Original Article

Increasing Serum Levels of Nephronectin Based on Exposure Duration of Marble Dust in Industry Workers

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Abstract

Background: Exposure to silica dust is still an occupational health problem worldwide. The marble industry is one of the industries at risk of causing respiratory disease in its workers. Exposure to marble dust in the airways triggers pulmonary fibrosis via nephronectin (Npnt) as an α8β1 integrin ligand, which is an extracellular matrix protein. The purpose of this study is to look at how serum nephronectin (NPNT) levels change over time after being exposed to marble dust.

Methods: This was a cross sectional analytical study of marble industry workers. A significant difference test is carried out on 4 groups of subjects (n=50), including marble industry workers with exposure durations of 1-5 years (n=12), 6-10 years (n=14) and >10 years (n=14), as well as non-marble industry workers (unexposed) as control subjects (n=10). A correlation test was performed to see the relationship between duration of exposure and serum Npnt levels.

Results: The median age value in the exposed group was 40.5 (20-67) years. There was a significant difference (*P*=0.012) in the median Npnt level of the exposed group [1.699 (0.22–5.27) ng/mL] and the non-exposed group [0.678 (0.21–1.96) ng/mL]. The median value of nephronectin levels in the 10 years exposed group [2.4710 (1.74–5.27) ng/mL] were significantly different with both the 1–5 years exposed group (*P*=0.0001) with a median value of 0.6960 (0.22–2.27) ng /mL and the 6–10 years exposed group (*P*=0.039) with a median value of 1.0480 (0.27–4.29) ng/mL. There was a significant (*P*=0.0001) positive relationship (r=0.633) between the length of exposure and the level of Npnt.

Conclusion: The duration of marble dust exposure had a significant effect on serum Npnt levels. The longer the marble industry workers were exposed to marble dust, the higher the serum Nephronectin level.

Keywords: nephronectin, silica, marble

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INTRODUCTION

Silica dust exposure is still an occupational health problem worldwide. It was estimated that 2 million people in the United States and 3 million people in Europe were exposed to silica dust in their work environment.[1] In Asia, it was estimated that more than 23 million people in China and 10 million people in India were exposed to silica dust in their work environment. Currently, there is no national data on the prevalence of occupational diseases due to the inhalation of silica dust in Indonesia. Studies conducted in a cement factory showed a radiological suspicion of silicosis of 0.5%. A study conducted at a cement factory in West Java showed that the incidence of silicosis was 2.06% in 1990–2003.[2]

Marble is also a raw material for tiles, tables, and floors. This industry produces fine dust, which is a source of occupational health problems worldwide. Marble industry workers are most at risk of exposure to marble dust, which contains calcium carbonate and silica.[3] Continuous exposure to marble dust can reduce lung function and cause various lung diseases, such as chronic obstructive pulmonary disease (COPD) and silicosis.[4]

Silicosis is caused by the chronic inhalation of large amounts of dust from an environment containing silica particles. Pathological changes in silicosis include the formation of irreversible silicosis nodules and excessive extracellular matrix (ECM) deposition, leading to pulmonary insufficiency. Although the etiology of silicosis remains unclear, various emerging studies have shown that several specific types of cells and cytokines play an essential role in the process of silicosis.[5]

Nephronectin (Npnt) is an α8β1 integrin ligand, which is an extracellular matrix protein. Nephronectin is expressed in various tissues and organs: kidney, lung, choroid plexus, tongue, jawbone, dental epithelium, and facial bone.[6] A study by Lee et al found that nephronectin (Npnt) levels were elevated in silicosis patients. This result indicates that Npnt plays a role in the initiation and progression of silica-induced pulmonary fibrosis. In addition, decreased lung function (%FEV_1) is also associated with high Npnt levels. Lee also found that Npnt was associated with the late phase of pulmonary fibrosis.[7] Based on the description above, this study aimed to analyze changes in nephronectin levels according to the duration of marble dust exposure in industry workers.

