Silica Dust Exposure Duration and Increasing of TGF-β1 Serum Level in Marble Industry Workers

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Abstract

Background: Marble industry workers are at risk of silica dust exposure. Inhaled silica dust causes injury, inflammation, and leukocytes entry. The recruited leukocytes secrete profibrotic cytokines such as TGF-β. This study observe TGF-β1 serum levels based on silica dust exposure duration. **Methods:** An analytical cross-sectional study was conducted on 10 non-exposed healthy participants as the control group, 40 silica dust exposed workers of marble industry as the exposed group, divided into categories based on exposure duration: 1-5 years(12), 6-10 years(14), and >10 years(14). Serum levels of TGF-β1 in each group were determined with ELISA. **Results:** The median TGF-β1 serum levels in exposed group [337.63 pg/ml (101.02-7505.24 pg/ml)] were significantly higher than control group [104.84 pg/ml (67.62-171.83 pg/ml)] (p=0.029). There was a significant difference in TGF-β1 serum levels between >10 years exposed group [6314.44 pg/ml (549.50-9857.35 pg/ml)] and control group (p=<0.001), 1-5 years exposed group [144.51 pg/ml (40.76-2337.82) pg/ml)] (p=0.003), 6-10 years exposed group [205.35 pg/ml (75.61-909.05 pg/ml)] (p=0.003). There was a significant positive correlation between silica dust exposure duration and TGF-β1 serum levels (r=0.465 ; p=0.002). **Conclusions:** Silica dust exposure can cause an increase in TGF-β1 serum levels in marble industry workers and exposure duration plays an important role.

Index Terms: marble, silica, TGF-β1 serum levels

I. INTRODUCTION

Silica dust exposure in the workplace is one of the oldest known causes of lung disease. Inhalation of very small crystalline silica particles causes various diseases, including silicosis, lung cancer, chronic obstructive pulmonary disease (COPD), kidney disease, autoimmune disorders, and cardiovascular disorders.[1] In 1995, the World Health Organization began a campaign to eliminate silicosis from the world by 2030, but silicosis remains a major health issue internationally. In 2016, the Global Burden of Disease Study estimated 10400 deaths per year, and 210000 years of life lost due to silicosis.[2]

Marble is a granular metamorphic rock that contains silica dust. It usually contains crystalline silica below 5% although can be as high as 30%.[3] The accumulation of silica dust exposure is influenced by the exposure duration.[4] Silica dust with a diameter of less than 5 µm can be breathed in which is called respirable crystalline silica (RCS).[2]

Inhaled respirable crystalline silica (RCS) causes intrapulmonary injury while nanoparticles, less than 0.1 µm in size, can be transported to other organs causing extrapulmonary injury.[5] The injury will initiate clotting and coagulation, followed by an inflammatory reaction and the entry of leukocytes (neutrophils, macrophages, and T cells). The recruited leukocytes secrete profibrotic cytokines such as TGF-β. TGF-β regulates the migration, proliferation, and activation of fibroblasts followed by tissue remodeling/resolution. If this process goes well, wound repair will be achieved, whereas if there is dysregulation or the injury stimulus persists, fibrosis can occur.[6] TGF- β has 3 isoforms, namely TGF-β1, TGF-β2, TGF-β3, where TGF-β1 is the isoform that obtained in the most quantities and expressed in various tissues.[7]

Tulungagung is known as one of the cities with the largest marble producers in Indonesia. The area of Tulungagung Regency has mountains which are a series of the South Mountains. They contain limestone that can be made into marble. Marble industry workers are at risk of exposure to dust containing crystalline silica in the workplace which can cause various health problems.[8]

The data of TGF-β1 serum levels based on silica dust exposure duration of marble industry workers are still very limited. Therefore, based on this background, research is needed to explore TGF-β1 serum levels based on exposure duration in marble industry workers, which aims to determine the risk of diseases associated with the silica dust exposure duration in marble industry workers, especially in Tulungagung.

