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Molecular Evaluation of the IFN γ +874, TNF α -308, and IL-1Ra VNTR Sequences in Silicosis

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ABSTRACT

Introduction: *to assess whether single nucleotide variation within regulatory sequences of cytokine or chemokine genes is associated with silicosis, this study was conducted for molecular evaluation of the IFN γ +874, TNF α -308, and IL-1Ra VNTR sequences in the patients with the silicosis.*

Materials and methods: *ASO-PCR technique was carried out for genotyping of IFN γ +874 and TNF α -308, and in the case of IL-1Ra VNTR, a PCR reaction was performed.*

Results: *our findings implied that: 1) IFN γ +874 T allele frequency was 0.44 in the cases and 0.48 in the controls; 2) IFN γ +874 A allele frequency was 0.56 in the cases and 0.52 in the controls; 3) TNF α -308 A allele frequency was 0.34 in the cases and 0.29 in the controls; 4) TNF α -308 G allele frequency was 0.66 in the cases and 0.71 in the controls; 5) the observed frequencies (%) of allele 1, allele 2, allele 3 and allele 4 were 65(72.2), 18(20), 2(2.22), 5(5.56) in the cases respectively, and 6) 68(75.6), 17(18.9), 2(2.22), 3(3.33) in the controls respectively. Genotypic and allelic frequencies were not significantly different between cases and controls (p value > 0.05).*

Conclusions: *it can be concluded that IFN γ +874, TNF α -308 and IL-1Ra VNTR are not associated with silicosis.*

Keywords: IFN γ +874, TNF α -308, IL-1Ra VNTR, silicosis

INTRODUCTION

Silicosis is an occupational lung disease that affects individuals exposed to silica particles in the dusty work places. Although history of dust exposure is the most important factor in the pathogenesis of silicosis, individual specific responses to dust exposure demonstrated that some genes and genes variations mainly influence the silicosis susceptibility (1). It is demonstrated that chronic inflammatory processes play critical role in the formation of pulmonary lesions and pathogenesis of silicosis (2-6). The exact mechanisms are still poorly known but it is documented that breathed silica particles are phagocytized via alveolar macrophages and alveolar macrophages became injured because of silica particles. Damaged alveolar macrophages are the source of stimulation, production and secretion of inflammatory cytokines (7,8). Several cytokine such as interleukin- 1,

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tumor necrosis factor-α, and leukotrienes, as well as, chemokines such as interleukin-8, macrophage inflammatory protein (MIP)-2, MIP-1α, MIP-1β, and monocyte chemoattractant proteins have important influence on inflammatory responses (7-13). Cytokines such as IL-1, IL-8, TNF- α, IL-18, IFN-γ and CXCR2 affect the gene expression level in human biological systems (14-17). It has been demonstrated that production, secretion and variety of gene expression regarding cytokines or chemokines have been associated to human diseases: sarcoidosis, scleroderma, and severe silicosis (18-21). Yucesoy et al (2001) showed that there is an association between the IL-1RA (+ 2018) variation and silicosis, and they suggested that the IL-1RA (+ 2018) may confer increased risk for the development of the silicosis (22). IL-1β +3953 (nt5887) C→T (TaqI) has not been associated with silicosis (22-24). Fan et al (2006) showed that IL-1α (-889) allele 2 is related to pneumoconiosis (25). Since the IFN γ +874, TNF α -308 and IL-1Ra VNTR cytokines play critical roles in human disorders, we aimed for molecular evaluation of the IFN γ +874, TNF α -308 and IL-1Ra VNTR sequences in silicosis. ❑