METHODS

This was a cross-sectional analytic study with the subjects of marble industry workers in Tulungagung, Indonesia. Samples were obtained through stratified random sampling that met the inclusion and exclusion criteria. Fifty subjects were obtained and divided into four treatment groups. Inclusion criteria included men aged 18–70 years who worked at least one year in the marble industry and signed the informed consent. Exclusion criteria were workers with a history of chronic lung disease and workers with malignancy, growth disorders, connective tissue diseases, as determined based on interviews and physical examinations.

The collected data were characteristics and clinical history of patients using patient data research forms, respiratory signs and symptoms using chest x-ray (CXR) photos as the tools for evidence, and serum Npnt levels using 3 ml of the subject's blood specimen. The Human Nephronectin ELISA Kit (Cat. No. E5745Hu) was utilized to measure serum Nephronectin levels. The plate has been pre-coated with Human Npnt antibody. The Npnt present in the sample was added and binded to antibodies coated on the wells. The biotinylated Human Npnt Antibody was then added and binded to Npnt in the sample. The next step was to add Streptavidin-HRP to the Biotinylated Npnt antibody. After incubation, unbound Streptavidin-HRP was washed away during a washing step. Substrate solution was then added, and color developed in proportion to the amount of Human Npnt. The reaction was finally terminated by the addition of acidic stop solution, and absorbance was measured at 450 nm.

A significant difference test was conducted for serum Npnt levels in 4 groups of subjects, namely marble industry workers with consecutive exposures of 1–5 years (12 subjects), 6–10 years (14 subjects), >10 years (14 subjects), and healthy subjects who were not marble industry workers (unexposed) as control subjects (10 subjects). Analysis of the difference was performed using one-way ANOVA if the data was normal, followed by post-hoc analysis with Bonferroni. If the data was not normal, a non-parametric test with the Kruskal-Wallis test was carried out, and it was significant if $P \leq 0.05$.

A correlation test of serum Npnt levels was also conducted in 4 groups of subjects using linear regression analysis. If the normality of the data was not fulfilled, a non-parametric test with an ordinal regression test was carried out, and the magnitude of the correlation was expressed by r (-1 to +1).

RESULTS

The mean age of the subjects was 40.08±10.99 years. Based on the length of exposure, the shortest exposure time was one year, and the longest was 39 years. Most workers had an exposure duration of 6–10 years (35%) and more than ten years (35%). Most of the workers (55%) were smokers with a mild Brinkman Index. Most subjects used masks as personal protective equipment (PPE), but they were not according to standards (45%).

In this case, the use of masks that met the standards was surgical mask covered with cloth mask. According to the subject's CXR, 90% of the subjects had normal CXR, while the other 10% had abnormal X-Ray. The abnormal CXR was in the form of chronic bronchitis in 5% of the subjects, hillar thickening in 2.5% of the subjects, and minimal pleural effusion in 2.5% of the subjects. Patient characteristics are described in Table 1.

Table 1. Characteristics of Subjects (n=40)

Characteristics	N (%)
Age, years [median (min-max)]	40.5 (20–67)
Working duration (years)	
1-5 years	12 (30.0)
6-10 years	14 (35.0)
>10 years	14 (35.0)
Cumulative duration of exposure (hours-years)	
Smoking	
Smokers	22 (55.0)
Ex-smokers	5 (12.5)
Non-smokers	13 (32.5)
Brinkmann Index (n=22)	
Mild (0–199)	20 (90.9)
Moderate (200–599)	2 (9.09)
Severe (>600)	0 (0.0)
Use of Personal Protective Equipment (PPE)	
Never use any masks	0 (0.0)
Sometimes use non-standard masks	8 (12.5)
Always use non-standard masks	17 (42.5)
Always use standard mask	18 (45.0)
Clinical Symptoms of Respiratory Disorders	
No symptoms	40 (100)
No symptoms for more than 2 weeks	0 (0.0)
Chest X-ray	
Normal lung	36 (90.0)
Abnormality findings	4 (10.0)

There was a significant increase (*P*=0.012) in Npnt levels among marble industry workers compared to non-marble industry workers (control subjects). The mean value of Npnt levels among marble industry workers (1.7517±1.20218 ng/mL) was significantly higher than the Npnt levels of control subjects (0.8014±0.61660 ng/mL). The mean serum Npnt levels are described in Figure 1.

