL-10 Polymorphisms: Genetic analysis of combined users at -819 with CC genotype showed less chance to be diagnosed as cancer against TT (OR=0.61; 95%CI 0.21-1.76) while other genotype TC and TC+ CC have shown no association. However, CC and AC+CC genotype at -592 showed risk (OR=1.30; 95%CI 0.47-3.60, OR=1.24; 95%CI 0.48-3.14) to be diagnosed as cancer in comparison of wild AA genotype.

Non Users and IL-10 Polymorphisms: Genotype analysis of Non users at -819 of IL-10 showed no risk for TC, CC and CC+TC against TT. However AC, CC and CC+AC genotypes at -592 conferred protective (less chance to be diagnosed) association against wild AA genotype (OR=0.37; 95%CI 0.12-1.08, OR=0.42; 95%CI 0.13-1.33 and OR=0.39; 95%CI 0.14-1.07) respectively.

Severity of carcinoma prostate with Genetic variants in tobacco exposed groups: The current study also explored the role of genetic polymorphisms of inflammatory cytokine with severity of disease in various tobacco exposed carcinoma patients as shown in Table 2.

Tobacco smoker's genetic variants and severity: The prostate carcinoma patients with GC and GC+CC at -137 of IL-18 have conferred more risk to be in advanced stage in comparison of GG genotype (OR=2.82; 95%CI 1.09-7.29 and OR=2.39; 95%CI 0.99-5.74), Although it was statistically significant for GC (GC Vs GG; $p < 0.05$). Other genotypes at -607 of IL-18 did not showed any risk for advancement in disease stage. Further CC and TC+CC genotype at -819 of IL-10 augmented risk of advanced stage than TT (OR=1.92; 95%CI 0.62-6.00 and OR=1.63; 95%CI 0.65-4.14) but statistically insignificant. Similarly AC and CC+AC genotype at -592 of IL-10 showed more risk to be diagnosed in advanced stage (OR=2.18; 95%CI 0.71-6.69 and OR=2.08; 95%CI 0.74-5.85) than AA.

Tobacco chewer's (smokeless users) genetic variants and severity: The prostate carcinoma patients with CC genotypes at -137 of IL-18 have shown less possibility of advancement in stage (OR=0.42; 95%CI 0.06-2.60) against GG genotype. CA genotypes at -607 of IL-18 showed increased in risk of stage advancement than CC (OR=2.14; 95%CI 0.53-8.68). Furthermore TC genotype at -819 of IL-10 showed more risk to be diagnosed in advanced stage of prostate carcinoma (OR=4.60; 95%CI 1.35-15.73), and this association was statistically significant when compared with TT genotype (TC Vs TT; $p < 0.05$). However AC genetic variants at -592 of

IL-10 also showed risk of disease advancement than AA (OR=1.67; 95%CI 0.42-6.69), although it failed to show any significant association.

Combined users (smokeless and smokers with alcohol) genetic variants and severity: Genotype CC at -137 of IL-18 showed protective association against advancement of stage when compared with GG (OR=0.30; 95%CI 0.04-2.10). Other variants of -607 of IL-18 ineffective in generating any significant association with advancement of disease stage. Genetic variants AC and CC+AC at -592 of IL-10 conferred to be diagnosed in advanced stage of prostate carcinoma (OR=5.04; 95%CI 1.08-25.43 and OR=4.80; 95%CI 1.21-18.98) and both of these genotype had shown significant (AC Vs AA; CC+AC Vs AA, $p < 0.05$) association in comparison of AA.

Non user's genetic variants and severity: Genetic variants CC at -137 of IL-18 showed minimal chance to be diagnosed with advanced stage of cancer, while GC showed risk to be diagnosed in advance stage of prostate carcinoma, when compared against wild genotype GG (OR=0.55; 95%CI 0.07-4.01; OR=1.96; 95%CI 0.50-7.69) respectively. Other variant AA at -607 of IL-18 have shown lesser chance (OR=0.38; 95%CI 0.05-2.77) with advancement of disease stage against CC. Genotype of AC, CC and AC+CC at -592 of IL-10 have shown risk to be in more progressed stage (OR=1.57; 95%CI 0.36-6.87, OR=1.40; 95%CI 0.28-7.02 and OR=1.50; 95%CI 0.39-5.77) but statistically insignificant.

Discussion

Cancer related inflammation is now regarded as one of the important hallmark of cancer, which has been found to be linked with various carcinomas such as lung, gastric, cervical, hepato-cellular, gall bladder, urinary bladder, pancreatic, esophageal, melanoma and prostate (Ebadi et al., 2014; Dwivedi et al 2011; 2012). It is also involved in the induction of genetic instability by inflammatory mediators, leading to accumulation of random genetic alterations in cancer cells (Colotta et al., 2009), Exposure of different exogenous agents as chemicals in working environment for a long time may influence the physiological and biochemical metabolism. It may influence the prostate gland, the principal that such chemicals can alter the enzymatic activity has been established (Lee et al., 2013). Moreover, animal studies demonstrated that prostate tumors can be induced by administration of chemicals (Nelles et al., 2011). Furthermore, many studies suggest various exogenous chemicals may affect hormone levels which may in turn, affect estrogen levels and androgenic stimulation of the prostate (Bostwick et al., 2004).

