NEOPTERIN VERSUS OXIDATIVE STRESS INDICATORS AMONG EGYPTIAN WORKERS IN MARBLE INDUSTRY

By

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Abstract:

Introduction: Neopterin concentrations in body fluids can be regarded as an indirect estimate of the degree of oxidative stress emerging during cell-mediated immune response thus elevated levels were observed in silicotic individuals. **Aim of work:** to study the neopterin levels among workers exposed to marble dust, and to assess the use of neopterin versus oxidative stress indicators as a screening tool for silica exposure and/or effect. **Materials and Methods:** The study was performed on 48 workers. They were divided into 24 workers exposed to marble dust compared to 24 non-exposed subjects as a control group. The studied group was subjected to a questionnaire, clinical examination, and laboratory investigations including neopterin levels in urine and oxidative stress indicators (malondialdehyde MDA, and total sulfhydryl group SH) in urine. Environmental measurements of air in the workplaces were done. **Results:** Comparison of neopterin levels between the studied groups revealed an elevated mean level among exposed group with a highly significant statistical difference at p-value < 0.001. As regards MDA, an elevated mean level in the exposed group with a highly statistically significant difference was found, while SH showed a decreased mean level in the exposed group in relation to the control with a non-significant statistical difference. Comparison in-between the biological parameters in urine, among exposed group, revealed a positive correlation of neopterin versus MDA levels, as well as a negative correlation of SH levels versus both neopterin and MDA levels. All correlations proved to be statistically non- significant. **Conclusion:** Neopterin levels in urine increase significantly in workers exposed to marble dust suggesting its use as a simple non-invasive quantitative biological indicator for exposure and effect.

Key Words: Marble industry, Neopterin, Oxidative stress, Silica.

Introduction

Marble is a commercial name for all types of hard compact carbonate rocks that attain surface polishing. The processing of marble in factories is known to produce huge amounts of wastes either solid (resulting from shaping of irregular blocks and cutting slabs, fractured blocks as well as rejection of broken or damaged slabs and tiles), or liquid (slurry, resulted from sawing blocks to slabs as well as grinding and polishing processes) (Mashaly et al., 2012).

Marble was commonly used in the sculpturing of statues as well as the construction of buildings and monuments, since ancient times, in tiles, countertops and indoor flooring. The disposal of marble wastes is a significant environmental problem around the world (Corinaldesi et al., 2010). In Egypt, marble quarry and production industry is considered one of the major waste generating industries because 70% of mineral is lost in mining, processing, and polishing procedures (El-Gammal et al., 2011).

Processing of marble produces marble dust, which becomes suspended in air and then inhaled by exposed workers. Silica is a well documented contaminant of marble industry at the work place showing strong association with chronic bronchitis, chronic obstructive pulmonary disease, silicosis, tuberculosis, and lung cancer (Altindag et al., 2003). Epidemiological studies indicates that workers in close contact with marble dust stand an increased risk of developing asthmatic symptoms, chronic bronchitis, nasal inflammation and impairment of lung function (Angotzi et al., 2005; Leikin et al., 2009).

Currently, Egypt has about 500 marble and granite factories. Around 70% of the industry is located in Shaq El Thoaban in Katameyya, Cairo, while the other factories are scattered all over Egypt (Kandil and Selim, 2006).

Preliminary studies indicating enhanced levels of autoantibodies and several cytokines reflect an involvement of the immune system in the pathogenesis of silicosis and its complications (Altindag et al., 2003). Neopterin was recently regarded as an early biomarker of the cellular immune response. This low molecular mass compound belonging to the class of pteridine (fig. 1) is a metabolite of guanosine triphosphate that is produced by the activated macrophages and dendritic cells after stimulation with γ-interferon (Shubhangi et al., 2008).

