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Genotype TNF-α(-308) and Silicosis on Factory Workers in Vietnam in 2020

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Abstract. The studFigy aims to determine the TNF-α single-nucleotide polymorphism TNF-α (-308) and assess the association of TNF-a(-308) SNP with the risk of silicosis among workers directly exposed to silica dust in Vietnam. A study was undertaken among 78 cases with silicosis and 103 controls without silicosis in Vietnam. Blood samples were collected for genomic DNA extraction from each subject. The phenotyping of TNF-α(-308) was performed using polymerase chain reaction-based restriction fragment length polymorphism (PCR-RFLP) and dye termination sequencing. Results: The average exposure time of the case group was slightly higher than that of the control group (12.46 ± 6.732 years vs. 12.09 ± 7.854 years). The majority of genotypes in both silicosis and non-silicosis was GG. When analyzing the concentration of TNF-α in the study participants' blood, it is shown that the average concentration of TNF-α in the case group was higher than that in the control group. The genotype AG in the case group was 1.368 times higher than that in the control group. The percentage of all A alleles in the case group with silicosis was 1.342 times higher than the control group without the disease, similar to previous studies. Conclusion: The majority of genotypes in both groups was GG. The average concentration of TNF-α in blood, genotype AG, and the percentage of all A alleles in the case group was higher than that in the control group.

Keywords: Silicosis, Genotype, TNF-α, Vietnam

1. Introduction

Silicosis (also known as Potters Rot) is the most widespread form of pneumoconiosis. Silicosis is an occupational lung disease among workers who inhale silica dust daily. The manifestation of the disease is an irreversible diffuse lesion, which continues to develop after cessation of silica dust exposure [1].

Many theories explain the mechanism of silicosis, such as voltage theory, protein absorption theory, mechanical theory, immunology, etc. Among those, the "macrophage" theory is the most widely recognized. Accordingly, when silicon crystals into the alveoli were inhaled, they will be phagocytized by alveolar macrophages, activating the inflammatory response network, which leads to the secretion of cytokines of the inflammatory reaction into the alveoli spaces. The consequence of a chronic inflammatory response is that silicosis is developed.

In this chronic inflammation, there are many cytokines such as IL-1, IL-6, IL-10, TNF-α,etc... Among them, TNF-α is a cytokine due to macrophages. Alveolar secretory cells play an essential role in both the inflammatory response and the direct regulating factor stimulating fibroblasts and the collagenization of damaged tissue to form pulmonary fibrosis in silicosis. TNF-α gene regulates TNF-α production at transcription level [2]. Studies have shown that single nucleotide polymorphism in the promoter region increased TNF-α production. Recently, many studies worldwide show that this single polymorphism is related to the formation and progression of silicosis. In 2012, Wang et al. conducted a case-control study that showed the association of single nucleotide polymorphism TNF-α (-308) with silicosis. However, the results were still inconsistent, which requires further research [3]. Polymorphism at -308 nucleotides upstream from the transcription initiation site in the TNF-α promoter is associated with elevated TNF-α levels and disease susceptibilities. The molecular epidemiological studies of silicosis estimate the risk that the TNF-α gene variant is associated with the increased risk of silicosis [3, 4, 5, 6, 7, 8]. Since then, it helps to build strategies for prevention, screening, and early diagnosis for workers.

Studies on molecular epidemiology of silicosis in the world and Vietnam have still been inadequate; thus, it requires more effort. Therefore, this study was implemented to determine the TNF-α single-nucleotide polymorphism TNF-α (-308) in workers directly exposed to silica dust and to analyze the association between nucleotide monomorphism TNF-a 308) and silicosis.

2. Materials and Methods

2.1 Study Design, Case and Control Selection and Specimen selection

A descriptive study with a control group was undertaken from October 2018 to October 2020. The selected study participants were categorized into two groups. The control group included 103 workers exposed to silica dust with no diagnosis of silica dust. The case group was 78 workers exposed to silica dust and diagnosed with silicosis, following Circular 15/2016-QD-BYT of Ministry of Health guidance [1]. The study participants were selected with TNF-α higher than the threshold of diagnosis were selected from the list of 800 employees who quantified TNF-α under the study. The study participants selected with TNF-α higher than the diagnosis threshold were chosen from the list of 800 employees who quantified TNF-α under the study. The inclusion criteria for study participants included 1) direct exposure to silica dust for at least one year; 2) written informed consent for participation. The exclusion criteria were composed of 1) pregnant women; 2) those who were suffering from any inflammatory disease or tumours; 3) administrative staff.