II. METHODS

This study was a cross-sectional observational study aimed to determine the TGF-β1 serum levels based on silica dust exposure duration in marble industry workers. Participants were 40 silica dust exposed workers of marble industry in district

Tjampurdarat, Tulungagung, East Java, Indonesia in June 2021 as the exposed group, divided into different categories based on exposure duration: 1-5 years (12), 6-10 years (14), and >10 years (14) and 10 non-exposed healthy participants as the control group.

Written informed consent was obtained from all the participants for being included in the study. All procedures were approved by the Institutional Ethics Committee of Faculty of Medicine, Universitas Brawijaya, Malang, Indonesia with ethical approval number 118 / EC / KEPK – PSPDS / 04 / 2021.

The inclusion criteria were men aged between 18 and 70 years old, who have been working for a minimal 1 year as craftsmen in open space areas for at least 40 hours a week, have agreed to join the study, and signed the informed consent. Whereas the exclusion criteria were workers with growth disorders, connective tissue diseases, history of neural, cardiovascular, renal, reproductive, hepatic, autoimmune diseases, malignancies, COPD, and fibrotic diseases that have been diagnosed before starting to work in the marble industry. The healthy control was the one who did not expose to silica dust, was a non-smoker, and have no comorbidities.

The independent variable was exposure duration while the dependent variables were TGF-β1 serum levels and chest x-ray results. The TGF-β1 serum levels in each group were measured by enzyme-linked immunosorbent assay using E-EL-0162 96T, El abscience, USA Kit. Data are presented as median (IQR).

Mann-Whitney and Kruskal-Wallis tests were used to investigate the comparison between different groups and categories. Spearman-test was used to determine the correlation between silica dust exposure duration and TGF-β1 serum levels. A P-value of less than 0.05 was considered significant.

III. RESULTS

Characteristics of subjects

The characteristics of the subjects are presented in Table 1. In this study, all subjects were men aged between 20 and 67 years old with a median age of 40.5 (31.25-47.75) years. The mean exposure duration was 12.80 ± 7.33 years. The longest exposure duration was 39 years and the shortest was 1 year with a median of 8.5 years. The number of subjects smokers are 22 people (55%), former smokers 5 people (12.5%) and the rest are not smokers 13 people (32.5%). Most of the subjects always use non-standards masks namely 18 people (45%), followed by sometimes use nonstandards masks as many as 17 people (42.5%) and never using masks 5 people (12.5%). On chest x-ray examination, 36 people had normal features, 2 people with chronic bronchitis pattern, 1 person with minimal traces of bilateral pleural effusion, and 1 person with thick hili, the suspected cause of the retention of secretions. All subjects did not experience any respiratory symptoms.

Table 1. Characteristics of Subjects

Characteristic	n	%	Median (IQR)
Age (year)			40.5 (31.25-47.75)
Working duration (year)			8.5 (5.00-20.50)
• 1-5	12	30	
• 6-10	14	35	
• >10	14	35	
Smoking status			
• Smoker	22	55	
• Ex-Smoker	5	12.5	
• Non-Smoker	13	32.5	
Brinkmann Indeks (n=22)			12.5 (0-75.00)
• Mild (0-199)	20	90.9	
• Moderate (200-599)	2	9.09	
• Severe (> 600)	0	0	
Personal protective equipment (PPE)			
• Never wear any kind of mask	8	12.5	
• Sometimes wear non-standard masks	17	42,5	
• Always wear non-standard masks	18	45	
• Sometimes wear standard masks	0	0	
• Always wear standard masks	0	0	
Clinical respiratory signs and symptoms			
• No respiratory signs and symptoms	40	100	
• Respiratory signs and symptoms	0	0	
Chest X-ray			
• Within normal limits	36	90	
• Pathological finding	4	10	

Serum Levels of TGF-β1

Serum levels of TGF-β1 in non-exposed healthy participants as the control group and marble industry workers who were exposed to silica dust as the exposed group are presented in Table 2. Table 3 presents TGF-β1 serum levels in the exposed group based on the exposure duration.