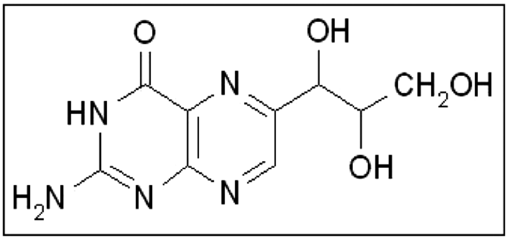

Fig. (1): Neopterin (D-erythro-1', 2', 3'-trihydroxypropylpterin)

Neopterin and its reduced form enhance the cytotoxic potential of activated macrophages (AC) and dendritic cells (DC); and modulate cytotoxic substances with generation of singlet oxygen, hydroxyl radical and NO (nitric oxide). In vivo, strong correlation was obtained between neopterin levels and the severity, progression and outcome of many diseases, such as malignancies, autoimmune diseases and viral infections, being explained by the influence of neopterin derivatives on the activation of cellular metabolism (Shubhangi et al., 2008).

In fact, elevated neopterin levels were observed in individuals with silicosis (Prakova et al., 2005), rheumatoid arthritis (Kullich, 1993), neuropsychiatric abnormalities (Zeuzem et al., 2000), Kaposi's sarcoma (Santelli et al., 1998), intrahepatic cholestasis of pregnancy (Zengping et al., 2004), as well as in pulmonary tuberculosis (Shubhangi et al., 2008) and severe burn sepsis (Yao et al, 1996). Accordingly, the use of serum neopterin as a marker for the effect of exposure to silica and other occupational diseases was acknowledged (Shubhangi et al., 2008).

Aim of work

This study was designed to scrutinize the levels of neopterin among workers exposed to marble dust, and to evaluate the possibility of using neopterin versus oxidative stress indicators as a screening tool for silica exposure and/or effect.

Materials and Methods

Study Design and Time

This is a cross sectional case control study which was conducted during the period from March to May 2013.

Study Population

The study was performed on a total of 48 workers randomly selected from different workshops. The study population was divided into 2 groups of 24 male workers, each. The workers included in the exposed group are involved with silica dust while cutting stone and marble for around 8 hours a day, 6 days per week. Exposed workers were selected from a number of workshops in Shaq El Thoaban, East of Cairo. In small workshops, cutting the stones was a very dusty operation. In larger workshops, the cutting process was done with the aid of water system thus controlling the dusty process.

Almost all exposed workers are living in El-Basateen district where cutting marble is the main work in nearby small workshops with additional environmental exposure to silica dust. Exposed population was further subdivided into three subgroups (8 workers each) according to different sites of environmental measurements; subgroup 1 (site A), subgroup 2 (site C) and subgroup 3 (site B).

The control group comprises workers who have never been exposed to any source of silica, being selected from car mechanics workshops. Both groups were selected so as to match each other in terms of age, socioeconomic standards and special habits of medical importance. The smoking index was calculated via multiplying the number of packs/day by the number of years smoked and expressed as "pack.year".

Study Methods

All individuals were subjected to the following:

1. **Questionnaire**

The questionnaire includes personal history with emphasis on residential history as a source of environmental exposure, smoking as a special habit of medical importance, and a detailed occupational history. The main goal of questionnaire is to exclude the presence of any chronic, inflammatory or autoimmune diseases that may be associated with elevation in the level of neopterin. It also includes inquiries about symptoms of respiratory and nasal irritation due to occupational exposure.

2. **Clinical examination**

The clinical examination is carried out to detect the signs of upper and lower respiratory tract irritation and affection.

3. **Urine Analysis**

Urine samples were collected and stored in a urine container (25 ml) away from heat and direct sunlight to determine the levels of neopterin and oxidative stress indicators. Neopterin was measured by ELISA (Enzyme Linked Immunosorbent Assay) technique. The intensity of the color developed after substrate incubation is inversely proportional to the amount of antigen in the sample. Results of samples can be determined directly using the standard curve.

Oxidative stress indicators, namely the Malondialdehyde (MDA) and total sulfhydryl (SH) group levels were determined. Urine MDA was assayed using the standard technique, while the total sulfhydryl groups as protein-bound and free sulfhydryl groups were determined in urine samples using DTNB (Ellman's reagent) (Stringer et al., 1989 and Bulaj et al., 1998).

