



Association of interleukin 1 beta (*IL-1β*) polymorphism with mRNA expression and risk of non small cell lung cancer[☆]



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ABSTRACT

Introduction: Interleukin 1 beta (*IL-1β*), a key proinflammatory cytokine encoded by the interleukin 1 beta gene, has been associated with chronic inflammation and plays an important role in lung inflammatory diseases including lung cancer. Elevated levels of Interleukin 1 proteins, in particular interleukin 1 beta greatly enhance the intensity of the inflammatory response.

Aim: To study the role of interleukin 1 beta-31C > T and -511 T > C polymorphism in the pathogenesis of non small cell lung cancer (NSCLC).

Materials and methods: One hundred and ninety non small cell lung cancer patients and 200 healthy age, sex, smoking and dwelling matched controls were used for polymorphic analysis by polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) followed by sequencing. Normal tissues of 48 histopathologically confirmed non small cell lung cancer patients were taken for mRNA expression analysis. Quantitation of interleukin 1 beta was carried out by quantitative real time PCR.

Result: The T/T genotype of interleukin 1 beta-31 gene was significantly associated with increased risk of NSCLC [(P = 0.001, OR = 2.8 (95%CI 1.52–5.26)]. The interleukin 1 beta –511 T > C does not show any difference between the NSCLC and control group (P = 0.3, OR = 0.72

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(95%CI 0.41–1.28). Quantitative analysis of mRNA showed significant association with interleukin 1 beta T allele as compared to the interleukin 1 beta-31C allele ($P = 0.006$).

Conclusion: We conclude that lung cancer risk genotype interleukin 1 beta-31TT results in increased expression of interleukin 1 beta mRNA in lung cancer patients. Our data suggest that this genotype (IL1 β -31TT) in the interleukin 1 beta regulatory region provide a microenvironment with elevated inflammatory stimuli and thus increasing the risk for lung cancer.

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Introduction

Lung cancer kills more people annually than the next four cancer types combined, and as many as one billion people may die worldwide from lung cancer this century (Jha, 2009). Tobacco smoking is considered to be the leading cause of lung cancer, with approximately 85% of deaths directly linked to smoking. It has been observed that all smokers did not develop lung cancer but other factors such as individual genetic susceptibility and inflammation may play a role in the development of lung cancer. Chronic inflammation has long been deemed to be an important factor in the pathogenesis of many human cancers, including lung cancer. Many lung cancer pathogens can cause inflammation in the respiratory tract and lung parenchyma. Elevated numbers of inflammatory cells and higher levels of pro-inflammatory cytokines are seen in lung cancer tumor microenvironments and their surrounding stromal tissue (Balkwill and Mantovani, 2001; Takizawa et al., 2000). Many pro inflammatory cytokines have been shown to be involved in the pathogenesis of lung cancer. However, the cellular and molecular mechanisms underlying this relationship are not well elucidated.

Interleukin 1 beta is an important cytokine in regulating the expression of several genes involved in various inflammatory processes (Dinarello, 1996, 2002a). Interleukin 1 beta plays an important role in various inflammatory diseases including lung cancer (Azad et al., 2008; Mayne et al., 1999). Sustained induction of interleukin 1 beta enhances the intensity of the inflammatory response and creates an inflammatory micro-environment in advantage of tumor initiation and/or promotion (Dinarello, 2006). The amount of interleukin 1 beta secreted to the microenvironment is regulated at the messenger RNA (mRNA) level and protein level. The processing of the pro-interleukin 1 beta precursor into active interleukin 1 beta molecules is mediated by the caspase-1 enzyme (Dinarello, 1996; Apte et al., 2006b). Different cell types including lung epithelial cells upon exposure to various chemicals and other environmental agents produce and secrete interleukin 1 beta (Apte et al., 2006a; Dinarello, 1996). It has also been shown that the interleukin 1 beta gene may be induced by cigarette smoke in human cells in vitro (Hellermann et al., 2002). The crucial role of interleukin 1 beta in susceptibility to 3-methylcholanthrene-induced carcinogenesis has been recently investigated in interleukin 1 beta knock-out mice. In the mice deficient in interleukin 1 beta, tumours developed slower and in fewer animals than in wild-type mice (Shirakawa et al., 1993; Apte et al., 2006a). Several single-nucleotide polymorphisms (SNPs) such as interleukin 1 beta-31C > T (rs1143627), interleukin 1 beta-511 T > C (rs16944), in the promoter region of the gene have been identified. The role of interleukin 1 beta in increasing the risk of lung cancer has been observed. In particular, the interleukin 1 beta-31 C > T polymorphism has been examined in several association studies. Some studies have reported an association between this SNP and risk for cancer and inflammatory diseases (Lind et al., 2005; Zienolddiny et al., 2004; Chen et al., 2006a; Haukim et al., 2002; Bidwell et al., 1999). Several studies have shown that both -31C/T and -511 T > C are significantly associated with the increased risk of non small cell lung cancer (Zienolddiny et al., 2004). The interleukin 1 beta-31 C > T is being considered as a potential candidate in regulating the expression of interleukin 1 beta due to the shift from T to C at position -31 that mediates a change from TATAAA to CATA AA and a potential disruption of the TATA box (Wobbe and Struhl, 1990). A study conducted in A549 cells showed that the expression of interleukin 1 beta was higher in cells carrying -31 T allele as compared to the normal wild -31 C allele (Lind et al., 2007) while studies have so far failed to show any functional relevance of -511 SNP to alter gene transcription (Chen et al., 2006b; El Omar et al., 2000).

