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Interleukin-18, interleukin-8, and CXCR2 and the risk of silicosis

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Abstract

Molecular mechanisms in the pathogenesis of silicosis are not fully understood. Exposure to crystalline silica leads to the activation of signaling pathways controlling the production and secretion of inflammatory mediators. Inflammatory cytokines are noted as important candidate genes for fibrotic lung diseases. Cytokines, chemokines, and variations of their genes have been associated with upregulation or downregulation of chronic inflammatory mediators. Variations in the interleukin (IL)-18, IL-8 and chemokine receptor CXCR2 genes are believed to influence the risk of silicosis in stone-grinding factory workers in Iran. Allele-specific oligonucleotide polymerase chain reaction (PCR) procedure was carried out for IL-18 −137 and IL-18 −607, meanwhile touchdown PCR was performed for IL-8 −251 and CXCR2 +1208 genotyping. Variation in genotypic and allelic frequencies was not statistically different among cases versus controls ($p > 0.05$). These findings indicated for the first time that IL-18 −137, IL-18 −607, IL-8 −251, and CXCR2 +1208 are suggested not to influence the risk of silicosis in tested occupational group.

Keywords

IL-18 (−137 G/C, −607 C/A), IL-8 (−251 A/T), CXCR2 (+1208 C/T), silicosis

Introduction

Silicosis is defined as lung disease that is caused by crystalline silica inhalation (Yucesoy and Luster, 2007). Chronic inflammation within the pulmonary system resulting in severe fibrotic changes in lungs (Wynn, 2008; Yucesoy and Luster, 2007) is a fatal, irreversible condition and is more common among occupational groups such as the workers of quarry and stone-grinding factories, miners, and sand blasters (Valiante et al., 2004; Wynn, 2008; Yucesoy and Luster, 2007). The pathobiological mechanism for silicosis is not elucidated completely, but it is understood that the silicosis is triggered when macrophages of alveoli in pulmonary system phagocytize silica and dust-related particles in order to remove them from the pulmonary system (Dubois et al., 1989; Hamilton et al., 2008). Subsequently, alveolar macrophages may be injured. Those macrophages containing silica or dust-related particles die and then release mentioned components in alveoli. In alveoli, rephagocytization of silica and dust-related particles via other alveolar macrophages corresponds to excess injuries (Fubini and Hubbard, 2003). This complex process is accompanied by a cascade of trafficking and chemotaxi of neutrophils and lymphocytes to the sites of injury and consequently resulting in chronic silicosis (Fubini and Hubbard, 2003; Gulumian et al., 2006; Huaux, 2007; Miller et al., 1990). Silica and dust-related particles stimulate alveolar macrophages to release tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, reactive oxygen species, reactive nitrogen species, and extra free radicals (Greenberg et al., 2007; Miller et al., 2012; Schmidt et al., 1984). Silica-stimulated monocytes release fibroblast proliferation factors identical to IL-1 (Schmidt et al., 1984). Inflammatory cytokines are noted as important candidate genes for fibrotic lung diseases. Recent studies

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have demonstrated that the variations in ethnicity, cytokines, and chemokines have been associated with wide range of human diseases such as sarcoidosis, scleroderma, and severe silicosis (Hollegaard and Bidwell, 2006; Luther and Cyster, 2001; Tangirala et al., 1997; Yamagami et al., 2004). Results of recent studies showed that the highly upregulated genes are *CCL2*, *CXCL8* (*IL-8*), *IL-6*, and *CXCL2* with stimulation by proinflammatory cytokines (Yamagami et al., 2003) and indicate that the amount of downregulated genes were smaller than upregulated genes (Yamagami et al., 2003). *CCR2* is mentioned as the most downregulated gene (Yucesoy et al., 2002). The level of CCR2 expression has been shown to be downregulated with the proinflammatory cytokines as well as CCL2 (Corbett et al., 2002). Large bodies of studies imply that the overexpression of IL-1β and TNF-α cytokines are associated with silicosis (Corbett et al., 2002; Kim et al., 2002; Nadif et al., 2006; Yucesoy et al., 2001a,b, 2002; Zhai et al., 1998; Zhang et al., 1993). In this study, we analyzed whether biallelic variation in the *IL-8* (−251), *IL-18* (−607 and −137), and *CXCR2* (+1208) genes, which alter promoter strength, were associated with the risk of silicosis in an occupational group (stone-grinding factory workers) in Iran.