4. Environmental measurements

Environmental measurements were performed at three different workshops (A, B, and C) in terms of degree of exposure to marble dust. The site (A) had cutting machines with water system placed in a closed area together with few other machines with a dusty process but in open air (figure 2). The highest exposure was detected at the site (B) where the workers used to hold the cutting machines manually in a very dusty process, though in open air (figure 3). Comparably, the site (C) is a big workshop with large cutting machines used to process large stones, yet a water system is available (figure 4).

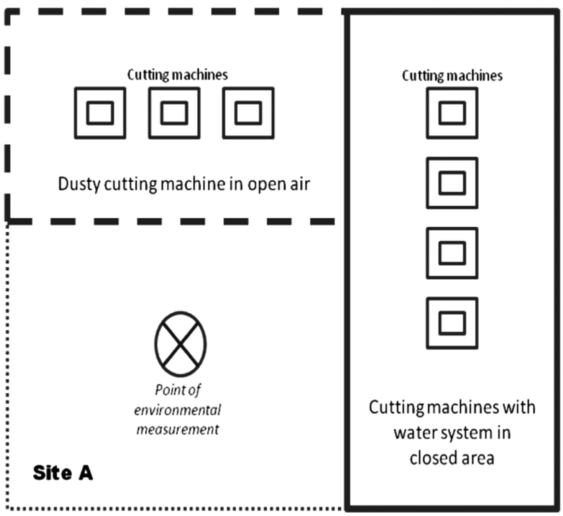

Figure (2): Site A had cutting machines with water system placed in a closed area together with few other machines with a dusty process but in open air.

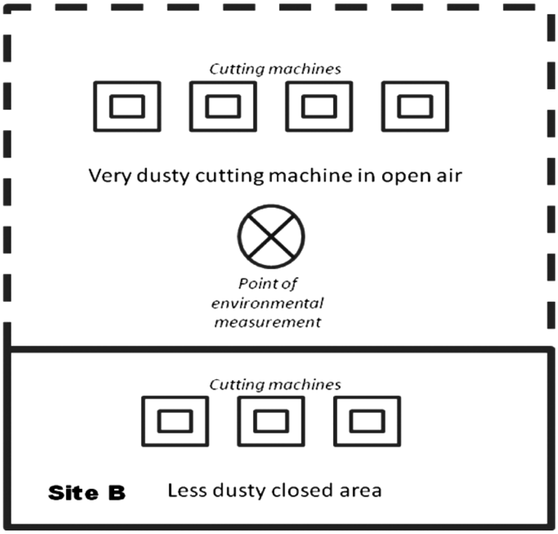

Figure (3): Site B had manual cutting machines with very dusty process in open air.

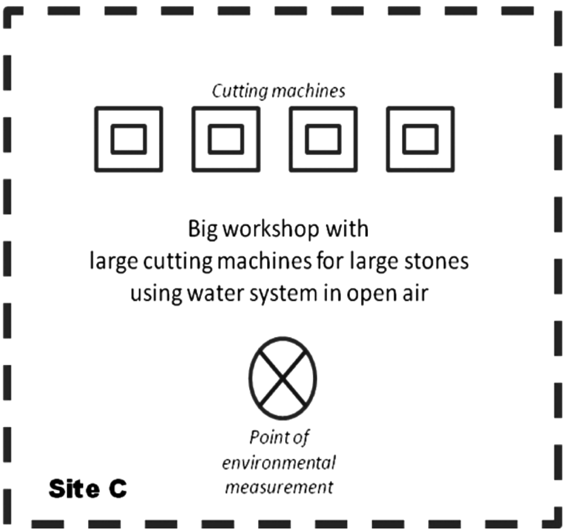

Figure (4): Site C is a big workshop having large cutting machines with water system for large stones.