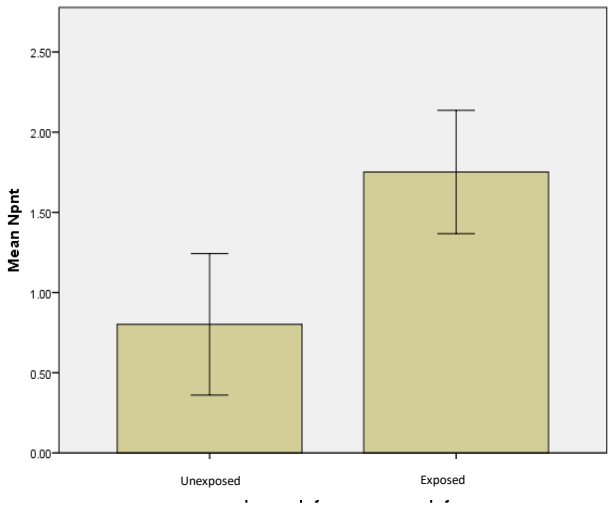

Figure 1. Comparison of the Mean Value of Serum Nephronectin Levels between the Exposed Group and the Unexposed Group

There was a significant result from the comparison of the mean Npnt level between the ten years exposed group and 1–5 years exposed group (*P*<0.05) and the 6–10 years exposed group (*P*=0.039). This result indicates that the mean Npnt level of the >10 years exposed group was significantly different (*P*=0.0001) from the other three groups, including the unexposed group.

Table 2. Nephronectin levels in the exposed groups and the control group

Exposure	N	Median (Min-Max)
Unexposed	10	0,6780 (0,21–1,96) ng/mL
Exposure 1–5 years	12	0.6960 (0.22–2.27) ng/mL
Exposure 6–10 years	14	1.0480 (0.27–4.29) ng/mL
Exposure >10 years	14	2.4710 (1.74–5.27) ng/mL

However, there was no significant result for the comparison of mean Npnt level between the 1–5 years exposed group and the 6–10 years exposed group (*P*=0.08), between the 1–5 years exposed group and control group (*P*=0.922), and between the 6-10 years exposed group and control group (*P*=0.089).

The results were shown to be significant only at >10 years of exposure compared to other groups. Nephronectin levels in the exposed groups and control group and the results of comparison test are described in Table 2 and Table 3.

Table 3. Comparison of Nephronectin Levels' Median between Four Exposure Groups

Comparison of Npnt levels		*P*
Control	Exposed 1–5 years	0.922
Control	Exposed 6–10 years	0.089
Control	Exposed ≥10 years	0.0001*)
Exposed 1–5 years	Exposed 6–10 years	0.080
Exposed 1–5 years	Exposed ≥10 years	0.0001*)
Exposed 6–10 years	Exposed ≥10 years	0.039*)

Note: **P*<0.05 are significant

The duration of exposure and cumulative duration of exposure had a very close relationship (*P*=0.0001) with Npnt levels. As a result, the duration of exposure to marble dust and the cumulative duration of exposure to marble dust had a significant impact on Npnt levels in marble industry workers. This study also showed that the duration of exposure and the cumulative duration of exposure were directly proportional to the Npnt levels (positive correlation value 0.633 for the duration of exposure and 0.633 for the cumulative duration of exposure).