Materials and methods

All steps and procedures of present investigation were approved by the ethical committee of Urmia University of Medical Sciences. Individuals were selected from a stone-grinding factory based on cases-control study that is conducted in the Occupational Medicine Center, Urmia University of Medical Sciences, Urmia, Iran. Inclusion and exclusion criteria were described previously by Mohebbi et al. (2010), Mohebbi and Abdi Rad (2007), Mohebbi et al. (2007), Mohebbi and Zubeyri (2007), Mohebbi et al. (2011) and according to the International Labor Office standards (International Labor Office, 1981, 2002). A total of 45 healthy volunteer controls and 45 patients with silicosis were entered at the study. All contributors were West Azarbaijani males and Iranian. Tested groups were matched for race, geographical region, work place, and history of exposure to occupational silica dust. Cases and controls with any confounding factors, such as history of systemic diseases (systemic lupus erythematosus and rheumatoid arthritis) and disorders in physical tests, and also by considering medical and familial history were excluded from study. Controls were healthy subjects with similar history of occupational silica dust exposure without any radiographic and respiratory functional findings in medical examinations regarding disease development. Information regarding important confounding factors was collected via detailed questionnaire by occupational medicine specialist.

With informed consent, approximately 2–3 mL of blood samples were collected into the EDTA-containing tubes and stored at −20°C until genomic DNA extraction. The genomic DNA was extracted by a standard method as described by Miller et al. (1988).

IL-18 −137

Common primer: 5′-AGGAGGGCAAAATGCACTG G-3′; G allele primer: 5′-CCCCAACTTTTACGGAA GAAAAG-3′; and C allele primer: 5′-CCCCAACTT TTACGGAAGAAAAC-3′.

Allele-specific oligonucleotide polymerase chain reaction (PCR) procedure was carried out for 2 min at 94°C, 20 s at 94°C, 60 s at 68°C, and 40 s at 72°C (five cycles) and 20 s at 94°C, 20 s at 62°C, and 40 s at 72°C (25 cycles) (Naeimi et al., 2006).

IL-18 −607

Common primer: 5′-TAACCTCATTCAGGACTTC C-3′; C allele specific primer: 5′-GTTGCAGAAAG TGTAAAAATTATTAC-3′; and A allele specific primer: 5′-GTTGCAGAAAGTGTAAAAATTATTAA-3′. Allele-specific oligonucleotide PCR procedure was carried out for 2 min at 94°C, 20 s at 94°C, 40 s at 64°C, and 40 s at 72°C (seven cycles) and 20 s at 94°C, 40 s at 57°C, and 40 s at 72°C (25 cycles) (Naeimi et al., 2006).

IL-8 −251

Common primer: 5′-TGCCCCTTCACTCTGTTAAC-3′; A allele specific primer: 5′-CCACAATTTGGTGA ATTATCAAT-3′; and T allele specific primer: 5′-CCACAATTTGGTGAATTATCAAA-3′.

A touchdown PCR procedure was carried out for 25 s at 95°C, 45 s at decreasing from 68°C for four cycles to 61°C for 20 cycles, and extension of 40 s at 72°C, annealing of 40 s at 58°C for five cycles, and extension of 40 s at 72°C. Finally, the annealing step for the remaining five cycles was carried out for 40 s at 58°C (Morris et al., 1992).