In view of the above findings, we conduct a case control study to investigate the polymorphic association of interleukin 1 beta-31C > T and interleukin 1 beta-511 T > C with non small cell lung cancer in our population. Also mRNA expression of interleukin 1beta was analysed in normal tissues of lung cancer patients by quantitative real time PCR.

Materials and methods

Materials

This study included 190 lung cancer patients and 200 age, sex, dwelling and smoking matched controls. All the lung cancer cases selected for study were histologically confirmed non small lung cancer cases. Patients were recruited from Out Patients Department, Department of Medical Oncology and Department of Cardiovascular and thoracic surgery, Sher-i-Kashmir Institute of Medical Sciences, Srinagar (SKIMS) between April 2010 and March 2012. All those patients who had prior history of cancer other than lung cancer and patients who had received any chemotherapy/radiotherapy were excluded from our study. All participants of the control group were selected from individuals receiving routine medical examinations in the same hospital, with no history of any malignancy, or any symptoms of other acute or chronic inflammatory lung diseases (i.e. COPD and asthma), peritonitis, rheumatoid arthritis, or late stage kidney diseases. Ethical approval was taken from the ethical committee of Sheri Kashmir institute of medical sciences Srinagar and World Medical Association (Declaration of Helsinki) protocol was followed. All the participants were informed and written consent was taken for participation in the study.

Peripheral blood samples (5 ml) were collected in EDTA vials from lung cancer patients and healthy controls and later stored at -80°C till further use. Tissue samples were stored in RNA later (Sigma Aldrich) and kept at -80°C till further use. A detailed questionnaire was completed for each patient and

Table 1

Selected demographic variables and risk factors of non small cell lung cancer cases and controls.

Variables	Cases N = 190 (%)	Controls N = 200 (%)	P value
All subjects			
<i>Age in years</i>			
≤50	59 (31.05)	65 (32.5)	0.8
>50	131 (68.95)	135 (67.5)	
<i>Sex</i>			
Male	163 (85.79)	170 (85)	0.88
Female	27 (14.21)	30 (15)	
<i>Smoking status</i>			
Nonsmoker	76 (40.0)	84 (42)	0.76
Smoker	114 (60.0)	116 (58)	
Dweller	127 (66.80)	129 (64.5)	
Rural	63 (33.15)	71 (35.5)	0.67
Urban			
<i>Stage</i>			
I & II	130 (68.42)	-	-
III & IV	60 (31.58)		
<i>Histology</i>			
SCC ^a	140 (73.69)		-
Others ^b	50 (26.32)	-	
Grade 1	71 (37.36)		
Grade 2	76 (40.0)		
Grade 3	43 (22.6)		

Adjusted for age, smoking, gender and dwelling.

G1 - well differentiated, G 2- moderately differentiated, G3- poorly differentiated.

^a Squamous cell carcinoma.