MATERIALS AND METHODS

The ethical committee of Urmia University of Medical Sciences approved the present study. A total of 90 males including 45 patients with silicosis and 45 healthy controls were enrolled in the study. All participants were Iranian and West Azerbaijani males. Diagnosis of silicosis was made on the basis of clinical findings, chest radiological criteria in accordance with the International Labour Organization (ILO) International Classification of Radiographs of Pneumoconiosis, an unequivocal history of substantial silica dust exposure and an appropriate interval of time after exposure (26-30). For each subject, the detailed histories of work occupations were recorded including any dusty workplaces and the age of initial diagnosis. Individuals with any accompanying disorders in physical examinations, chest radiological criteria, and also, by considering history of silica dust exposure were excluded. Cases and controls were matched regarding age, geographical region, work place (stone-grinding factory workers) and history of exposure to occupational silica dust. All participants or their guardians signed written constant forms. 3-5 mL of peripheral whole blood was drawn and poured in ethylenediaminetetraacetic acid (EDTA)-containing 15-mL falcon tubes and stored at -20°C until DNA isolation. The genomic DNA was isolated using method as described previously by Miller et al (31).

PCR profiles:

IFN γ +874:

Common primer: 5′-tcaacaaagctgatactcca-3′, T allele specific primer: 5′-ttcttacaacacaaaa tcaaatct-3′ and A allele specific primer: 5′-ttcttacaacacaaaatcaaatca-3′.

Allele-Specific Oligonucleotide PCR procedure was carried out via 10 cycles: 94°C 30 sec, 62°C 50 sec, 72°C 40 sec, and 20 cycles: 94°C 20 sec, 56°C 50 sec, 72°C 40 sec (32).

TNF α -308:

Common primer: 5′-tctcggtttcttctccatcg-3′, G allele specific primer: 5′-ataggttttgaggggcatgg-3′ and A allele specific primer: 5′-ataggttttgaggggcatga-3′.

Allele-Specific Oligonucleotide PCR procedure was carried out via 30 cycles: 94°C 30 sec, 61°C 2.5 min, 72°C 1 min (33).

IL-1Ra VNTR

5′-ctcagcaacactcctat-3′ and 5′-tcctggtctgcaggtaa-3′. PCR procedure was carried out via 30 sec at 95 oC, 30 sec at 58 oC and 30 sec at 72 oC for 30 cycles (34).

The expected amplified PCR products were 261 and 184 bp for IFN γ +874 and TNF α -308, respectively. In the cases of IL-1Ra VNTR, the expected alleles are including allele1 with 4 repeats and 410 bp, allele 2 with 2 repeats and 240 bp, allele 3 with 5 repeats and 500 bp, allele 4 with 3 repeats and 325 bp and allele 5 with 6 repeats and 595 bp fragments.

The electrophoresis of all PCR products was performed using 2% agarose gel that was stained with ethidium bromide and visualized via a UV transilluminator. ❑

STATISTICAL ANALYSIS

Statistical power was determined about 70% (two-tailed, α = 0.10) for a minimum sample size of 37. The frequencies of alleles and genotypes were calculated in the cases and the

controls by direct counting, and compared to each other by chi-square test. P value, odds ratio (OR), and 95%C.I. were calculated by SPSS version 16 and excel 2007. The level of statistically significance was considered at 0.05. ❑

RESULTS

In this case-control study, 45 patients with silicosis (mean age: 38±14) and 45 healthy controls (mean age: 45±14) were studied. Exposure duration was 36.20± 14.68 (month) in cases and 36.98±12.26 (month) in controls. History of tobacco consumption and occupational silica dust exposure without any findings in medical tests were similar in cases and controls ($p > 0.05$). A summary of the characteristics data in patients with silicosis and controls was reported previously by Mohebbi et al (2010) (26). Representative images of gel analysis are shown in Figures 1-3. our analysis showed: 1) IFN γ +874 T allele frequency was 0.44 in the cases and 0.48 in the controls; 2) IFN γ +874 A allele frequency was 0.56 in the cases and 0.52 in the controls; 3) TNF α -308 A allele frequency was 0.34 in the patients and 0.29 in the controls; 4) TNF α -308 G allele frequency was 0.66 in the cases and 0.71 in the controls; 5) the observed frequency (%) of allele 1, allele 2, allele 3 and allele 4 was 65(72.2), 18(20), 2(2.22), 5(5.56) in the cases respectively; and 6) 68(75.6), 17(18.9), 2(2.22), 3(3.33) in the controls respectively. The frequencies of IFN γ +874, TNF α -308 and IL-1Ra VNTR genotypes/alleles in the healthy males (controls) and the patients with silicosis and a comparison between cases and controls were reported in Table 1. Genotypic and allelic frequencies were not significantly varied between cases and controls (OR (95% C.I.), χ^2 and p value are reported in Table 1). ❑