The Total Suspended Particulate matter (TSP) was determined at the atmosphere of selected sites using the “Filtration Method”. Air was aspirated by a calibrated vacuum pump and its volume calculated from the dry gas-meter readings. The membrane filter was weighed in the laboratory, mounted on filter holder, and then transported to the sites of measurements. The weight of suspended particulate matter was calculated as the difference in weight before and after sampling to estimate the concentration of suspended particulate in mg/m^3.

The respirable dust sampling technique was used to evaluate the workers exposure to free silica (crystalline). Dust was collected by a personal sampler positioned in the worker’s breathing zone. A portable battery operated pump drew 3.4 liters of air per minute for at least 8 hours. For each sample, respirable dust concentration was determined using the difference in filter weight, after calculation of sampling volume.

The samples were analyzed by X-ray diffraction (XRD) to determine the silica content. Sample analysis also revealed the presence of Calcite ($CaCO_3$) and Dolomite [$Ca\ Mg\ (CO_3)_2$] as the main constituents. Other environmental samples were collected by a dust fall jar left in the workplace for one month and then analyzed by X-Ray Fluorescence (XRF). The samples showed a high weight percentage for CaO 20-30% and SiO_2 15-20% with minor weight percentage 2-4% of Al_2O_3, fe_2O_3, and MgO.

Data Management

Computer based statistical package for social sciences (SPSS) version 20 was used. Unpaired student t-test in addition to Pearson correlation and ANOVA (analysis of multiple variances) were applied for quantitative parameters.

Consent:

Verbal consent was obtained from those who accepted to participate in the study.

Ethical Approval:

Reviewing the proposal was carried out before starting data collection via the Ethical Review Committee of department of Occupational and Environmental Medicine, Faculty of Medicine, Cairo University. The aim of the study was explained to each participant before filling the questionnaire.

Results

Environmental measurements in the form of total suspended particulates (mg/m3) and free silica (μg/ m3) at three different sites (A, B, and C) revealed the presence of 6.2 and 65 at site (A), 8.6 and 212 at (B), as well as 3.4 and 117 at (C). The respirable suspended particulates were calculated for exposed workers at the sites A, B and C and were found to be 1.054, 1.143 and 0.705mg/ m3 (Table 1).

Table (1): Environmental measurements at different exposure sites

	TSP	Free Silica	RSP
Site A	6.2	65	1.054
Site B	8.6	212	1.143
Site C	3.4	117	0.705

TSP: Total Suspended particulates (mg/m^3); Free Silica (μg/ m^3);
RSP: Respirable suspended particulates (mg/m^3)

The study population (48 workers) was divided into an exposed group of male workers involved with the processing of marble being exposed to silica particles (24), and a control group (24) involved in different types of work but not exposed to silica. The duration of work among the exposed group ranged from 1 to 30 years with a mean and standard deviation values of 11.3 ± 7.6 years.

The main complaint among the exposed workers was related to respiratory tract affection in the form of cough, expectoration, chest wheezes, and shortness of breath on exertion; in addition to nasal irritation and symptoms of sinusitis. The symptoms related to respiratory tract affection were detected among (9) exposed workers, thus representing 37.5 % of the exposed group; those with nasal symptoms (4 workers) represented 16.7 % of the group.

Table (2): Comparison of means between both Exposed and Control Groups (n = 48) in terms of Age, Smoking Index (SI), as well as the Biological Parameters Measured in Urine

		Exposed N = 24	Control N = 24	t-value	p-value
Age	*Mean ± SD*	33.75 ± 11.8	36.75 ± 12.64	0.85	0.4
	Range	12 – 59	14 - 55		
SI	*Mean ± SD*	11.84 ± 14.26	8.88 ± 12.14	0.77	0.44
	Range	0 - 45	0 - 40		
Neopterin	*Mean ± SD*	240.05 ± 41.23	123.51 ± 21.8	12.24	□0.000
	Range	142.6 – 301.5	102.9 -184.7		
MDA	*Mean ± SD*	0.14 ± 0.004	0.09 ± 0.06	5.78	□0.000
	Range	*0.013 – 0.26*	*0.006 - 0.02*		
SH	*Mean ± SD*	0.12 ± 0.09	0.16 ± 0.16	-0.98	0.33
	Range	*0.01 - 0.32*	*0.02 - 0.51*		

**Highly significant difference at p-value < 0.001*

Age (years); SI : Smoking Index (pack.year); Neopterin (µmol/mol creatinine);

MDA: Malondialdehyde (mmol/gm creatinine); SH (mmol/mg protein).