^b Others include adenocarcinoma, large cell carcinoma, bronchiogenic carcinoma.

control. The questionnaire included information on the number of cigarettes smoked daily/quantity of tobacco smoked every day and the number of years the subject had been smoking. For smoking status, a person who had smoked (cigarette/Hokka) at least once a day for > 1 year was regarded as smoker. The cases included 163 (85.79%) males and 27 (14.71%) females with age distribution of ≤ 50 years 59 (31.05%) and > 50 years of age were 131 (68.95%) with mean age of 57.80 ± 10.97 . The control group had 170 (85%) males and 30 (15%) females with age distribution ≤ 50 years 65 (32.5%) and > 50 years 135 (67.5%) with mean age of 56.69 ± 12.22 . Smokers in cases and controls were 114 (60%), 116 (58%) and non smokers were 76 (40%) and 84 (42%) respectively. Non small cell lung cancer included 130 (68.42%) cases who had stage I and stage II and 60 (31.58%) cases who had stage III and IV. Squamous cell carcinoma patients were 140 (73.69%) while others which included large cell carcinoma, bronchogenic carcinoma and adenocarcinoma were 50 (26.32%). Out of 190 patients 71 had developed grade 1, 76 had developed grade 2 and 43 presented with grade 3 non small cell lung cancer (Table 1).

Interleukin 1 beta genotyping

DNA extraction was performed according to the manufacturer's protocol for Qiagen DNA extraction kits (Qiagen, Hilden, NRW, Germany). DNA content was quantified by spectrophotometric absorption (Nanodrop Spectrophotometer, BioLab, Scoresby, VIC, Australia). Polymerase chain reaction (PCR) was performed using an iCycler Thermal Cycler (Bio- Rad, Hercules, CA, USA). Interleukin 1 beta-31C > T (rs1143627) and interleukin 1 beta-511 T > C (rs16944) genotypes were determined using polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) method followed by DNA sequencing. Amplification of the target region was carried out by polymerase chain reaction using the specific forward and reverse primers. Primers were designed and selected using Primer3, version 0.4.0 software. For interleukin 1 beta-31 C > T, the primers were forward 5'-AGAAGCTTCCACCAATACTC-3' and reverse 5'-AGCACCTAGTTGTAAGGAAG-3'. For interleukin 1 beta-511 T > C, the primers were forward 5'-TGG CAT TGA TCT GGT TCA TC-3' and reverse 5'-GTT TAG GAA TCT TCC CAC TT-3'. The PCR reaction mixture consisted of Taq 1.5 U (Ferments), sense and antisense primers (0.5 μmol/l), MgCl₂ (50 mmol/l), dNTP (0.2 mmol/l), and DNA template (1 μg) and was subjected to an initial denaturing step of 4 min at 95 °C, then 35 cycles of denaturing for 30 s at 95 °C, annealing for 30 s at 56 °C, extension for 30 s at 72 °C, and a final extension step of 10 min at 72 °C. Digestion of the amplified products of interleukin 1 beta-31C > T and interleukin 1 beta-511 T > C was done by using 10 units restriction endonucleases *Alu1* (New England Biolabs) and *Ava1* (New England Biolabs) respectively and incubated at 37 °C for 16 h. The digested products were checked on 3% agarose gel, the RFLP picture for interleukin 1 beta – 31 genotype was identified as (C/C - 239 bp), (T/T- 137/102 bp), (T/C- 239/137/102 bp) and interleukin 1 beta-511 was identified as (T/T- 304 bp), (C/C -190/114), (T/C- 304/190/114 bp) Fig. 1. RFLP results were later confirmed by DNA sequencing Fig. 2.

Interleukin 1 beta (IL-1β) mRNA expression

Total RNA was extracted by using TRIZOL (Sigma Aldrich, USA) from normal tissues of lung cancer tissue. Integrity of the mRNA was checked on 1% agarose gel and quantified at 260/280 ratio. RNA was converted to cDNA by using first strand cDNA synthesis kit according to manufactures protocol (Fermentas, USA). Dilution of the cDNA was performed to get uniform quantity of cDNA in all samples. PCR primers were designed for interleukin 1 beta and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) over introns to avoid the amplification of possible traces of genomic DNA contamination by using Primer Express 3.0 software, Applied Biosystems and glyceraldehyde 3-phosphate dehydrogenase mRNA (GenBank accession number NM-002046). Glyceraldehyde 3-phosphate dehydrogenase was used as an internal control. For interleukin 1 beta primer sequence were as follows- forward 5'-GCACGATGCACCTGTACGAT and reverse 5'-CACCAAGCTTTTTGCT GTGAGT-3' and for glyceraldehyde 3-phosphate dehydrogenase, the primers were as follows: forward 5'-GATCCGCATAATCTGCATGTT-3' and reverse 5'-GATCCGCATAATCTGCATGTT-3'. Quantitative Real time PCR (Agilent Biotechnologies, Germany) was performed for the detection of interleukin 1 beta mRNA by recruiting Applied Biosystems Inc StepOne software v2.0. PCR was performed containing Maxima® SYBR Green qPCR Master Mix (2X) and all the samples (unknown and standards) were run in triplicates and accompanied by non template control (NTC). Thermal cycling conditions included 40 cycles of 30s at 55 °C. The melting curves of all