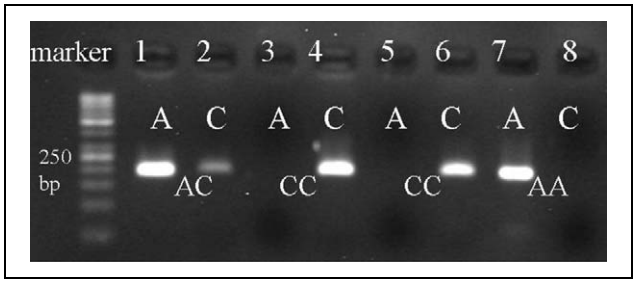

Figure 1. Detection of IL-18 −607 SNPs by ASO-PCR in four samples (eight alleles). Lane marker: a 50-bp DNA ladder. Presence or absence of a 196-bp fragment representative for A or C allele. Sample1: heterozygote for A and C alleles (AC); samples 2 and 3: homozygote for C allele (CC); sample 4: homozygote for A allele (AA). SNP: single-nucleotide polymorphism; ASO-PCR: allele-specific oligonucleotide-polymerase chain reaction; IL-18: interleukin-18.

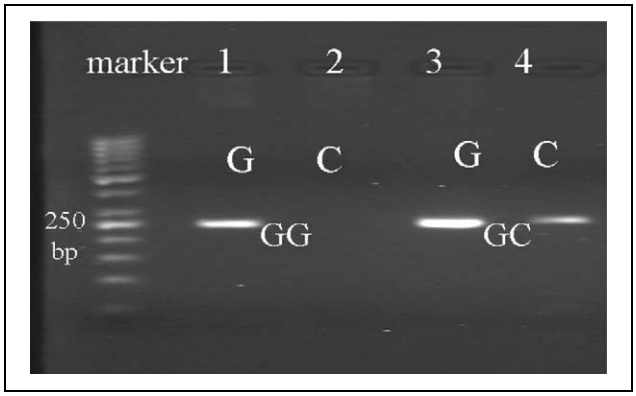

Figure 2. Detection of IL-18 −137 SNPs by ASO-PCR in two samples (four alleles). Lane marker: a 50-bp DNA ladder. Presence or absence of a 261-bp fragment representative for C or G allele. Sample1: homozygote for G allele (GG); sample 2: heterozygote for C and G alleles (CG). SNP: single-nucleotide polymorphism; ASO-PCR: allele-specific oligonucleotide-polymerase chain reaction; IL-18: interleukin-18.

CXCR2 +1208

Common primer: 5′-GTCTTGTGAATAAGCTGC-TATGA-3′; C allele specific primer: 5′-CCATTG TGGTCACAGGAAGC-3′; T allele specific primer: 5′-CCATTGTGGTCACAGGAAGT-3′. A touchdown PCR procedure was carried out for 25 s at 95°C, 45 s at decreasing from 70°C for four cycles to 65°C for 20 cycles, and extension of 40 s at 72°C, annealing of 40 s at 55°C for five cycles, and extension of 40 s at 72°C (Renzoni et al., 2000).

The amplified PCR products were then visualized and analyzed using 2% agarose gel electrophoresis that is stained with ethidium bromide. All statistical