A sample of blood (3mL) was collected in EDTA (Ethylene Diamine Tetracetic Acid) from each participant, and DNA was extracted using silica-res in method (Thermo Fisher Scientific, USA). The quality and quantity of genomic DNA samples were examined using Spectrophotometer.

2.2 Polymerase Chain Reaction-Based Restriction Fragment Length Polymorphism of TNF-α Fragments

Polymerase Chain Reaction-Based Restriction Fragment Length Polymorphism (PCR-RFLP) assays were designed to determine TNF-α genotypes. The primers sequences for TNF-α(-308) were described by Wang et al. (2012) with forwarding primer 5'-GAGGCAATAGGTTTTGAGGGCCAT-3', reverse primer 5'- CATCAAGGATACCCCTCACACT- 3' (Thermo Fisher Scientific, USA) [3]. The polymerase chain reaction (PCR) was performed on Eppendorf™ Mastercycler™ Nexus Thermal Cycler (Eppendorf, Germany) in a 25 µL reaction volume, which contained 12.5 µL Dream Taq Green PCR (Thermo Fisher Scientific, USA), the paired primers for TNF-α(-308), H2O and 3 µL genomic DNA. DNA was heated to 95°C for 5 mins, and subject to 35 cycles of PCR at 94°C for 30 s, at 57°C for 40 s and 72°C for 30 s, finally extension at 72°C for 5 mins. PCR products were digested with NcoI at 37°C for 8 hours, and 3% agarose gel electrophoresis (Thermo Fisher Scientific, USA) was performed to check the status of the digested fragments. The results of electrophoresis assays were explained as follows, homozygous GG had one band sizing 114bp of restriction digested fragment PCR product on a gel, heterozygous GA had two bands sizing 114bp and 135 bp, respectively, and homozygous AA had one band sizing 135bp. Genotypes of TNF-α(-308) for selected samples were confirmed by dye termination sequencing method on ABI PRISM® 310 (Thermo Fisher Scientific, USA) in manufacturing. The representative sequencing results of ABI PRISM® 310 for the selected samples from each genotype of SNP were found in our study, which was shown in Figure 1.

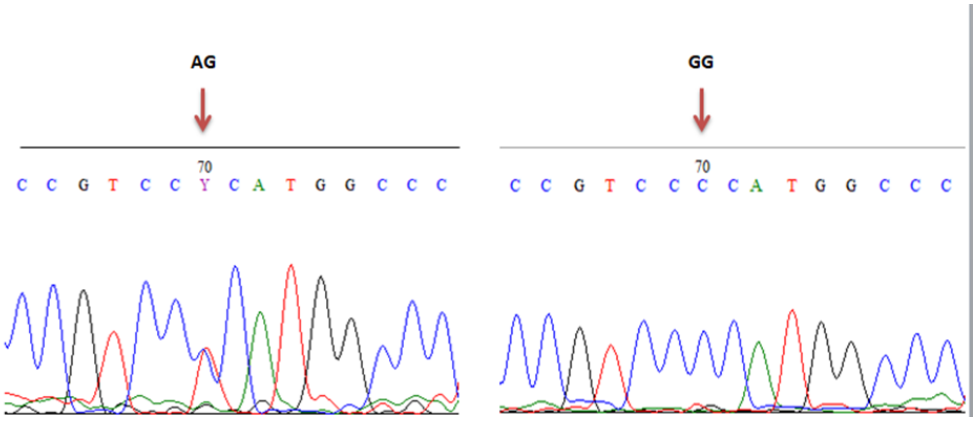

Fig. 1. The presentative sequences of TNF(-308) SNP genotypes.

2.3 Data analysis

Data were analyzed with SPSS 16.0 software (IBM, USA), and Review Manager 5.4 (Clicktime.com, Inc, USA) was used to evaluate the allele rate, genotypes, and their correlation with silicosis. The data are presented as frequencies and percentages for categorical variables and as mean ± standard deviation (SD). $\chi 2$ test or Fisher exact test was used to assess differences between the variables of workers with and without silicosis. Use the t-test to compare the mean age of the two disease groups and the control group.

To examine the association between the SNP TNF-α (-308) single polymorphism and the risk of silicosis, the Chi-Square test ($\chi 2$) was applied to investigate the differences between genotype ratio and OR ratio and 95% CI. Since the genotype AA was not detected in this study, we only analyze AG and GG. P values < 0.05 were considered indicative of statistical significance.