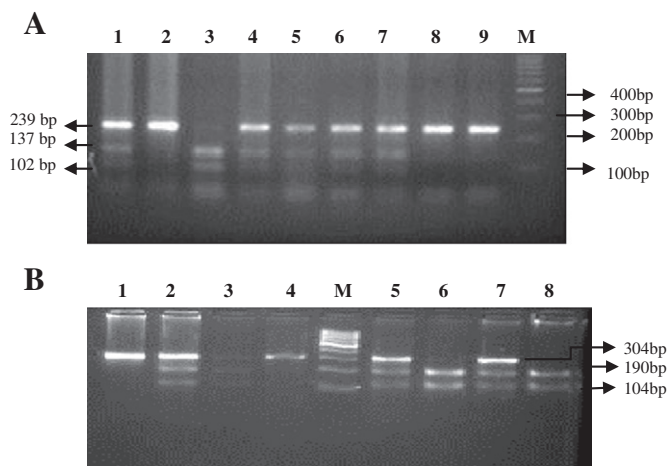


Fig. 1. A: Three percent electrophoresis results for the *IL-1β* -31 genotype. Lane M represents the 100 bp DNA marker; lane 3 represents the *IL-1β* -31 T/T genotype; lanes 1, 4, 5,6,7 represent the heterogeneous T/C genotypes; and lane 2,8,9 represents the C/C genotype. B: Three percent electrophoresis results of the *IL-1β* -511 genotype. Lane M represents the 100 bp DNA marker; lane 1,4 represents the *IL-1β* -511 T/T genotype; lanes 2, 3, 5,7 represent the heterogeneous C/T genotype; and lane 1,4 represents C/C genotype.

final real time PCR products were analysed for determination of genuine products and contamination of non specific products and primer dimer. All amplified products of real time polymerase chain reaction were subjected for separation on 2% agarose gel electrophoresis for ensuring the correct amplification products. Delta CT (Δ CT) method was used to check the interleukin 1 beta mRNA expression in normal lung tissue of lung cancer patients by normalization against glyceraldehyde 3-phosphate dehydrogenase used as a reference gene.

Statistical analysis

The distribution of the genotypes in controls was compared with that expected from Hardy-Weinberg equilibrium (HWE) by the chi square (χ^2) test. Odds ratios (ORs) and their 95% confidence intervals (CIs), with adjustments for age, sex dwelling and smoking were calculated by Fisher's exact test/Chi square test as appropriate. Students unpaired *t* test was used to compare the means and standard deviation. All reported P values were based on two-sided tests. Significance level was taken at $p \leq 0.05$. Statistical tests were performed using the software SPSS 16.0 (SPSS Inc., Chicago, Illinois).

Results

Interleukin 1 beta SNPs

A total of 190 Lung cancer cases and 200 healthy controls were successfully evaluated for genotyping of interleukin 1 beta-31C > T and interleukin 1 beta-511 T > C. The frequencies of tested genotypes in cases and controls are given in (Table 2). The observed genotypes for the controls population was in complete accordance with the Hardy Weinberg equilibrium ($P > 0.05$). The distribution of genotypes of interleukin 1 beta-31 C/T in controls were -31C/C(29.5%), -31C/T (55.5%), -31TT (15%) while in cases the distribution was -31 C/C (17.8%), -31C/T (56.3%) and -31 T/T (25.7%). The frequency of interleukin 1 beta genotypes was higher in cases with odds ratio of 1.6 (95% CI 1.01-2.7) in -31 CT and 2.8 (95% CI- 1.52-5.26) in -31TT respectively (Table 2). The distribution of both -31CT and -31TT observed in cases showed a strong significant association with non small cell lung cancer ($P < 0.05$) (Table 2). The interleukin 1 beta -31 T allele occurred more significantly in the non small cell lung cancer group than in the control group ($P = 0.002$) (Table 3). The distribution of genotypes of interleukin 1 beta-511 T/C in controls was -511 T/T(23.5%), -511 T/C(45%),-511C/C(31%) and in cases genotype distribution was -511 T/T(22.63%), -511 C/T(55.26%) and -511 C/C (22.10%). We found odds ratio of 1.27(95% CI 0.77-2.10) and 0.72 (95% CI 0.41-1.28) in -511 CT and TT genotypes respectively ($P > 0.5$)