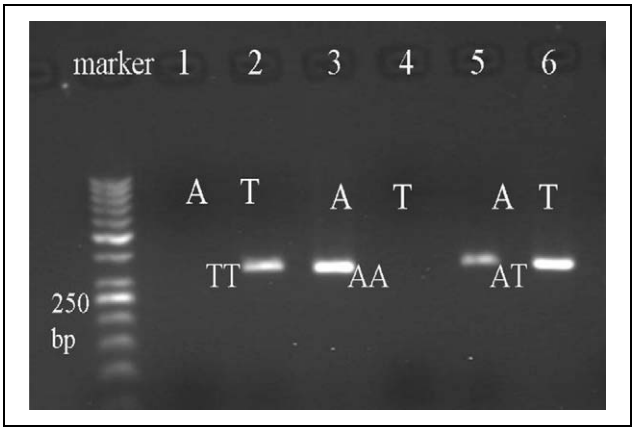

Figure 3. Detection of IL-8 −251 SNPs by ASO-PCR in three samples (six alleles). Lane marker: a 50-bp DNA ladder. Presence or absence of a 336-bp fragment representative for A or T allele. Sample1: homozygote for T allele (TT); sample 2: homozygote for A allele (AA); sample 3: heterozygote for A and T alleles (AT). SNP: single-nucleotide polymorphism; ASO-PCR: allele-specific oligonucleotide-polymerase chain reaction; IL-18: interleukin-18.

analyses were carried out by the SPSS ver. 16.0 and Microsoft Excel 2007. Allelic and genotypic frequencies were computed by direct counting. A comparison was made between cases and controls using χ^2 test or Fisher's exact test regarding IL-18 −607, IL-18 −137, IL-8 −251, and CXCR2 +1208. To minimize the genotypic errors rate and therefore improve data quality, the expected genotype frequencies were calculated and then compared with those of observed genotype frequencies (Hardy-Weinberg equilibrium (HWE)). The χ^2 and p value, the odds ratio (OR), and 95% confidence interval (CI) were calculated for statistic analysis. Two-sided tests ($\alpha = 0.10$) with power analysis (1-β) of 70% for a minimum sample size of 37 was performed and $p < 0.05$ was noted as statistically significant.

Results

A total of 90 males including 45 patients with silicosis and 45 healthy controls were enrolled in the study. Allelic and genotypic frequencies were compared by χ^2 test and confirmed for the examination of their fitness to the HWE test for IL-18 −607, IL-18 −137, IL-8 −251, and CXCR2 +1208. Representative images of gel analysis are shown in Figures 1–4. The distributions of all genotypes were fit to HWE in our tested groups: IL-8 −251 (patients group: $\chi^2 = 1.73 < 3.84$, $p = 0.419 > 0.05$, T allele frequency = 0.53, A allele frequency = 0.47; controls group: $\chi^2 = 2.65 < 3.84$,

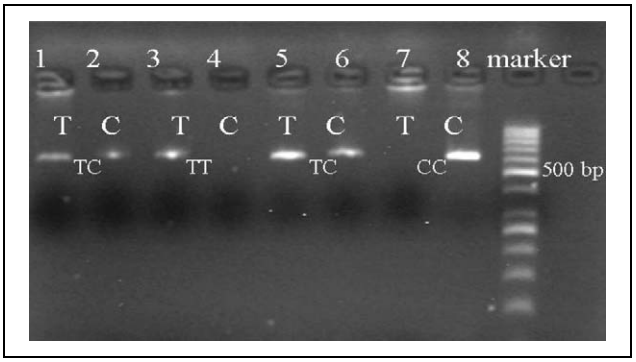

Figure 4. Detection of CXCR2 (+1208 C/T) SNPs by ASO-PCR in four samples (eight alleles). Lane marker: a 50-bp DNA ladder. Presence or absence of a 627-bp fragment representative for T or C allele. Sample 1: heterozygote for T and C alleles (TC); Sample 2: homozygote for T allele (TT); sample 3: heterozygote for T and C alleles (TC); sample 4: homozygote for C allele (CC). SNP: single-nucleotide polymorphism; ASO-PCR: allele-specific oligonucleotide-polymerase chain reaction.

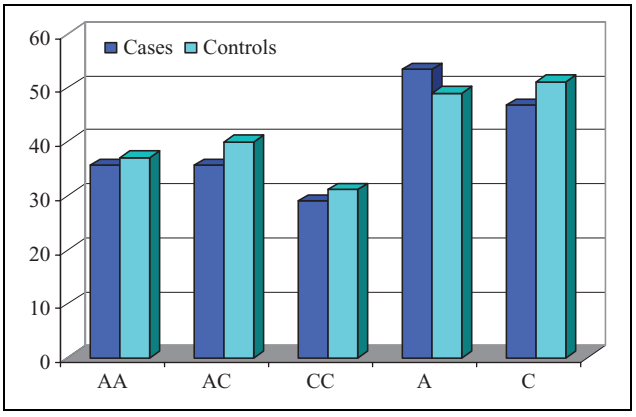

Figure 5. Distribution of IL-18 −607 AA, AC, CC, A, and C genotypes/alleles (%) in the present study.