3. Results

Baseline characteristics of the 78 silicosis patients and 103 workers without silicosis were described in Table 1. Most of the participants were male (92,3% in the case group and 88,3% in control). The mean age of the case group was 43.81 ± 10,086, which was higher than that in the control group (39.46 ± 7,543). The average exposure time of the case group was 12.46 ± 6.732 years; it was similar in the control group (12.09 ± 7,854 years). There was a non-significant difference between gender, age group, exposure time between the two groups of study participants.

Tab. 1. Characteristics of the study participants.

Variables	Cases (n,%)	Controls (n,%)	p values
Gender			0.457
Male	72 (92.3)	91 (88.3)	
Female	6 (7.7)	12 (11.7)	
Total	78 (100)	103 (100)	
Age group			0.195
>45 year-old	12 (15.4)	33 (32.0)	
35-45 year-old	38 (48.7)	45 (43.7)	
<35 year-old	28 (35.9)	25 (24.3)	
Age (Mean±SD)	43.81 ±10.086	39.46±7.543	
Exposure time			0.714
>10 years	11 (14.1)	25 (24.3)	
5-10 year	24 (30.8)	23 (22.3)	
<5 year	43 (55.1)	55 (53.4)	
Average year of exposure (Mean±SD)	12.46 ± 6.732	12.09±7.854	

Characteristics of the TNF-α was presented in Table 2. It is shown that the average concentration of TNF-α in the case group was higher than that in the control group (Tab. 2). The mean blood concentration TNF-α in the case group was 15.81 pg/mL. The minimum and maximum values of TNF-α in this group were 0.17 pg/mL and 120.97 pg/mL. Whereas in the control group, the mean blood concentration TNF-α was 15.38 pg/mL with its minimum value being 2.56 pg/mL and 77.23 pg/mL, respectively. However, the difference in the average concentration of TNF-α in comparative groups was non-significant (p value > 0.05).

Tab. 2. Characteristics of the TNF-α concentration.

Characteristics of the TNF-α concentration (pg/mL)	Cases (n=78)	Controls (n=103)	p values*
Mean	15.81	15.38	0.2
Median	6.8182	8.358	
Standard deviation	22.34	17.12	
Max	120.97	77.23	
Min	0.17	2.56	

*t-test, comparing the mean of TNF-α concentration between case and control groups

Table 3 shows that GG genotype was the most common (87.2% in the case group and 90.3% in controls), following AG genotype. None of the study participants was detected with an AA genotype.

As for the allele ratio of the gene locus, in the disease group, the G allele was dominant with 93.6%. The A allele only accounts for 6.4%. Likewise, the control group's G and A alleles were 95.1% and 4.9%, respectively.

Tab. 3. Genotype ratio and allele ratio of locus SNP TNF-α (-308).

	Cases (n,%)	Controls (n,%)
TNF-α (-308) Genotype		
AA	0 (0)	0 (0)
AG	10 (12.8)	10 (9.7)
GG	68 (87.2)	93 (90.3)
Total	78 (100)	103 (100)
Allele ratio of the gene locus TNF-α (-308)		
G	146 (93.6)	196 (95.1)
A	10 (6.4)	10 (4.9)
Total	156 (100)	206 (100)

The risk of silicosis between genotypes AG and GG was evaluated in Table 4. The study found that the genotype AG in the case group was 1.368 times higher than that in the control group (OR = 1.368; 95% CI: 0.539-3.469). However, the correlation was non-significant, with a p-value > 0.05.

Tab. 4. Association of TNF-α (-308) . with risk of silicosis.

Allele	Cases (n,%)	Controls (n,%)	OR (95%CI)	p value
AG	10 (12.8)	10 (9.7)	1.368 (0.539-3.469)	0.508
GG	68 (87.2)	93 (90.3)		

When analyzing the A and G alleles of SNP TNF-α (-308) G/A on two groups of cases and controls, we found that the percentage of all A alleles in the case group with silicosis was 1.342 times higher than the control group without the disease (Tab. 5). However, this result was not statistically significant.

Tab. 5. Assessing the risk of silicosis between the alen A and alen G.