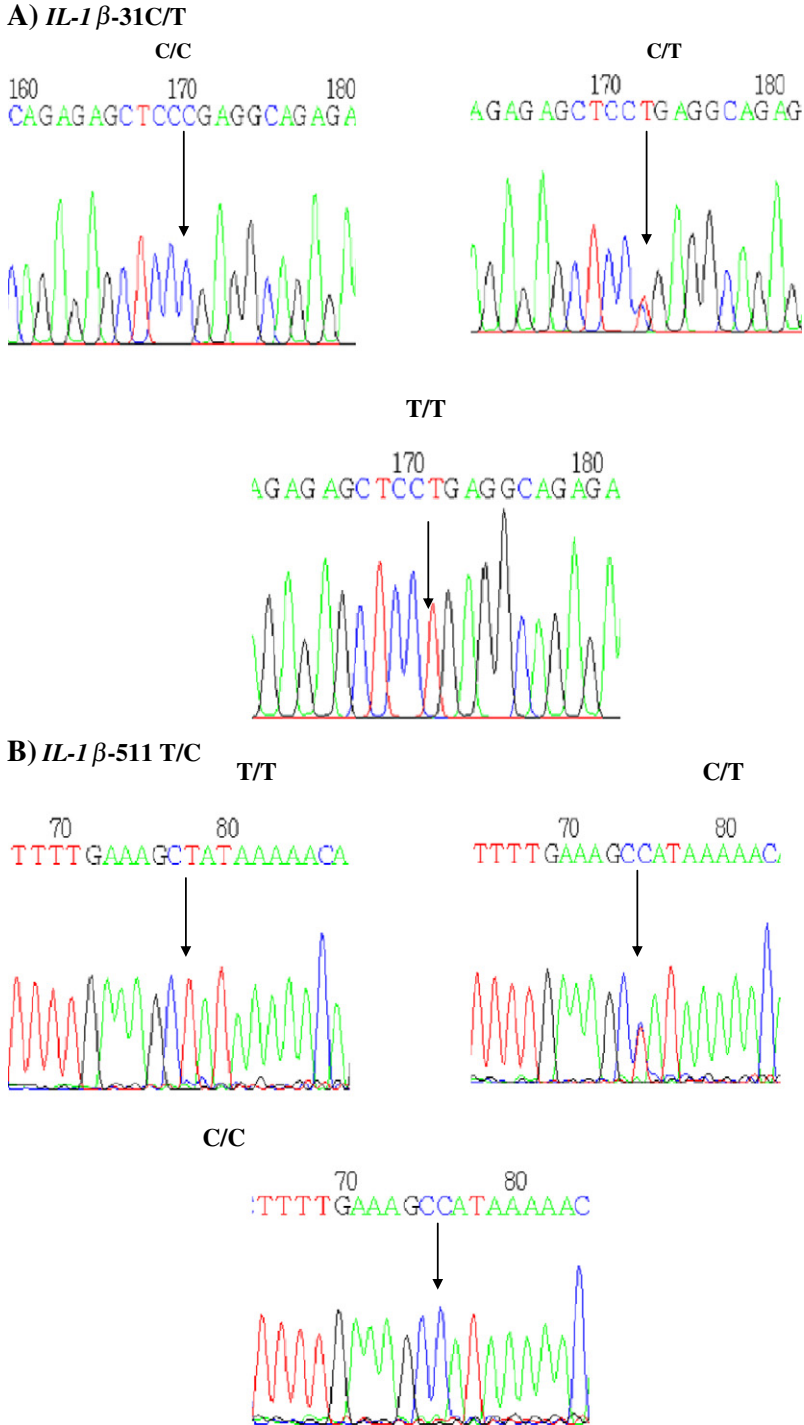


Fig. 2. A. IL-1β – 31 sequencing results. Arrow indicates the SNP location. B. IL-1β – 511 sequencing results. The arrow indicates the SNP location.

Table 2Association between Interleukin1 beta (*IL-1β*) genotypes and non small cell lung cancer.

