Original Article

Increase in Neopterin Serum Levels Based On Exposure Duration of Silica Dust in Marble Industry Workers

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Abstract

Background: Workers in the marble industry face the risk of exposure to dust containing silica crystals in their workplace, a substantial contributor to most occupational lung diseases. Neopterin, an early biomarker of the cellular immune response, is recognized for its association with silica dust exposure. According to a previous study, neopterin levels significantly increased in workers with silicosis compared to controls with no exposure to marble dust. This study aims to analyze changes in neopterin levels based on the duration of silica dust exposure among marble workers.

Methods: The study design is an analytical cross-sectional study with a subject pool comprising 32 marble industry workers, categorized into 4 groups based on the duration of exposure: 1-5 years, 5-10 years, >10 years, and control. A comparative analysis was conducted with a control group. Neopterin levels were assessed through the utilization of the Human Neopterin ELISA kit. Analysis using T-tests, ANOVA, and Pearson correlation tests, providing a comprehensive evaluation of the impact of exposure duration.

Results: Chest X-ray shows normal results in a routine procedure for most workers. When compared to the control groups, there was a significant increase in serum neopterin levels in all workers. A significant increase was also obtained in neopterin levels among workers with over 5 and over 10 years of exposure. Also, a significant positive correlation between neopterin levels and the duration of exposure. But, among smoker workers, neopterin levels and the Brinkman index level do not show a significant increase. Notably, workers who use nonstandard face masks demonstrate no significant difference in neopterin levels.

Conclusion: As marble industry workers are exposed to silica dust for an extended period, there is a corresponding increase in their serum neopterin levels. The duration of silica dust exposure, specifically exceeding five years, significantly influences the elevation of serum neopterin levels.

Keywords: marble worker, neopterin, silica

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INTRODUCTION

Industrial workers are at risk of exposure to dust containing crystalline silica in the workplace, which can cause various health problems. Previous research shows that pulmonary function disorders are experienced by home industry workers, with a weak relationship found in age, nutritional status, and length of service. A moderately strong relationship was found between exposure time and lung function disorders and a strong relationship was found between the use of masks.[1,2]

Regarding the marble industry in Tulungagung, East Java, Indonesia, a 2016 study showed that the average concentration of marble dust produced is still within threshold limits. However, the dust concentration can change, both due to internal factors such as production capacity, and external factors such as wind direction and atmospheric stability.[3]

Silica dust is a respirable silicon dioxide (SiO2) crystal and is one of the most common and serious occupational hazards for stone workers. Workers' exposure to silica dust can occur when cutting, sawing, grinding, crushing, and crushing stone, marble, concrete, brick, blocks, and mortar.[4]

Silica dust contributes to the majority of cases of occupational lung disease. It is known that long-term exposure to silica dust has long caused one of the oldest known industrial diseases, namely silicosis. In addition to silicosis, respirable silica dust has been linked to autoimmune diseases, non-malignant kidney disease, COPD, and lung cancer.[5]

Biological markers (biomarkers) are cellular, biochemical or molecular alterations that are measurable in biological media such as human tissues, cells or fluids.[6] Neopterin is a marker of

immune activation and has been studied in various conditions, including silicosis, a type of pneumoconiosis caused by inhalation of silica dust.[7] Neopterin as a biomarker showing risks of developing pathology in the bronchi and lungs among workers who have occupational contact with industrial aerosols.[8]

An initial study assessing serum neopterin in workers exposed to silica dust and silicosis conducted in Belgium, involving 92 exposed workers, showed a significant increase in serum neopterin, namely 3.22 ng/ml vs 1.6 ng/ml in the control group.[8] Another study in Turkey also produced similar findings, namely high levels of neopterin in a group of workers exposed to silica dust in a group of sandblasting workers.[9]

However, there are still no studies regarding serum neopterin levels based on the duration of exposure to silica dust by marble industry workers. Therefore, based on this background, further research is needed that explores serum neopterin levels based on length of exposure in marble industry workers, which aims to determine the risk of disease associated with long exposure to silica dust in marble industry workers, especially in Tulungagung, East Java, Indonesia.

METHODS

The study design is an analytical cross-sectional study with a subject pool comprising 32 marble industry workers in Tulungagung, East Java, Indonesia, categorized into 4 groups based on the duration of exposure. A comparative analysis will be conducted with a control group consisting of nine healthy individuals. Serum neopterin levels were assessed through the utilization of the Human Neopterin ELISA kit by Primacu™ ELISA.

Group differences will be examined using T-tests, ANOVA, and Pearson correlation tests, providing a comprehensive evaluation of the impact of exposure duration. This study received ethical approval from the Health Research Ethics Commission, Faculty of Medicine, Brawijaya University (No. 330/EC/KEPK/11/2021).

RESULTS

In this study, 36 subjects met the inclusion and exclusion criteria and agreed to participate in the study. All research subjects who participated were male. Subject data collection was carried out in Campurdarat District, Tulungagung, East Java, Indonesia. Neopterin data analysis was carried out at the Clinical Pathology Laboratory of Saiful Anwar Hospital. Data on the characteristics of research subjects in the form of age, exposure group, and chest X-ray can be seen in Table 1 below.