The present case-control study sought to determine the existence of a possible association between the polymorphisms of cytokine genes with tobacco exposed prostate cancer patients. IL-18 is pro-inflammatory cytokine and it has been earlier investigated that levels of IL-18 are up-regulated in prostate cancer patients (Dwivedi et al., 2011). Furthermore in our previous study

the levels of Interleukin 18 have shown varied results in different modes of tobacco exposure viz. smokers, chewers and both products combined users in patients of prostate cancer (Dwivedi et al., 2015, Dwivedi et al., 2014). So this study correlated the risk of susceptibility and severity by exploring combined effect of indigenous factor (promoter genetic variants) with exogenous factor (tobacco exposure). Our results illustrated that IL-18 promoter's having CC genotype at -137 have shown (protective effect; as $p > 0.05$) very less chance to be diagnosed for cancer prostate especially in smokeless and smokers group than GG. The results also demonstrated that C allele carrying patients were less susceptible (protective) for prostate carcinoma. Previous studies suggested that that G to C substitution at -137 position abolishes a histone 4 transcriptional factor- 1 nuclear factor binding site and have an impact on IL-18 gene activity (Giedraitis et al., 2001).

Recent study demonstrated that IL-18-primed human NK cells, including from directly within the human cancer environment, can enhance type-1 immune responses by selectively inducing high DC expression of Teff cell-recruiting chemokines, including CXCL9, CXCL10, and CCL5, without inducing the Treg cell-attracting chemokine CCL22. Thus our study is compliment to study of Wong et al., and also has confirmed its role as anti-cancerous and immune protective (Wong et al., 2013). Further, the effect of polymorphisms on severity of the disease showed that the patients carrying heterozygous GC genotype at -137 locus of IL-18 in tobacco smokers have risk to be diagnosed in advanced stage of the diseases.

IL-10 polymorphisms have shown varied results for susceptibility but overall insignificant. However presence of TC at -819 in tobacco smokers, AC and AC+CC at -592 of IL-10 in combined tobacco users patients have shown more risk to be diagnosed in advanced stage of the cancer. The polymorphism on promoter regions have their role in regulations of gene activity and its circulatory levels and our previous study on prostate carcinoma have shown its aggravation in circulatory levels (Dwivedi et al., 2011a; Dwivedi et al., 2011b, Dwivedi et al., 2014; Dwivedi et al., 2015). The immunosuppressive cytokines, such as interleukin (IL)-10, are secreted from tumor cells and inhibit the maturation of dendritic cells (DC) and T cell function through regulatory T cells (Berger et al., 2005). Thus it weakens the immune system of the patients and this may be a reason for their advanced stage. Further, the exogenous tobacco exposure in various modes may also influence the overall inflammatory and redox status of the patients so it may build-up a cumulative effect with endogenous genetic variants on regulatory region (promoter's) of pro- and anti-inflammatory cytokine. Recently, Few studies have linked, these two pathways as first in the intrinsic pathway, genetic events causing neoplasia initiate the expression of inflammation-related programs that guide the construction of an inflammatory microenvironment [e.g. RET and various cytokine signalling pathways (Mantovani, 2005; Rius et al., 2008). In the extrinsic pathway (pollutants, tobacco exposure, microbial infections etc) inflammatory conditions facilitate cancer development. Ultimately both extrinsic

and intrinsic factors trigger various transcription factors and mount chronic inflammation that may influence the susceptibility as well as severity of the cancer (Balkwill, 2009). Our results clearly reveal that IL-10 and IL-18 gene polymorphism with tobacco exposure play an important role in susceptibility and severity of the prostate cancer. To the best of our understanding and extensive literature search, this is perhaps the first study depicting the association of pro-inflammatory (IL-18) and anti-inflammatory (IL-10) polymorphisms with susceptibility and severity of prostate carcinoma in tobacco exposed population.

Conclusions: The findings of this study, thus indicated that genetic variants with C alleles at -137 in tobacco exposed (smokers and smokeless) populations have shown less susceptibility (protective) to prostate cancer and heterozygous GC genotype at the same locus of IL-18 pro-inflammatory cytokine may augment the severity of the patients and likely to be diagnosed in advanced stage than GG wild homozygous genotype. Our results further illustrated that anti-inflammatory cytokine (IL-10) genetic variants, although have shown profound effect on severity of the cancer. Cancer patients with -819 TC and -592 AC in tobacco chewers and combined users (both chewers and smokers) respectively, have more chance to be diagnosed in advanced stage than other variants.

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