Genotype	Cases N = 190(%)	Control N = 200(%)	P value OR(CI 95%)
<i>IL1β</i> 31 C > T			
CC	34 (17.8)	59 (29.5)	Reference
CT	107 (56.3)	111 (55.5)	0.04, 1.6 (1.01-2.7)
TT	49 (25.7)	30 (15)	0.001, 2.8 (1.52-5.26)
Allele			
C	175	229	0.002, 1.57 (1.18–2.08)
T	205	171	ref.
<i>IL1β</i> 511 T > C			
TT	43 (22.63)	47 (23.5)	0.37, 1.27 (0.77-2.10)
CT	105 (55.26)	90 (45.0)	0.3, 0.72 (0.41-1.28)
CC	42 (22.10)	63 (31)	0.25, 0.84 (0.64- 1.12)
Allele			
T	191	184	
C	189	216	

IL1β -31 -CC homozygous wild, CT heterozygous, TT homozygous variant.

IL1β -511 TT homozygous wild, CT heterozygous, CC homozygous variant.

Adjusted for age, smoking, gender, and dwelling.

(Table 2). Also in our study group, we could not find any significant association of -511C allele with non small cell lung cancer ($P = 0.2$) (Table 3). Interleukin 1 beta-31TT genotype did not show any significant association with various clinical parameters such as age and gender ($P > 0.05$) while smoking, histology, dwelling and stage showed significant association with -31TT genotype ($P < 0.05$) (Table 4). Interleukin 1 beta-511CC genotype showed significant association with gender and dwelling ($P < 0.05$) while other clinical parameters such as smoking, pathological stage and histology did not show any association with -511 C/C genotype ($P > 0.05$) (Table 4).

Interleukin 1 beta mRNA expression in non small cell lung cancer

We included 48 lung cancer patients for detection interleukin 1 beta mRNA expression in adjacent normal lung tissues of lung cancer patients. We analysed the effect of interleukin 1 beta-31 T SNP which is located in the core promoter of interleukin 1 beta gene with the mRNA expression. The mRNA expression was checked by ΔC_T method with expression ratio of (amount of interleukin 1 beta mRNA/amount of glyceraldehyde 3-phosphate dehydrogenase mRNA) by applying the formula $\Delta C_T = (C_T \text{ GAPDH} - C_T \text{ IL1B mRNA})$. The ΔC_T values of normal tissues of lung cancer patients was significantly higher in cases carrying interleukin 1 beta-31 T allele with a mean \pm standard deviation (SD) of 6.16 ± 3.48 as compared to cases carrying interleukin 1 beta-31C allele with a mean \pm standard deviation (SD) of 2.89 ± 2.21 ($P = 0.006$) (Table 5). However, we could not find any significant association between various clinicopathological characteristics and interleukin 1 beta mRNA expression in normal lung tissues of non small cell lung cancer patients.

Discussion

Epidemiological studies have supported the concept that chronic inflammation frequently precedes the development of lung cancer in adults (Tockman et al., 1987). Interleukin-1beta (*IL-1β*), a key proinflammatory cytokine encoded by the interleukin 1 beta gene, has been associated with chronic inflammation and plays an important role in lung inflammatory diseases, including lung cancer (Coussens and Werb, 2002; Ballaz and Mulshine, 2003; Smith et al., 2006). Together with tumor necrosis factor alpha (*TNFα*), *IL-1α* and interleukin 1 beta are defined as “alarm cytokines” that are secreted by macrophages and initiate inflammation (Dinarello, 1996, 2002b; Apte and Voronov, 2002). Interleukin 1 alpha and Interleukin 1beta cause inflammation but more importantly they induce the expression of pro-inflammatory genes which particularly include cyclooxygenase type 2 (*COX-2*), inducible nitric oxide synthase (*iNOS*), interleukin- 6 (*IL-6*) and other chemokines/cytokines (Apte et al., 2006b).

Table 3
Clinicopathological relevance of *IL-1β* genotypes in non small cell lung cancer.