$p = 0.264 > 0.05$, T allele frequency = 0.48, A allele frequency = 0.52); IL-18 −607 (patients group: $\chi^2 = 3.67 < 3.84$, $p = 0.159 > 0.05$, A allele frequency = 0.53, C allele frequency = 0.47; controls group: $\chi^2 = 1.79 < 3.84$, $p = 0.408 > 0.05$, A allele frequency = 0.49, C allele frequency = 0.51); IL-18 −137 (patients group: $\chi^2 = 1.06 < 3.84$, $p = 0.587 > 0.05$, G allele frequency = 0.87, C allele frequency = 0.13; controls group: $\chi^2 = 0.32 < 3.84$, $p = 0.852 > 0.05$, G allele frequency = 0.92, C allele frequency = 0.08); CXCR2 +1208 (patients group: $\chi^2 = 1.30 < 3.84$, $p = 0.521 > 0.05$, T allele frequency = 0.32, C allele frequency = 0.68; controls group: $\chi^2 = 0.06 < 3.84$, $p = 0.969 > 0.05$, T allele frequency = 0.31, C allele frequency = 0.69).

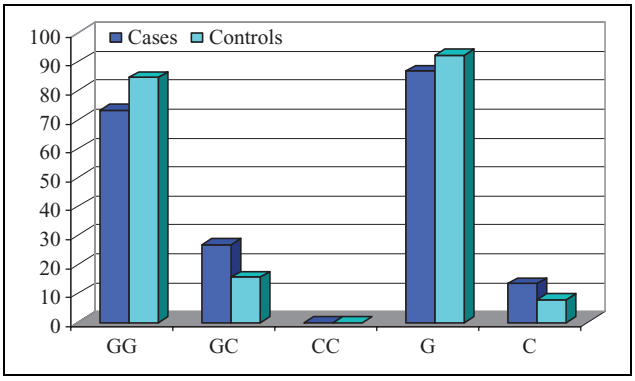

Figure 6. Distribution of IL-18 −137 GG, GC, CC, G, and C genotypes/alleles (%) in the present study.

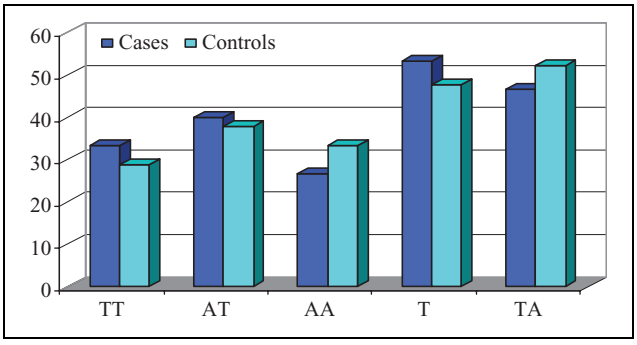

Figure 7. Distribution of IL-8 −251 TT, AT, AA, T, and A genotypes/alleles (%) in the present study.

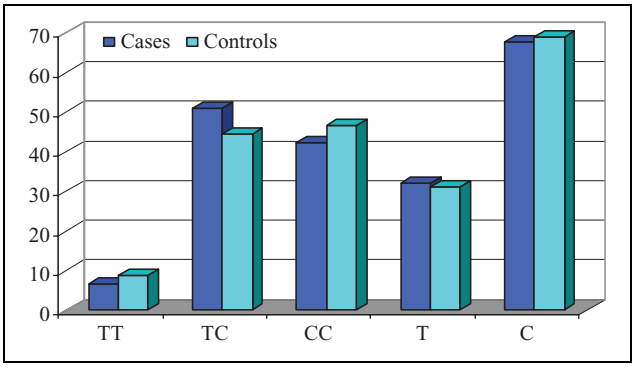

Figure 8. Distribution of CXCR2 +1208 TT, TC, CC, T, and C genotypes/alleles (%) in the present study.