Table 2. Serum TGF-β1 levels in the Non-Exposed Group and The Exposed Group

Exposed	n	Mean ± SD (pg/ml)	Median (IQR) (pg/ml)
Non-exposed group	10	117.65 ± 64.92	104.84 (67.62-171.83)
Exposed group	40	3069.46 ± 4247.09	337.63 (101.02-7505.24)

Table 3. Serum levels of TGF-β1 in The Exposed Group Based on The Exposure Duration

Exposure Duration (year)	n	Mean ± SD (pg/ml)	Median (IQR) (pg/ml)
1 – 5	12	1773.18 ± 3331.88	144.51 (40.76-2337.82)
6 – 10	14	1570.23 ± 3399.32	205.35 (75.61-909.05)
> 10	14	5679.78 ± 4674.91	6314.44 (549.50-9857.35)

Increased TGF-β1 serum levels in Marble Industry Workers

As shown in Figure 1. the median TGF-β1 serum levels in exposed group [337.63 pg/ml (101.02-7505.24 pg/ml)] were significantly higher than control group [104.84 pg/ml (67.62-171.83 pg/ml)] (p=0.029).
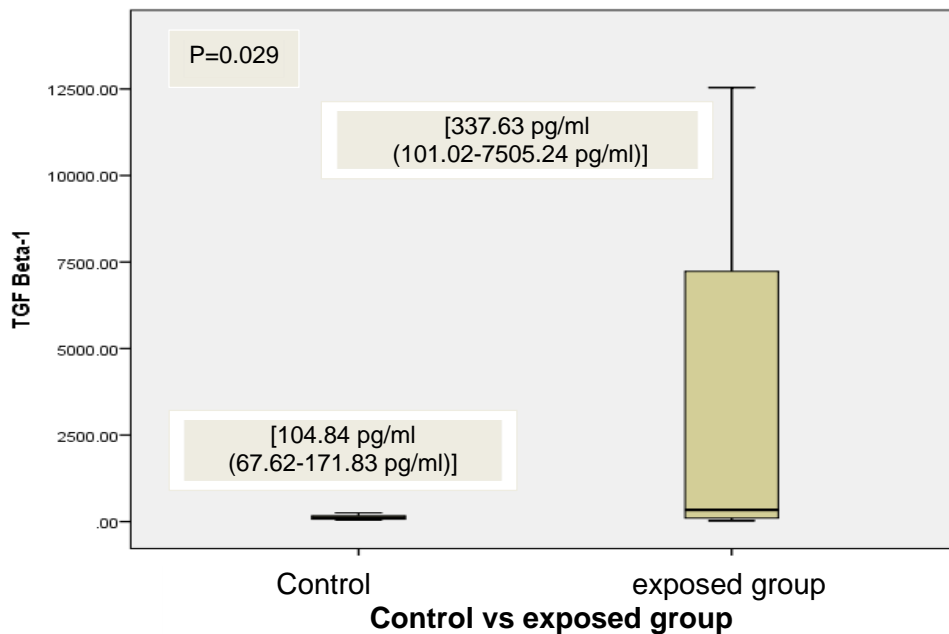

Figure 1. The difference in the TGF-β1 serum levels in the non-exposed group and the exposed group

The Differences in TGF-β1 serum levels Based on Silica Dust Exposure Duration in Marble Industry Workers