Parameters	Genotype (<i>IL-1β</i> 31 C > T) N = 190			P value	Genotype (<i>IL-1β</i> 511 T > C) N = 190			P value
	CC	TC	TT		TT	TC	CC	
<i>Age</i>								
≤50 years	09	30	20	0.22	13	34	12	0.8
>50 years	25	77	29		30	71	30	
<i>Gender</i>								
Male	31	93	39	0.30	43	83	37	0.00
Female	03	14	10		00	05	22	
<i>Smoking satus</i>								
Nonsmoker	11	38	27	0.04	19	43	14	0.5
Smoker	23	69	22		23	61	28	
<i>Histology</i>								
Sq.CC	29	82	29	0.01	33	75	32	0.7
Others ^a	05	25	20		10	30	10	
<i>Dweller</i>								
Rural	27	22	28	0.01	26	74	27	0.01
Urban	07	35	21		17	31	30	
<i>Pathological stage</i>								
I & II	18	81	31	0.03	25	72	33	0.12
III & IV	16	26	18		18	33	09	
<i>Pathological grade</i>								
G1	17	35	19	0.47	20	39	12	0.17
G2	11	45	20		12	47	17	
G3	06	27	10		11	19	13	

G1- well differentiated, G2- moderately differentiated, G3- poorly differentiated

^a Others include adenocarcinoma, large cell carcinoma, bronchogenic carcinoma.

Interleukin 1 beta is expressed in 90% of invasive breast carcinomas and to a lesser extent in ductal in situ carcinomas (DISC) and benign lesions (Jin et al., 1997). In advanced breast carcinomas, high interleukin 1 beta content correlated well with other parameters of aggressive tumors (estrogen receptor negativity, high tumor grade, presence of *p53*, absence of *bcl-2* and expression of pro-inflammatory cytokines, i.e., interleukin 1 alpha and interleukin-8) (Jin et al., 1997; Miller et al., 2000). The elevated expression of interleukin 1 beta has been reported in various cancers and patients with increased expression were related with bad prognosis (Lewis et al., 2006). Inflammation in the lung may be modulated by host genetic factors such as polymorphisms in inflammatory genes. Identification of polymorphisms in inflammatory genes may help understanding interindividual differences in susceptibility to lung cancer. Polymorphism of a specific gene can have effects on gene transcription, the stability of the mRNA, or the quantity and activity of the resulting protein. Thus, screening and risk evaluation is important for purposes of cancer prevention or detection. A specific interleukin 1 beta haplotype -3893 G, -1464 G, -511 C and -31 T in the promoter region of interleukin 1 beta was found to be associated with

Table 4
IL-1β mRNA expression in normal lung tissue samples of non small cell lung cancer cases in relation to *IL-1 β* 31 T > C genotypes.

Genotypes	<i>IL1β</i> mRNA Expression (mean ± SD)	Number	P value
CC	2.89 ± 2.21	10	0.006
CT + TT	6.18 ± 3.48	38	

IL1 β -31 -CC homozygous wild, CT heterozygous, TT homozygous variant.

Table 5The relationship between clinicopathological variables and *IL-1 β* mRNA expression in lung cancer.

Clinical parameters	<i>IL1β</i> mRNA Expression (mean \pm SD)	Number	P value
Age			
≤ 50 years	5.59 \pm 4.32	16	0.8
> 50 years	5.42 \pm 3.11	32	
Sex			
Male	5.32 \pm 3.38	38	0.5
Female	6.08 \pm 4.13	10	
Smoking status			
Nonsmoker	5.90 \pm 3.81	15	0.58
Smoker	5.29 \pm 3.42	33	
Histology			
SCC ^a	5.41 \pm 3.68	35	0.8
Others ^b	5.66 \pm 3.17	13	
Dwelling			
Rural	5.76 \pm 3.77	30	0.48
Urban	5.02 \pm 3.09	18	
Pathological stage			
I & II	6.16 \pm 3.64	31	0.06
III & IV	4.20 \pm 2.95	17	
Pathological grade^c			
G1	5.23 \pm 3.93	21	0.67
G2 + G3	5.67 \pm 3.22	27	

^a Squamous cell carcinoma.^b Others include adenocarcinoma, large cell carcinoma, bronchiogenic carcinoma.^c G1- well differentiated, G2- moderately differentiated, G3- poorly differentiated.

increased interleukin 1 beta mRNA and increased risk of risk of non small cell lung cancer (Landvik et al., 2009). The two functionally important polymorphisms in the promoter region of interleukin 1 beta-31 T/C (rs1143627) and interleukin 1 beta-511C/T (rs16944) have been observed to be associated with the risk of lung cancer (Kai-song et al., 2010).