Allele	Cases (n,%)	Controls (n,%)	OR(A/G) (95%CI)	p
A	10 (6.4)	10 (4.9)	1.342 (0.545-3.310)	0.521
G	146 (93.6)	196 (95.1)		

4. Discussions

Silicosis is pneumoconiosis of lung fibrosis caused by inhalation of silica dust, usually for an extended period [9]. It is a common occupational disease among workers exposed to silica particles [9, 10]. The development of disease is related to the total dose and intensity of exposure to silica particles. Still, to date, the scientist cannot answer why some workers would get silicosis, while others did not in the same working environment. Many authors proposed strong evidence for individual variation in susceptibility to silicosis both in humans and in experimental animals [10, 11, 12, 13]. Despite the contribution of environmental factors, but in animal studies, it is shown that different strains have different susceptibility to disease, implying that susceptibility to silicosis is at least partly determined by genetic factors [12].

In our study, we investigated exposure time that is an essential factor in the pathogenesis of silicosis. Whereby 78 workers in the case group had an exposure time between 2 and 31 years, and the average exposure time of the case group was 12.46 ± 6.732 years. As for 103 subjects in the control group, their exposure time was ranged from 1 to 31 years with a mean of 12.09 ± 7.854. The average exposure time of all the participants in our study is quite similar to R. Nadif's but lower than other studies carried out in South Africa, China, and Iran. This difference in exposure time between these studies may result from the epidemiological differences between Vietnamese people and others. It may also be influenced by the total amount of silica dust produced in the workplace due to the type of work with silica dust exposure in Vietnam that was different from other regions [3, 15, 16].

In silica dust-exposed persons and experimental model of fibrosis, TNF-α produced by macrophage is involved in the initial of chronic inflammation [9]. TNF-α is an inflammatory cytokine that plays a significant role in the pathogenesis of the pulmonary inflammatory disease. This hypothesis was demonstrated by some studies in animal experiments and population-based studies. In a case-control study in Chinese carried out with three different groups which are healthy group exposed to silicon dust with TNF-α concentration = 47.86 ± 16.52 pg/mL, another group diagnosed with silicosis having TNF-α = 109.11 ± 31.08 pg/mL, and silicosis patient group with the concentration TNF-α = 216.35 ± 51.03 pg/mL. This study shows that is not only significantly elevated TNF-α in exposed healthy donors and silicosis patients compared to healthy not exposed donors but also the TNF-α concentration of silicosis patients is higher than exposed healthy donors [17]. When we investigate the TNF-α concentration in silicosis, there is no significant difference between case and control. Still, it reflects the trend which is TNF-α concentration is higher in silicosis patients. These findings are similar to E. Slavov's study. It was implemented on Bulgarian populations with three groups which are the healthy population who were not exposed to silicon dust have concentration TNF-α = 14.8 ± 8.8 pg/mL, the healthy group with a history of silica dust exposure at concentrations TNF-α = 22.4 ± 11.1pg/mL, and the case group diagnosed with silicosis with the concentrations TNF-α = 20.9 ± 12.9 pg/mL [18].

The regulation of TNF-α production occurred significantly at the transcription level [19]. Many authors proposed that the variant in the promoter of the TNF-α gene affected to enhance TNF-α production. The SNP TNF-α(-308) located in the promoter was thought to play an elevated TNF-α production. A case-control study in Indonesia with 336 workers shows that the AA/AG genotype of TNF-α(-308) affects elevated TNF-α concentration. Furthermore, this study also proved the AG genotype in silicosis patients higher than control donors, and AG genotype had a higher risk of silicosis than GG genotype in our study [7]. The results showed that the genotype AG in the case group was 1.368 times higher than that in the control group (OR = 1.368; 95% CI: 0.539-3.469). However, this finding has no significant meaning that the genotype AG increases the risk of silicosis in the study group. Our results are similar to those of Wang Yong Wei (2012) when the silicosis risk of AG genotype compared with GG was not statistically significant, or the study of Elizabeth L. Corbett on a South African population with 121 cases and 120 controls also showed no increased risk of silicosis of genotypes AA, AG compared with GG genotype. This result is similar to the study of author Yusesoy carried out on the European population and the author Isa Abdi RAD on the Iranian people [3, 4, 16, 20].

When analyzing the risk distribution of silicosis between subjects with the AG genotype and the subjects carrying the GG genotype in our study on the Vietnamese population with other studies in the world, we can see the AG genotype detection rate in the disease group was 1.37 times higher than that in the control group. This rate was similar to other studies in the world (Fig. 2).