Table (2) shows that the exposed and control groups were chosen so as to match each other, in terms of age, sex, smoking index, socioeconomic status and special habits of medical importance. Assessment of neopterin levels among the studied population revealed the presence of elevated level in the exposed than the control group with a highly significant statistical difference. Similarly, malondialdehyde levels (MDA) showed a highly significant statistical elevation among the exposed. On the other side, the level of total sulfhydryl groups (SH) was lower in among exposed workers in relation to the control group with a non-significant statistical difference.

Table (3): Analysis of Variance (ANOVA) test among the three subgroups (n=8 each) and control group (n=24) regarding the urinary levels of neopterin, MDA and SH

	Subgroup 1 *Site A*	***Subgroup 2*** *Site C*	***Subgroup 3*** *Site B*	***control***	***F***	***P***
Neopterin	241.01±36.37	230.01±54.57	249.13±32.79	123.51 ± 21.8	49.67	0.00*
MDA	0.10±0.06	0.10±0.08	0.06±0.04	0.09 ± 0.06	13.66	0.00*
SH	0.10±0.09	0.14±0.10	0.12±0.08	0.16 ± 0.16	0.41	0.75

**Highly significant difference at p-value < 0.001*

Table (3) shows further subdivision of the exposed population into 3 subgroups according to the different sites of environmental measurements. The comparison between the 3 subgroups and the control group as regards the different parameters assessed in urine revealed a highly significant statistical difference for neopterin and MDA, but not for the SH level.

Table (4): Multiple Comparisons (post Hoc test) in-between the 3 subgroups and control group regarding the urinary levels of neopterin, MDA and SH at 95% confidence interval.

Subgroups		**Neopterin**		**MDA**		**SH**	
		Post Hoc	**P value**	**Post Hoc**	**P value**	**Post Hoc**	**P value**
Control	**1**	-117.5	0.00*	-0.088	0.00*	0.055	0.87
	2	-106.5	0.00*	-0.085	0.00*	0.019	0.99
	3	-125.6	0.00*	-0.045	0.00*	0.033	0.98
1	**2**	11.00	0.985	0.003	1.00	-0.036	0.994
1	**3**	-8.11	0.997	0.044	0.243	-0.022	1.00
2	**3**	-19.11	0.819	0.04	0.316	0.014	1.00

**Highly significant difference at p-value <0.001.*

Table (4) shows multiple comparisons (post Hoc test) in-between the 3 subgroups and control group regarding the urinary levels of neopterin, MDA and SH at 95% confidence interval. The multiple comparisons showed a highly significant statistical difference in neopterin and MDA only between each subgroup and the control, but not in-between the subgroups. While non-statistically significant results were obtained as regards the SH-group.

Table (5): Pearson Correlation of Age and Duration of Exposure to Silica versus the Biological Parameters as well as in-between the Different Biological Parameters measured in Urine among the Exposed Group (n = 24)

		Age	Exposure	Neopterin	MDA
Neopterin	*r value*	0.02	0.13	---	---
	P value	0.94	0.55		
MDA	*r value*	0.11	0.16	0.13	---
	P value	0.6	0.47	0.56	
SH	*r value*	0.32	-0.2	-0.30	-0.04
	P value	0.13	0.35	0.15	0.84

Age (years); Exposure: Duration of Exposure to Silica (years); Neopterin (µmol/mol creatinine); MDA: Malondialdehyde (mmol/gm creatinine); SH: Sulfhydryl (mmol/mg protein).