Distribution of studied genotypes and alleles in the present study are indicated in Figures 5–8. The frequencies of IL-8 −251, IL-18 −607, IL 18 −137, and CXCR2 +1208 genotypes/alleles in healthy males (controls) and cases as well as the results of a comparison between the mentioned groups are reported in Table 1. Variation in genotypic and allelic frequencies was not statistically

Table 1. The frequencies of IL-18 −607, IL-18 −137, IL-8 −251, and CXCR2 +1208 genotypes/alleles in healthy males (controls) and patients with silicosis and a comparison between cases versus controls.

SNPs	Genotypes/alleles	Cases *F* (*F*%)	Controls *F* (*F*%)	OR (95% CI)	χ^2	*p* Value
IL-8 −251	TT	15 (33.3)	13 (28.9)	1.2308 (0.503–3.01)	0.207	0.6488
	AT	18 (40)	17 (37.8)	1.098 (0.47–2.564)	0.047	0.8288
	AA	12 (26.7)	15 (33.3)	0.7273 (0.294–1.799)	0.476	0.4902
	T	48 (53.3)	43 (47.8)	1.2492 (0.696–2.243)	0.556	0.456
	A	42 (46.7)	47 (52.2)	0.8005 (0.446–1.437)	0.556	0.456
IL-18 −607	AA	16 (35.6)	13 (37.1)	0.934 (0.373–2.338)	0.021	0.884
	AC	16 (35.6)	18 (40)	0.828 (0.353–1.943)	0.189	0.664
	CC	13 (28.9)	14 (31.1)	0.9 (0.365–2.217)	0.053	0.818
	A	48 (53.3)	44 (48.9)	1.195 (0.666–2.145)	0.356	0.551
	C	42 (46.7)	46 (51.1)	0.837 (0.466–1.502)	0.356	0.551
IL-18 −137[a]	GG	33 (73.3)	38 (84.4)	0.507 (0.179–1.437)	1.668	0.197
	GC	12 (26.7)	7 (15.6)	1.974 (0.696–5.598)	1.668	0.197
	G	78 (86.7)	83 (92.2)	0.548 (0.205–1.464)	1.471	0.225
	C	12 (13.3)	7 (7.78)	1.824 (0.683–4.871)	1.471	0.225
CXCR2 +1208	TT	3 (6.67)	4 (8.89)	0.732 (0.154–3.476)	0.155	0.694
	TC	23 (51.1)	20 (44.4)	1.307 (0.57–2.994)	0.401	0.527
	CC	19 (42.2)	21 (46.7)	0.835 (0.363–1.92)	0.18	0.671
	T	29 (32.2)	28 (31.1)	1.053 (0.562–1.973)	0.026	0.873
	C	61 (67.8)	62 (68.9)	0.95 (0.507-1.78)	0.026	0.873

SNP: single-nucleotide polymorphism; IL-18: interleukin-18; *F*: frequency; OR: odds ratio; CI: confidence interval.
[a]IL-18 −137 CC genotype was not found in our cases and controls.

different among cases versus controls (OR (95% CI), χ^2 value, and *p* value are reported in Table 1).

Discussion

Biallelic variations have been identified within the promoter region or other regulatory sequences of cytokines, chemokines, and chemokine receptors genes that greatly influence the strength of the promoter cause to mediate transcription and expression (Yucesoy et al., 2001; Yucesoy and Luster, 2007; Zhai et al., 1998). It has been demonstrated that the interactions of gene–environmental factors such as cytokine/chemokine genetic variations play an important role in silicosis (Yucesoy et al., 2001; Yucesoy and Luster, 2007; Zhai et al., 1998).