As shown in Figure 2. there was a significant difference in TGF-β1 serum levels between >10 years exposed group [6314.44 pg/ml (549.50-9857.35 pg/ml)] and control group (p=<0.001), 1-5 years exposed group [144.51 pg/ml (40.76-2337.82) pg/ml)] (p=0.003), 6-10 years exposed group [205.35 pg/ml (75.61-909.05 pg/ml)] (p=0.003).
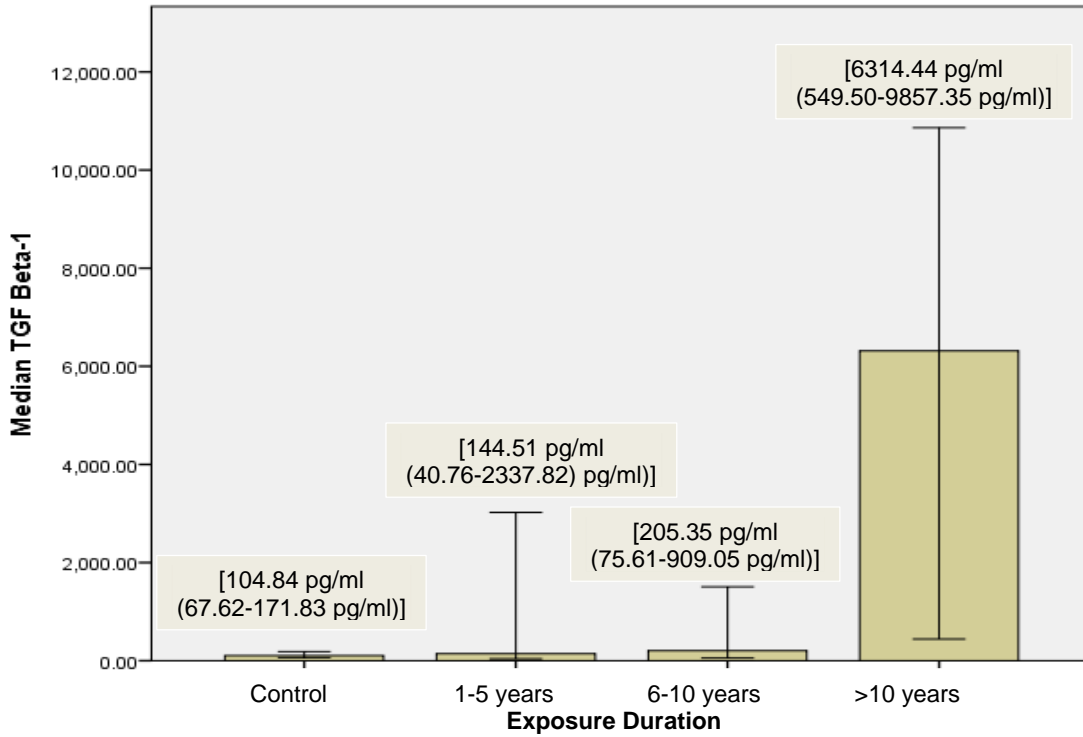
[6314.44 pg/ml
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(40.76-2337.82) pg/ml)]

[205.35 pg/ml
(75.61-909.05 pg/ml)]

[104.84 pg/ml
(67.62-171.83 pg/ml)]

Figure 2. The difference in the TGF-β1 serum levels in the unexposed group and the exposed group based on exposure duration

Correlation Between Silica Dust Exposure Duration and TGF-β1 serum levels in Marble Industry Workers