As a result, the longer a worker is exposed to marble dust, the higher the Npnt serum level of the worker. Conversely, the shorter a worker is exposed to marble dust, the lower the serum Npnt level. The duration of exposure and the cumulative duration of exposure are depicted in Figure 2 and Figure 3.

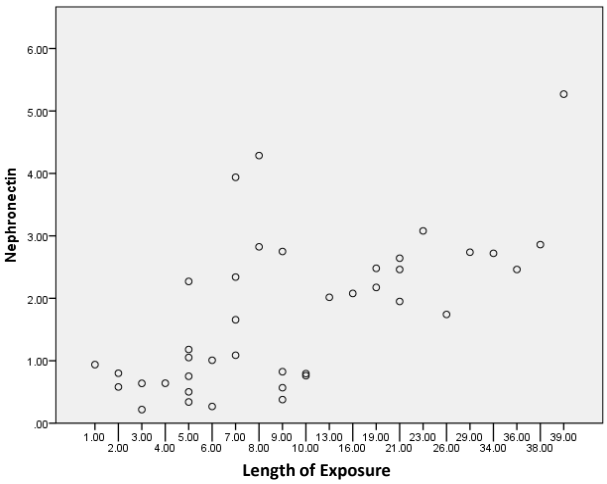

Figure 2. Scatter Plot of Length Exposure to Nephronectin Levels

To remove the potential bias within this study, we analyze the 3 confounding variables of age, smoking behavior, the use of PPE, and duration of exposure as independent variables. The findings revealed that all four combined variables had a significant impact (*P*=0.0001) on Nephronectin levels in marble industry workers. The duration of exposure and smoking behavior significantly (*P*=0.0001 and *P*=0.048 with R=0.470) affected Npnt levels in marble industry workers, where the duration of exposure had a stronger effect than smoking behavior.

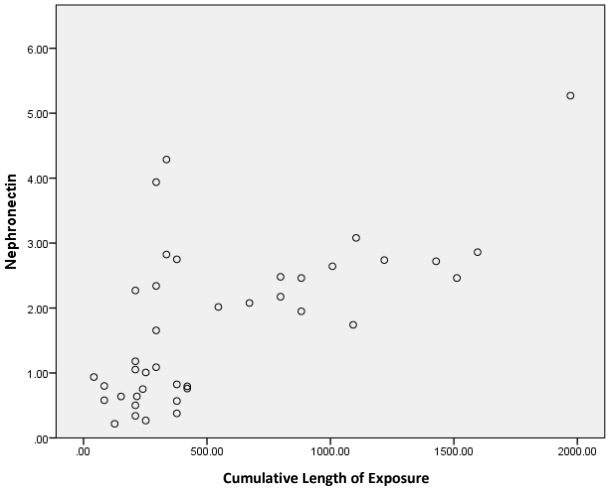

Figure 3. Scatter Plot of Cumulative Length of Exposure to Nephronectin Level

Thus, the four independent variables in the multiple regression model influenced 47% of the data diversity on Nephronectin levels, while other factors outside the model only influenced 53%. On the other hand, the age of workers and the use of PPE had no significant effect on Npnt levels (*P*=0.0795 and *P*=0.582).

In this study, we also want to know whether changes in nephronectin levels are followed by abnormalities in the CXR of marble industry workers. Based on logistic regression analysis, the significance value of the simultaneous test was 0.481, and the partial test had a significance value of 0.505. Therefore, the levels of Npnt had no significant effect on the CXR of the marble industry workers.

The duration of exposure did not significantly affect the CXR of the marble industry workers in this study. The logistic regression analysis results found that the significance value of the simultaneous and partial test of the variable length of exposure was 0.511 and 0.499. Therefore, it could be concluded that the duration of exposure had no significant effect on the CXR of the marble industry workers.