Table 1. Characteristics of The Research Subject

Variable	n (%)
Age (mean±SD)	39.19±10.20
Duration of exposure	
Control/no exposure	9 (25.0%)
1–5 years	9 (25.0%)
5–10 years	9 (25.0%)
>10 years	9 (25.0%)
CXR	
Normal	34 (94.4%)
Abnormal	2 (5.6%)
Smoking Status	
Active Smoker	21 (58.3%)
Ex-Smoker	5 (15.9%)
No Smoker	10 (27.8%)
Brinkman Index	
Mild (0–199)	34 (94.4%)
Moderate (200–599)	2 (5.6%)
Severe (>600)	0 (0.0%)
Mask	
Do not use	2 (5.6%)
Sometimes used but not standard	10 (27.8%)
Always used but not standard	15 (41.7%)
Sometimes wearing a standard mask	0 (0.0%)
Always wearing a standard mask	0 (0.0%)

Note: CXR=chest X-ray; SD=standard deviation

In this study, the influencing variable was the length of exposure, which was divided into four groups. Collectively, our analysis showed that all exposure groups control with neopterin levels of 4.63±1.85 nmol/L, a group with 1–5 years with theirs neopterin levels 6.25±1.96, 5–10 years group with 7.08±2.08 neopterin levels, and >10 years group with 8.43±1.49 neopterin levels showed a significant increase (P<0.05). Neopterin levels between controls and the entire group of workers showed significant differences (P<0.05). In Figure 1, it can be seen that

there is a trend of increasing neopterin levels, which is in line with the increase in the exposure group. The correlation test showed that there was a positive and significant correlation between neopterin levels and exposure time (*P*=0.001; r=0.615).

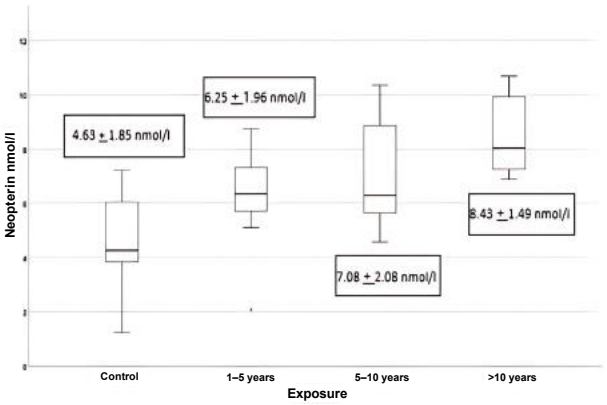

Figure 1. Graph of Duration of Exposure (years) and Neopterin Levels (nmol/l)

Furthermore, there are significant differences in neopterin levels between the control group and the groups with exposure periods of 5–10 years and >10 years. The smoking history of subjects in this study was assessed using the Brinkman index. The Brinkman index in the mild category means a Brinkman score of 0–199, the Brinkman index in the moderate category means a Brinkman score of 200–599, while the Brinkman index in the severe category means a Brinkman score >600.[10]

In this study, there were 34 subjects included in the mild Brinkman index and 2 subjects included in the moderate Brinkman index. Meanwhile, there were no subjects included in the heavy Brinkman index. There were 21 active smokers, 5 former smokers, and 10 subjects who had never smoked. Subjects who were active smokers showed higher neopterin levels than former smokers. Ex-smoker subjects showed higher neopterin levels than non-smokers. However, the difference between groups in smoking status was not significant (*P*>0.05). Subjects with a moderate Brinkman index showed higher neopterin levels than subjects with a mild Brinkman index, although the difference between Brinkman index groups was not significant (*P*>0.05), as present in Table 2. The data suggest that secondary smoke exposure could be a factor, as smokers interact with others of different smoking intensities.

Table 2. Neopterin Levels Based on Smoking Status and Brinkman Index

Characteristic	Neopterin levels (nmol/l; mean±SD)	*P*
Smoker Status		
Active smoker	6.94±1.61	
Ex-smoker	6.70±3.56	0.443
Non-smoker	5.82±2.74	
Brinkman Index		
Mild	6.51±2.23	0.363
Moderate	8.03±3.23	

Note: *P*<0.05, significant value; statistic analysis using T-test and ANOVA

There is a variety of uses of masks in the form of masks for various subjects. The masks used are cloth masks and surgical masks, which do not meet standards. The use of masks in the form of masks was also not always used by some of the subjects in this study. There were no significant differences in neopterin levels between various groups using masks (*P*>0.05). This can be seen in the table below.