As shown in Figure 3. there was a significant positive correlation between silica dust exposure duration and TGF-β1 serum levels (r=0.465; p=0.002). The longer the silica dust exposure, the higher the TGF-β1 serum levels in marble industry workers.
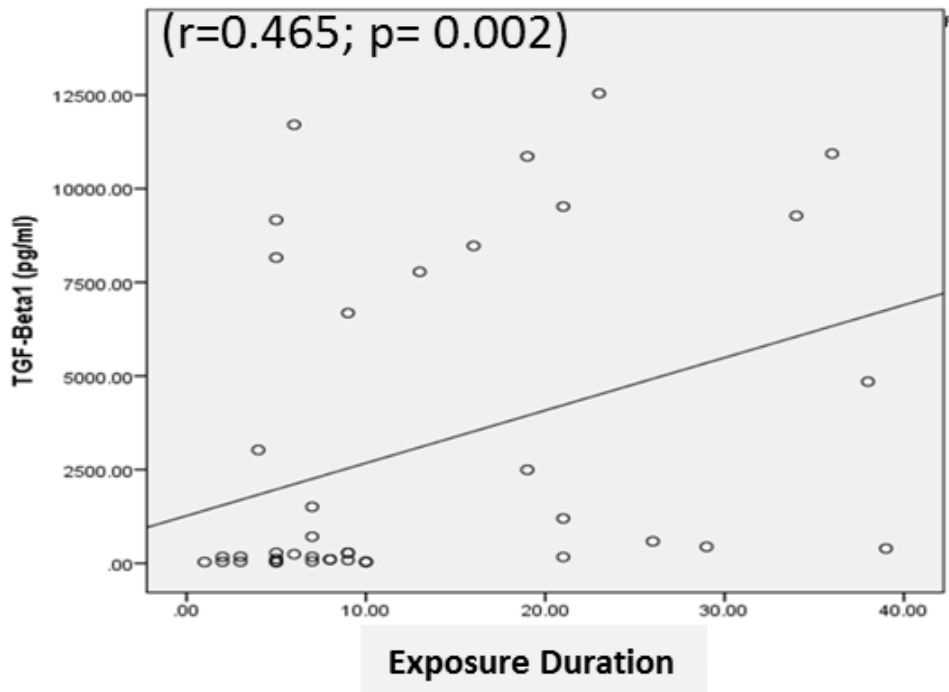

(r=0.465; p= 0.002)

Figure 3. Scatter plot correlation between exposure duration and TGF-β1 serum levels in marble industry workers
The Correlation Between Independent Variable (Silica Dust Exposure Duration) and Confounding Variables (Age, Smoking, and Use of Personal Protective Equipment) Towards The Dependent Variable (TGF-β1 serum levels)

In this study, the correlation between the independent variable (silica dust exposure duration) and confounding variables (age, smoking, and use of personal protective equipment) towards the dependent variable (TGF-β1 serum levels) was also analyzed. As shown in Table 4. there was no significant correlation between age and TGF-β1 serum levels (p=0.953), as well as between smoking habits and TGF-β1 serum levels (p=0.538). There was a significant relationship between the use of PPE with TGF-β1 serum levels (p=0.007).

Table 4. Multiple Regression Analysis Partial Test Results

Model	Standardized Coefficients Beta	t	Sig.
(Constant)		1.164	0.252
Exposure duration	0.414	2.030	0.050
Smoking	-0.091	-.621	0.538
Age	-0.011	-.058	0.954
PPE	0.395	2.877	0.007

IV. DISCUSSION

Our data suggest that TGF-β1 serum levels are elevated in silica dust exposed subjects as compared to non-exposed healthy individuals. All of the silica dust exposed workers of the marble industry who participated in this study are men with the mean age is 40.08 ± 10.99 years, similar to other studies, the majority of marble industry workers are around 46-50 years old.[9] The WHO state that the productive age ranges from 15 to 64 years, which indicated that the workforce in the marble industry is in productive age. They have been working for 1-39 years. This is in line with other study that the worker at marble workshops have been working for 5-35 years.[10] Most of the workers were smokers (55%), similar to other studies, 66.7% of workers were smokers. [9] However, in contrast to the study of Resende, et al with 47% were non-smokers, 28.5% were former smokers and the remaining 23.8% were smokers.[11]

Personal protective equipment, commonly referred to as "PPE", is equipment worn to minimize exposure to hazards that cause serious workplace injuries and illnesses.[12] NIOSH recommends the use of half-facepiece particulate respirators with N95 or better filters for airborne exposures to crystalline silica at concentrations less than or equal to 0.5 mg/m3. [13] In this study, 45% of workers are always using non-standard PPE, in line with the study by Wijaya, et al that 56,4% of stone quarry workers are using PPE.[14]