DISCUSSION

The subjects in this study were men aged 18 to 67 years with a mean age of 40.08±10.99 years. In a previous study conducted by Khoiroh, who also studied marble workers as subjects, the mean age of marble industry workers was around 46–55 years old.[8] However, study by Ahmed et al obtained that the average age of marble industry workers was 29,92±6,19 years old.[9] Productive human resources in Indonesia have entered the working age or productive age range that is 15 to 64 years. This data shows that the workers in the marble industry are classified as being of productive age.

Workers with 1–5 years of exposure to marble dust comprised 12 subjects (30%) of the subjects, those with 6–10 years of exposure comprised 14 subjects (35%), and those with >10 years of exposure also comprised 14 subjects (35%). These results are consistent with study by Khoiroh, where most marble industry workers have worked for 13 to

19 years, and in study by El-Gammal et al for 5 to 35 years.[3,8]

The number of smokers among the subjects were 22 subjects (55%). Eryani in 2015 showed that several factors affected lung function capacities: age, gender, years of service, length of work, work history, disease history, nutritional status, smoking habits, and exercise habits.[10] This study follows study by Wijayain 2019, where 83.6% of stone processing workers exposed to silica dust had a smoking habit.[2]

Research from Fathmaulida in 2013 also obtained that smoking habits were found in limestone processing workers, who smoked 13 cigarettes per day. However, the smoking habit and lung disorders in these workers did not reveal a significant relationship.[11] Several findings have shown that smokers exposed to silica dust were more likely to develop clinical silicosis than non-smokers exposed to the same dose.[12] Thus, education for workers about smoking cessation is important to reduce the adverse effects of silica dust exposure on health.

The majority of study subjects (45%) always wore masks, but not in accordance with the standard. This finding is similar to the research conducted by Hutomo in 2016 to see the level of knowledge regarding the use of PPE for furniture industry workers in Jepara, Indonesia. In this study, it was observed that most of the respondents used masks as PPE (47.6%), although their knowledge of the types of masks was not good (46%).[13]

All subjects did not experience clinical symptoms of respiratory distress. Unlike what Sahrun discovered in 2018 that mining, metal, and ceramic workers who spend about 8 hours per day inhaling 3500L of air, including dust particles or other contaminants at work, will be exposed to clinical manifestations of lung disease.[14]

There was a significant increase in Npnt levels among marble industry workers compared to control subjects (P=0.012). These findings are consistent with study from Lee, et al., who discovered that subjects exposed to silica dust had higher levels of Nephronectin than normal patients (who were not exposed). Nephronectin also plays an essential role in inducing and developing pulmonary fibrosis due to silicosis.[7]

There is a relationship between the differences in serum levels of Npnt according to the duration of exposure to silica dust in marble workers. In this study, the results were shown to be significant at >10 years of exposure. The main factors that play a role in the pathogenesis of silicosis are dust particles and the body's response, especially the respiratory tract, to these dust particles. Chemical composition, physical properties, dose, and duration of exposure determine whether or not silicosis can occur easily. The amount of inhaled crystalline silica depends on the concentration and particle size (<5μm) as well as individual susceptibility.[10]

The most common crystalline forms of silica in the workplace include quartz, tridymite, and cristobalite. Quartz contains the highest free silica, so workers exposed to these crystals experience a fast latency period.[15] Workers with high silica exposure categories are 30 times more likely to die than workers with low or no crystalline silica exposure.[16]

These results showed that the duration of exposure and the cumulative duration of exposure to marble dust significantly affected Npnt levels in marble industry workers. This study also showed that the longer a worker is exposed to silica dust, the higher the Npnt serum level of the worker. This follows research from Alonso, et al. in Spain, which pointed out that the duration of exposure to silica dust for 15–20 years had a significant effect on the incidence of silicosis.[17] According to procedures, improper PPE rules may be to blame for the shorter duration of marble workers in Indonesia. Thus, in order to have high levels of Npnt as an indicator of silicosis, the exposure time is shorter, starting at the 10th year of exposure as opposed to the study from Alonso, which began at the 15th year.[17]