Positive correlation was detected between neopterin level and the duration of exposure to marble dust among the exposed group. Similarly, positive correlation was obtained between the MDA level and duration of exposure. However, the correlation of SH groups to the duration of exposure was found to be a negative correlation. All correlations appeared to be statistically non-significant (P value > 0.05). Comparison in-between the biological parameters in urine revealed a positive correlation of neopterin versus MDA levels and a negative correlation of SH levels versus both neopterin and MDA levels. All correlations related to biological parameters proved to be statistically non-significant (Table 5).

Discussion

Marble is a metamorphic rock composed of re-crystallized carbonate minerals, most commonly calcite or dolomite (Kearey, 2001). All types of stone cutting as sawing, abrasive blasting, chipping and grinding, are important sources of dust containing calcium carbonate and silica (Carson et al., 2010). Egypt occupies the fifth position among the stone producing countries and has the fourth world ranked marble and granite industrial zone in Shaq El Thoaban, East of Cairo (Mashaly et al., 2012).

Prolonged exposure to respirable crystalline silica has long been known to cause one of the oldest known industrial diseases, silicosis (Tjoe-Nij et al., 2003). The neopterin level was suggested to be used as a marker for the effect of exposure to silica (Prakova et al., 2005). The increased level of neopterin concentration increase in urine or blood reflects the activation of cellular immunity and endogenous release of INF-γ (Shubhangi et al., 2008). Neopterin is a low molecular weight substance which is biologically and chemically stable in body fluids, and it can be applied without difficulty for routine measurements in laboratory diagnosis. Neopterin is eliminated by the kidney, and changes of neopterin concentrations in serum are reflected by urine levels (Fuchs et al., 1994) as long as renal function is not impaired. In fact, there is an equal sensitivity of neopterin measurements either in serum or in urine (Überall et al., 1994).

The aim of the present work was to study the alteration of neopterin in relation to silica exposure, and to assess the possibility of using neopterin level versus oxidative stress indicators as a screening tool for occupational exposure to silica particles.

The area of Shaq El Thoaban includes around 400 workshops involved in the processing and preparation of specific dimensional marble obtained from quarries using specialized mills equipped with cutting saws, polishing machines, and others. Accordingly, large amounts of dust are produced when using the dry method. The wet method is another cutting method involving the continuous use of water to decrease the amount of dust produced.

Environmental sampling of marble dust from workshops at Shaq El

Thoaban industrial zone and analysis of dust samples showed the presence of calcite [CaCO3] and dolomite [Ca. Mg(CO3)2]. This was consistent with the results obtained by Mashaly et al., 2012, who found that the main bulk composition of limestone sludge was CaCO3 and MgCO3. In fact, the content of CaCO3 and MgCO3 greatly affects the size of the produced powder. The higher the content of CaCO3 in the processed stone is, the finer is the produced powder and vice versa. In Table (1), the total suspended particulates measured at the different sites (A, B and C) were found to be 6.2, 8.6 and 3.4 mg/m3 respectively. The results of environmental assessment were found to be above the current exposure standard for dust exposure (5mg/m3) (El-Gammal et al., 2011) at the sites A and B if compared to site C which proved to be within the normal limits.

Estimation of neopterin levels among the studied groups revealed a highly significant elevation of urinary neopterin levels among workers than control groups ($p<0.001$) (Table 2). These results were consistent with those obtained by Altindag and his colleagues (2003), who suggested the use of neopterin as a new biomarker for the evaluation of occupational exposure to silica dust. The potential role of neopterin as a biomarker for silicosis, being high in silicotic workers, and as an early indicator for cellular immune response activity and macrophage participation in disease pathogenesis, has also been confirmed.

In fact, neopterin level could be used in the diagnostic criteria of the disease, together with the typical radio-morphological changes (Prakova et al., 2005). In the current study, this fact could be supported by the multiple comparisons done between the subgroups with different levels of silica exposure, which revealed a highly significant statistical difference in neopterin for each subgroup and the control, but not in-between the subgroups (Table 4).