IL-18, a pleiotropic contributor in chronic inflammation (McInnes et al., 2000), was defined as an interferon-γ inducing factor and belongs to the IL-1 family (Ushio et al., 1996). IL-18 is produced by a wide variety of cells such as activated monocytes and macrophages, keratinocytes, adrenal cortex cells, intestinal epithelial cells, microglial cells, synovial fibroblasts, Kupffer's cells, osteoblasts, and articular chondrocytes, dendritic cells, as well as pituitary gland (Dinarello, 1999; McInnes et al., 2000; Ushio et al., 1996). IL-18 regulates both the innate and acquired immunities and plays an important role in inflammation (McInnes et al., 2000). IL-18 has a unique function which differentiates either Th1 or Th2 subsets (McInnes et al., 2000). Synergistically, IL-18 and IL-12 lead to an increased level of production of TNF-α and IL-1 by macrophages (Nakahira et al., 2002) and upregulates the expression of adhesive molecules (Klein et al., 1999; Kohno et al., 1997; Nakahira et al., 2002). Nitric oxide production became induced in the site of chronic inflammation (Klein et al., 1999; Kohno et al., 1997; McInnes et al., 2000). Expression of IL-18 gene is regulated by two single-nucleotide polymorphisms at positions −607 and −137 in the promoter of the gene (Giedraitis et al., 2001). In this report, significant associations of IL-18 gene polymorphism (IL-18 −607 and −137) with the risk of silicosis were not observed in tested population. IL-8 is prominent for its function in recruitment/activation of neutrophils during inflammatory responses, leukocytes trafficking to the central nervous system, as well as development of central nervous system, neuronal functions, and neuroimmune interactions (Bacon and Harrison, 2000; Danik et al., 2003). Recent studies imply that IL-8 −251 T<A and its receptor CXCR2 +1208 T<C have been

associated with human disease by different mechanisms such as infectious disease (Jiang et al., 2003; Yamagami et al., 2004), prostate (McCarron et al., 2002), and lung cancer (Yamagami et al., 2004). The presence of A allele in the promoter sequence of IL-8 −251 has been associated with higher production of IL-8 (*in vitro*) (Hull et al., 2000). The results of several investigations indicating that CXCR2 +1208 T<C has critical role in the risk of chronic inflammatory disorders (Renzoni et al., 2000). The role of IL-8 −251 T<A and CXCR2 + 1208 T<C single-nucleotide variation has been studied in human diseases (Renzoni et al., 2000; Yamagami et al., 2004). Our results indicate that the differences among the cases and controls were not statistically different regarding the allelic/genotypic frequencies of IL-8 −251 T<A and CXCR2 + 1208 T<C.

Therefore, these findings are reported for the first time and showed that IL-8 (−251), IL-18 (−607 and −137), and CXCR2 (+1208) have no role in pathogenesis of silicosis in studied population. Results of present investigation may be considered for designing new studies with more details in the future. These findings help us to increase our understanding of the complex mechanism of silicosis pathogenesis. It has been demonstrated that the information about the role of environmental factors for silicosis is more than the genes predisposing disease. Not only it is possible to role out an independent role for a gene on pathogenesis of silicosis but also another gene(s) or environmental factors increase the risk of silicosis in different ethnic groups. Based on our knowledge, the present article is the first study in its kind and had some limitation such as sample size. Although our sample size is small, the present study is the first study and was well performed. Study in larger groups with more details may be carried out to validate these findings.

Conclusion

It can be concluded that based on the findings of the present study, we failed to suggest that IL-8 (−251), IL-18 (−607 and −137), and CXCR2 (+1208) have been associated with the risk of silicosis in an occupational group from Iran.

Authors' Note

The Urmia University of Medical Sciences had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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