The four independent variables, namely duration of exposure, age, history of smoking, and use of PPE, significantly affected Npnt levels in marble industry workers (P=0.0001). The duration of exposure and smoking had a significant effect on the levels of Npnt in marble industry workers, and the

duration of exposure had a stronger effect than smoking (*P*=0.0001 and *P*=0.048 with R=0.470). These findings are supported by study from Lee, et al. in Japan, which revealed a link between Npnt levels in subjects exposed to silica dust, but no relationship between age and Npnt levels.[7] Wijaya, et al. observed that 83.6% of stone processing workers exposed to silica dust had smoking habit. However, there were no significant results in the same study between smoking habits and serum TGF-β1 as a biomarker of silica in the blood. This was due to the small proportion of subjects in the study.[2]

In contrast, smoking can cause an increase in serum TGF-β1 levels due to the immunosuppressive effect of TGF-β1 on the immune system. Smokers who smoke 20 cigarettes per day have higher mean serum TGF-β1 level than non-smokers and smokers who smoke less than 20 cigarettes per day. Serum TGF-β1 levels increase in tandem with cigarette consumption.[18]

This study concluded that the Npnt levels had no significant effect on the CXR of marble industry workers. The CXR is one of the essential tools in detecting pneumoconiosis (asbestosis, silicosis, and pneumoconiosis in coal miners). On exposure to silica dust, the development of opacity with a diameter of more than 1 cm will be seen. The standard of the CXR interpretation method has been determined by the International Labour Organization (ILO).[19]

Even though the standard has been used, there is still variability between readers of the CXR results. Radiographs may also be less sensitive to early-stage changes produced by exposure to dust. For example, it was estimated that about 20% of asbestos-exposed workers with pulmonary fibrosis on pathological examination do not show any abnormal changes detected on radiographs. CXR alone is inadequate to serve as a surveillance tool or to detect occupational lung disease. Bronchitis is difficult to be detected on CXR. Emphysema is accurately detected only at an advanced stage.[19]

In a study conducted by Lopes in 2008, there was a more significant difference between specialist doctors who read CXR based on the results of small opacity readings. The inter-reader variability is lower at significant opacity. However, despite these limitations, CXR is still an efficient tool for follow-up evaluation of workers exposed to silica, as it is an inexpensive procedure and subjects are exposed to only low doses of radiation. This study obtained that the diagnosis of silicosis using a CT scan is better than a CXR for the early detection of the early phase of the disease and detection of progressive massive fibrosis.[20]

A study conducted by Austin in 2021 also stated that CXR alone was not sufficient to detect occupational lung disease. It was recommended to use CT (Computed Tomography) scan to diagnose occupational lung disease because CT scan sensitivity was higher for early detection of disease and had better accuracy for determining disease patterns.[21]

This study proved that the length of exposure did not significantly affect the CXR of marble industry workers. However, this could be explained because the assessment of CXR did not follow the ILO standard and used regular assessment in the hospital. This result was not in line with the study conducted by Mitra in 2015 on stone crushing factory workers in Lakshmi, India, which revealed that the longer the duration of exposure, the higher the prevalence of CXR with statistically significant positive silicosis (*P*<0.05).[22]

LIMITATIONS

This study had several limitations, namely that the number of sample subjects used was still small. Moreover, the recommended radiological examination for the diagnosis of pulmonary fibrosis as part of the ILO standards has not been carried out. In addition, a pulmonary function test was not executed in this study where the lung test is a better approach to describing the subject's respiratory function, because the study was conducted during the second wave of the pandemic. Finally, the

acquired risk factors, such as a history of smoking and the use of PPE, were not homogeneous, which could affect the results.

CONCLUSION

The duration of exposure to marble dust had a significant effect on serum Nephronectin levels. The longer the marble industry workers were exposed to marble dust, the higher the serum Nephronectin level.

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CONFLICT OF INTEREST

None.

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