Table 3. Neopterin Levels Based on the Use of Masks

Mask	Neopterin levels (nmol/l; mean±SD)	*P*
Not use	6.69±0.91	
Sometimes used but not standard	7.27±1.60	0.922
Always use, but not standard	7.31±2.39	

Note: *P*<0.05, significant value; statistic analysis using ANOVA

DISCUSSION

A significant increase in neopterin levels was found in this study along with an increasing duration of exposure group. The correlation test showed that there was a positive and significant correlation (*P*=0.001; r=0.615), which means that the longer the exposure, the higher the neopterin levels. This finding is in line with the findings in the study of Khalifa et al, which shows a positive, strong and significant correlation between work duration and neopterin levels.[8]

In this study, Receiver Operating Characteristic (ROC) analysis was also carried out between neopterin levels and the diagnosis of silicosis. It was found that neopterin levels had good diagnostic ability (AUC >0.7) in diagnosing silicosis. The optimal limit of neopterin levels found for the diagnosis of silicosis in this study was 17 nm/L with

a sensitivity of 86% and a specificity of 87%.[8] While the study correlates the duration of exposure with neopterin levels, it does not account for the intensity of exposure (i.e., how concentrated the silica dust was in the environment). Variations in exposure intensity could influence immune system activation and neopterin levels, potentially confounding the findings.

The relationship between accumulated exposure to silica dust and neopterin levels is thought to be related to the reaction mechanism in lung tissue, which occurs when silica crystals accumulate in lung tissue. The accumulated silica crystals will cause a chronic immune system activation reaction in the lung tissue. Activation of the immune system leads to an inflammatory process and causes the appearance of fibrotic tissue. Another study conducted by Akbar et al shows the potential of another biomarker, namely nephronectin, as a biomarker that is significantly associated with silica dust exposure.[11]

In this study, significant differences in serum nephronectin levels were shown in the exposed group compared to those not exposed, as well as in the group exposed for a duration of 1–5 years compared to 6–10 years. This study also showed that there was a positive and significant correlation between the duration of exposure to silica dust and serum nephronectin levels.

The study conducted by another researcher in 2022 shows the potential of another biomarker, namely transforming growth factor beta 1 (TGF-β1).[12] There were significant differences between groups in terms of exposure and TGF-β1 levels. A positive and significant correlation was also found between the duration of exposure to silica dust and serum TGF-β1 levels. Neopterin, which is a marker of immune system activation, will increase in this condition.[8]

Neopterin is a biomarker for inflammation and response to oxidative stress. Neopterin was found to be a biomarker of Interferon-gamma (IFN-γ) synthesis, macrophage activation, and the overall cellular immune system.[13] Neopterin is effective in preventing lipid peroxidation and the formation of protein hydroperoxides. Protein peroxidation can cause enzyme inhibition, affect protein turnover, and cause the accumulation of damaged and difficult-to-degrade proteins. Neopterin has been proven to be able to react and break down protein hydroperoxides. Neopterin is also known to inhibit the formation of superoxide mediated by nicotinamide adenine dinucleotide phosphate in mouse peritoneal macrophages.[14]

This is also done on COPD sufferers. These various findings indicate that smoking history is significantly associated with neopterin levels, but not with smoking index. This finding means that there is a more significant immune system activation process in smokers. However, among smokers, there was no significant difference in immune system activation between light and moderate smokers.[15] Social dynamics in active smokers, for example, the occurrence of routine interactions between smokers of various intensities (light, moderate, and heavy smokers), which culminate in the occurrence of secondary smoking, could be one of the factors that can explain this finding.[15]

In this study, 2 subjects did not wear masks, 10 subjects who did not always wear masks, and 15 subjects who always wore masks. Subjects included in the control group were not analyzed due to inappropriate exposure to silica dust compared with other groups of subjects. The highest neopterin levels were found in the group of subjects who always wore masks (7.31±2.39). Then the second highest neopterin levels were in the group of subjects who did not always wear masks (7.27±1.60), and finally in the group of subjects who did not use masks (6.69±0.91). However, the difference in neopterin levels between the three groups using PPE was not significant.

The masks used by the subjects in this study while working were cloth masks and surgical masks. According to National Institute for Occupational Safety and Health (NIOSH) recommendations, the recommended personal protective equipment (PPE) in the context of silica exposure is an N95 mask with NIOSH standards.[16] The mask used by the subject did not comply with these recommendations, so it

was classified as not meeting standards. There have been no previous studies examining the relationship between neopterin levels and mask use in the context of exposure to silica dust.

In this study, it can be seen that the use of masks that do not comply with standards does not have a significant impact on serum neopterin levels. These findings demonstrate the importance of using a mask that meets standards in the context of silica dust exposure. However, it should be remembered that neopterin is only one of many markers of activation of the body's immune system, so collaboration with various subsequent studies is needed to obtain conclusive results regarding this study.

LIMITATION

In this study, there are various limitations related to the subject and additional variables that we hope can be explored in future studies related to marble industry workers, exposure to silica dust, and neopterin levels, First, exposure to silica dust levels in the subject's workplace has not been measured. Silica dust levels can be an additional variable that provides insight into neopterin levels and other variables. Second, a pulmonary function examination has not been carried out. Various lung function variables can be additional variables that provide insight into neopterin levels and various other variables. Lastly, all subjects' work hours were not explored.

CONCLUSION

Marble industry workers are exposed to silica dust for an extended period, there is a corresponding increase in their serum neopterin levels. The duration of silica dust exposure, specifically exceeding five years, significantly influences the elevation of serum neopterin levels.

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CONFLICT OF INTEREST

Authors declare no conflict of interest.

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