Exposure to crystalline silica particles has also been associated with increased level of malondialdehyde resulting from lipid peroxidation processes through repeated phagocytosis of silica and enhanced generation of reactive oxygen species (Liu et al., 2009). In some studies, the malondialdehyde

(MDA), which is an indicator of lipid peroxidation was introduced as another indicator of oxidative stress resulting from cell-mediated immune activation due to respirable silica dusts exposure (Altindag et al., 2003 and Prakova et al., 2005). Consistently, serum malondialdehyde and urinary neopterin levels among glass sandblasters exposed to crystalline silica aerosols, showed significantly higher levels than that of control population ($p<0.001$). Accordingly, malondialdehyde of blood serum and urinary neopterin were regarded as biomarkers of exposure to crystalline silica aerosols (Azari et al., 2011).

In this study, there were statistically significant higher levels of MDA in the exposed than control group (Table 2), which was in consistence with the results obtained by Jun-Ling et al. (2011), who studied the serum oxidative stress status among silicosis patients. Exposure to SiO2 dust was suggested to be a trigger for the body-defending antioxidant mechanisms. However, elevated concentration of MDA indicated that, the stimulation of the body's antioxidant agents failed to cope with the overproduction of reactive oxygen species in silicosis patients.

Furthermore, the concentration of antioxidants showed marked variations during the different stages of silicosis. Orman et al. (2005) showed a decreased erythrocyte glutathione levels and increased plasma MDA levels in workers with cement dust-exposure silicosis, which was also consistent with our findings. Multiple comparisons between the subgroups, with different levels of exposure, and the non-exposed control group revealed a highly significant statistical difference in MDA for each subgroup and the control group, but not in-between the subgroups (Table 4). Glutathione is a major non-protein sulfhydryl compound present in tissue to be released by pulmonary alveolar macrophages in response to silica particle exposure (Boehme et al., 1992).

A positive correlation was detected between neopterin as well as MDA levels and the duration of silica exposure among exposed group (Table 5). This finding was partly consistent with the results of other studies that proved the direct relationship between the increase in urinary neopterin levels and both the extent of exposure to silica dust and the working years (Altindag et al., 2003). A more recent study on serum neopterin among workers exposed to inorganic dust rich in free crystalline silicon dioxide excluded the influence of either duration or level of exposure on the level of neopterin in serum. A statistically high level of neopterin was evident only among workers serving for less than 10 years (Prakova et al., 2009).

The correlation studied between the duration of exposure at work and SH levels proved to be inversely proportionate (Table 5). Recently, the study of serum oxidative stress status in silicotic patients revealed a negative correlation between GSH and the disease duration. According to the free radical theory proposed to explain the mechanism of silicosis, lung alveolar macrophages were activated then released various reactive oxidative species (ROS) in long-lasting exposure to silica dust. They suggested that ROS continually consumed the antioxidant agents present in the body, thereby indicating the failure of the total antioxidant defense mechanism (Jun-Ling et al., 2011).

Conclusion

The presence of increased neopterin levels in urine of workers exposed to marble dust confirm the possibility of its being used as an early biomarker for stimulation of macrophage activity and cellular immunity overstimulation before the development of overt silicosis. It may be used as a quantitative marker either for the extent of exposure or the severity and progression of disease. However further large-scale studies are to be considered.

Recommendations

Urinary neopterin level should be considered as an affordable non-invasive screening test in the periodic medical examination of workers exposed to crystalline silica in their workplace for the early detection of cellular immunity overstimulation before development of silicosis.

Engineering control methods such as the use of wet processes, enclosure of dusty processes under negative pressure and exhaust ventilation, should be introduced.

Personal protective equipment should always be used for respiratory protection and not as a substitute for proper dust control which is not always effective or adequate during marble production and processing.

Conflict of interests

Authors have declared that no conflict of interests exists.

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