All subjects did not experience respiratory symptoms. Early pneumoconiosis can be asymptomatic, but the advanced disease often leads to disability and premature death.[15] This data was different from a study by Rezende, et al among the marble industry workers that the frequency of isolated respiratory symptoms found was (19%) while the sum of symptoms (cough, dyspnea, and wheezing), most reported symptoms (57%).[11]

Most of the chest x-ray results were within the normal limits (90%). The chest radiograph remains the primary mode of screening for pneumoconiosis in the United States and elsewhere. However, the chest radiograph is relatively insensitive for detecting early pneumoconiosis.[16] The chest radiograph is a relatively insensitive and nonspecific tool for diagnosing pneumoconiosis. In addition, the results may cause underestimation or overestimation of the extent of disease. Moreover, normal chest radiographs do not rule out interstitial fibrosis.[17]

The results of this study are in line with the research of Wijaya, et al among stone quarry workers who were exposed to silica dust. The results showed that the mean TGF-β1 serum level was 63878.3 (±9228.2) pg/ml where the normal value was 18289–63416 pg /mL with a mean of 39592 pg/mL (Wijaya et al., 2019). [14]

In line with the study of Mioa, et al that compared control group (23.28±12.24) pg/ml and exposed group (29.31±14.52) pg/ml, serum levels of TGF- β1 in silicosis group was much higher (P < 0.01). [18] In this study the TGF-β1 serum levels in the exposed group were significantly higher than in the control group. Just as exaggerated or prolonged inflammation can lead to tissue destruction and loss of function, increased TGF-β activity can lead to exaggerated scar formation and loss of function.[19]

The risk of developing silicosis is closely linked to the accumulated exposure of individuals to crystalline silica during their working lifetime.[20] The accumulated exposure is influenced by the duration, characteristics, and level of the exposure. [4] This study revealed that TGF-β1 serum levels in the >10 year-exposed group were significantly different from the other three groups. While TGF-β1 serum levels between the three other groups (non-exposed, exposed 1-5 years, and exposed 6-10 years) were not significantly different.

There was a significant positive correlation between silica dust exposure duration and TGF-β1 serum levels. The longer the silica dust exposure, the higher the TGF-β1 serum levels in marble industry workers. There is a clear relationship between the silica dust exposure duration and the incidence of silicosis. Research by Xia, et al found 3,665 cases of pneumoconiosis in Hubei Province with the average duration of exposure being 17 years.[21]

There was no significant correlation between age and TGF-β1 serum levels, similar to a study by Wjaya, et al among stone quarry workers. [14] The age-related decline in TGF-β1 serum levels were observed in a study by Lim, et al, suggests that decreased production of TGF-β1 might be involved in the aging process.[22]

A study by Lim, et al found that a high amount of tobacco consumption was associated with elevated TGF-β1 serum levels among men. One possible explanation for this positive association relates to the immunosuppressive effect of TGF-β1 on the immune system. Chronic inhalation of cigarette smoke alters a wide range of immunological functions, including both innate and adaptive immune responses.[22] Whereas this study revealed that there was no significant correlation between smoking habits and TGF-β1 serum levels, This might be due to the small proportion of subjects who smoke with mild and moderate Brinkman Index.

In this study, there was a significant correlation between the use of PPE and TGF-β1 serum levels, where workers who always used non-standards PPE had higher serum levels of TGF-β1 compared to workers who sometimes used non-standards PPE

or never used PPE. Further analysis, most of the subjects who always used non-standards PPE were from the group who had been exposed for >10 years, while the subjects who sometimes used non-standards PPE or never used PPE came from the exposed group 1-5 years and 6-10 years. This indicated that always using non-standards PPE cannot prevent an increase in serum levels of TGF-β1. Further research is needed to determine the correlation between the use of PPE and TGF-β1 serum levels based on exposure duration. The other study found that there was no correlation between the use of PPE and TGF-β1 serum levels among stone quarry workers, which might be because the non-standards masks were not reducing dust exposure.[14]

V. CONCLUSION

Silica dust exposure can cause an increase in TGF-β1 serum levels in marble industry workers and exposure duration plays an important role.

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