DISCUSSION

Occupational exposure to silica or other dust related materials is the most important risk factor for silicosis. It has been demonstrated that interactions of several genes and environmental factors play an important role in the development of silicosis (35). We studied the presence or absence of IFN γ +874 A/T, TNF α -308 A/G, IL-1Ra VNTR (A1,A2,A3,A4 and A5 alleles) genotypes/alleles in the patients with silicosis and controls in the tested population. IFN γ shows several genetic variations (36,37) which have been associated with human disorders such as atopic asthma in Japanese children (38), increased severity of graft-versus-host disease (39), insulin-dependent diabetes mellitus (40). Our findings implied that there are not significant differences between cases and controls regarding the frequencies of IFN γ +874 A/A, A/T and T/T genotypes and IFN γ +874 A and T alleles (p value > 0.05) (see Table 1). The findings of this study were supported by Wu et al (2008) (41). TNF α shows several single nucleotide variations (15, 41-46). It has been demonstrated that TNF α −238 allele 2 has been associated with low level of expression; but TNF α −857T, −509T, −244 (in certain cell lines), −238 (in certain cell lines), −863A, -308A allele (or haplotype) have been associated with increased expression level cytokines (15, 41-46). TNF α −308 is well studied in several human diseases such as silicosis with controversial findings (15, 18, 22), We failed to show any significant differences between cases and controls regarding the frequencies of TNF α -308 A/A, A/G and G/G genotypes and TNF α -308 A and G alleles (p value > 0.05) (see Table 1). The results of this study are in agreement with those of Basotho tribe group (Fisher's exact p value = 0.15) (47), but are inconsistence with the others (22, 35, 47,48). IL-1Ra gene has several genetic variations in intron 2 based on variable number of a repeated sequence of 86-bp fragment (34). They are including: 1) Allele1 with frequency of 0.736 which contains 4 repeats and provide a 410 bp fragment PCR product. 2) Allele 2 with frequency of 0.214 which contains 2 repeats and provides a 240 bp fragment PCR product. 3) Allele 3 with frequency of 0.036 which contains 5 repeats and provides a 500 bp fragment PCR product. 4) Allele 4 with frequency of 0.007 which contains 3 repeats and provides a 325 bp fragment PCR product. 5) Allele 5 with frequency of 0.007 which contains 6 repeats and provides a 595 bp fragment PCR product (34). It has been emphasized that the frequency of allele 2 is increased in several human inflammatory disease and that is correspondence to high level of IL-Ra production. Carriers for allele 2 have higher level of IL-Ra production than the non carrier individuals (49). In the present study, the frequencies of allele 1,2,3,4 and 5 was 75.6%, 18.9%, 2.22% and 3.33% in the controls and 72.2%, 20%, 2.22% and 5.56% in the cases respectively. Al-

lele 2 frequency has been increased in our cases versus controls, but this variation was not statistically different (20% vs. 18.9%)(p value >0.05). The prevalence of the IL-1RA VNTR allele 2 was increased in our patients with silicosis (0.20) compared to the controls (0.189) OR (95% C.I.): 1.0(0.51-2.24). Our finding is in consistence with other studies (13, 41). Our study had some limitations such as small number of participants and poor medical documentations. ❑

CONCLUSION

This report as the first study of its own kind in Iranian Azeri Turkish patients, implied that IFN γ +874, TNF α -308 and IL-1Ra VNTR have not been associated with silicosis in the tested population. ❑

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