In this study we examined the risk of non small cell lung cancer association with two polymorphisms interleukin 1 beta-31C > T (rs1143627) and interleukin 1 beta-511 T > C (rs16944) and effect of interleukin 1 beta -31TT on its mRNA expression. We found both heterozygous (-31CT) and homozygous (-31TT) carriers of the interleukin 1 beta -31 T/C had a significantly higher risk of lung cancer ($P < 0.05$). Our results were in accordance with the previous studies which have shown association of interleukin -31C > T and interleukin 1 beta-31 T/T with the non small cell lung cancer (Zienolddiny et al., 2004; Haukim et al., 2002; Asada et al., 2006). We also observed that -31 T allele is significantly associated with non small cell lung cancer as compared to controls ($P = 0.002$). The heterozygous and homozygous subjects were particularly at higher risk of lung cancer with -31TC and -31TT genotype having 1.6 and 2.8 fold increased risk for the non small cell lung cancer respectively (Table 2). Thus our report supports the role of interleukin 1 beta-31C > T in predisposing the risk of lung cancer in our population. However we did not observe any significant role of interleukin 1 beta-511 T > C polymorphism in risk of non small cell lung cancer as has been previously reported (Bidwell et al., 1999; Kai-song et al., 2010; Asada et al., 2006). Thus we found that in this group, the risk was more closely related to polymorphisms of interleukin 1 beta-31 T rather than interleukin 1 beta-511C. The discrepancy in our report with the previous studies that have shown association of both the SNP's (*IL-1 β* -31 C > T and *IL-1 β* -511 T > C) could be due to the ethnic variation. When clinicopathological characteristics were correlated with interleukin 1 beta-31 C > T and interleukin 1 beta-511 T > C polymorphism, interleukin 1 beta-31TT genotype showed significant association with smoking, histology, dwelling, pathological stage while as interleukin 1 beta-511 showed significant association with gender and dwelling only ($P < 0.05$).

We investigated interleukin 1 beta mRNA expression in normal lung tissues of non small cell lung cancer patients using qRT PCR. We observed an increasing trend of interleukin 1 beta mRNA expression in normal tissues of non small cell lung cancer patients with genotypes interleukin 1 beta-31CT and interleukin 1 beta-31TT as compared to -31CC genotypes ($P = 0.006$). We did not find any clinicopathological factor affecting interleukin 1 beta mRNA expression and thus our study may help in understanding the role of -31 T allele in affecting the expression of interleukin 1 beta mRNA. Functional studies of the SNP have also shown an increasing trend of mRNA expression in normal tissues of lung cancer patients. The decreased expression of the C allele observed in our report is already confirmed by previous study (Landvik et al., 2009). Accordingly the high expression of T allele as found in our study has been reported by Helge Lind et al in A549 cells in A549 cells (Lind et al., 2007).

Similarly Kent Hart, observed a higher expression of the interleukin 1 beta mRNA in lungs of the patients carrying the *SEPS1-105G/G- IL1 β - 31 T/T* genotype (Hart et al., 2008). This is an interesting observation since inflammatory cytokines such as interleukin 1 beta have been shown to induce DNA damage and inhibit DNA repair in vitro (Jaiswal et al., 2000). The decreased expression found in our study with interleukin 1 beta-31C allele has been already postulated due to differential binding patterns of nuclear proteins to oligonucleotide probes containing the interleukin 1 beta-31C allele compared to those with the interleukin 1 beta -31 T allele as a result of specific binding of the transcription factor Yin Yang 1 (YY1) (Landvik et al., 2012).

It has already been shown that the down-regulation of YY1 by the use of siRNA results in increased tumor necrosis factor alpha (TNF- α) induced interleukin 1 beta expression. Further YY1 has been shown to have inhibitory effects on transcription (Shi et al., 1997), thus YY1 when binds to the interleukin 1 beta core promoter (TAATA) promoter could inhibit gene expression, this may explain the previously observed lower promoter activity in reporter vectors with the interleukin 1 beta-31C promoter compared to -31 T promoter (Lind et al., 2007).

In conclusion, our work has indicated that the molecular status of interleukin 1 beta may play a significant role in the pathogenesis of non small cell lung carcinoma. Over expression of interleukin 1 beta mRNA was noted in non small cell lung cancer and more so in lung cancer with interleukin 1 beta-31 T allele. These findings imply that continued research into interleukin 1 beta mRNA expression and polymorphisms will be an important source of information in understanding the role of inflammation in the pathogenesis of lung cancer.

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