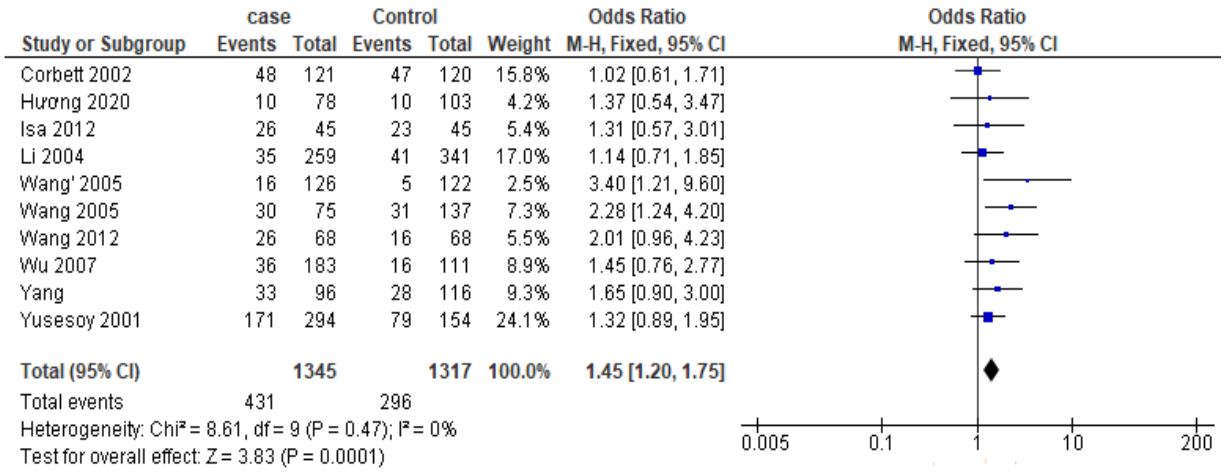

Fig. 2. Comparison of silicosis between genotypes AG and GG between our research and a number of studies around the world.

Regarding the study on the risk of allele A compared with the G allele in silicosis, different studies also show many inconsistent results. This study found that the percentage of all A alleles in the case group with silicosis was 1.342 times higher than the control group without the disease, which was similar to previous studies. Research by Kurniawidjaja shows that the rate of allele A in the disease group was statistically significantly higher than that in the control group ($p = 0.02$) [7]. Additionally, in the meta-analysis study of author Li Te Yang conducted on nine previous case-control studies of SNP TNF-α (-308) with risk of silicosis, it is identified that the allele A was 1.4 times more likely than the allele G to have a risk of silicosis (OR = 1.4; 95% CI: 1.11-1.78) [21]. In the study conducted by Yusesoy on the European population, the rate of allele A in the disease group was 1.32 times higher than that in the control group. However, some studies did not confirm the prominent allele A in silicosis patients. A study of the author Elizabeth L. Corbett did not prove that allele A increases the risk of silicosis compared to the allele G ($p = 0.15$). Our results can be easily compared with the results of Li Te Yang et al., where it shows that the association of SNP TNF-α (-308) with silicosis varies among various populations [4, 20].

As SNP TNF-α (-308) is in the promoter area of the cytokines protein TNF-α gene, there have been many studies on how allele changes at this SNP site affect the binding site of transcription initiation factors as well as cis factors, and the trans factor enhances the transcriptional enhancement of the TNF protein-encoding mRNA. -α in lymphocytes and macrophage cells can increase the concentration of TNF-α, essential cytokines in the pathogenesis of silicosis and pulmonary fibrosis diseases. However, studies on the role of this SNP in silicosis are still inconsistent. In our study, the percentage of allele A in the case group was higher than that in the control group. Although this difference was not statistically significant, it is still likely that this SNP is susceptible to silicosis because the SNP locus TNF-α (-308) is a gene with low permeability [22].

Our study had some strengths and limitations. It is the first study to determine the average concentration of TNF-α among the worker population directly exposed to silica dust. However, we cannot conclude the higher risk of TNF-α with silicosis that further study with larger sample size is needed.

5. Conclusions

Our study illustrates the majority of the genotype of TNF-α in both silicosis and non-silicosis was GG. When analyzing the concentration of TNF-α in the study participants' blood, it is shown that the average concentration of TNF-α in the case group was higher than that in the control group. The genotype AG in the case group was 1.368 times higher than that in the control group. The percentage of all A alleles in the case group with silicosis was 1.342 times higher than the control group without the disease, similar to previous studies. However, the result has not shown a statistically significant difference. Therefore, further studies with larger sample sizes are recommended.

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