



Neopterin as assessment marker for susceptibility of silicosis in dental technician

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ABSTRACT

Background: Dental technician expose to large amounts of crystalline silica that increase their risk of developing silicosis. Neopterin are produced upon stimulation with the cytokine interferon- γ . Neopterin is produced by activated monocytes, macrophages, and dendritic cells upon stimulation by interferon gamma produced by T-lymphocytes. Neopterin production provides prognostic information in patients with autoimmune diseases. **Objective:** Assessment of serum neopterin, inflammatory cytokines, enzyme activities and trace elements in smokers and non smokers dental technicians and to compare them with the corresponding levels in a control group. **Subjects and Methods:** Serum was examined in 45 dental technician, 15 subjects as a control group and 30 subjects exposed to silica. The surveyed dental technician were divided into three groups: 1st group (control group), 2nd group (smokers dental technician) and 3rd group (non smokers dental technician). Serum neopterin, some trace elements, LDH, ALP, ACP, TNF- α , and IL-8 were determined. **Result:** Higher levels of neopterin were established in smokers dental technician exposed to crystalline silica (16.48 ng/ml) by comparison with non smokers dental technicians (9.85 ng/ml) and the control group (4.24 ng/ml). Significant increase in the levels of silica, calcium, copper, enzymatic activities, TNF- α , IL8 and a significant decrease in Zn levels in serum of smokers subject compared with both non smokers and control subjects. **Conclusion:** Research on biomarkers for silicosis is still at its nascent stage. The concentration of serum neopterin could be used as a biomarker in the diagnostic criteria for silicosis moreover it is suitable for introduction into the routine clinical laboratory practice.

INTRODUCTION

Silicosis is one of the various occupational lung diseases that induced by inhalation of dust such as crystalline silica for a long period, silicosis involves chronic inflammation of lung due to accumulation of various inflammatory mediators and fibrogenic factors in the airways.⁽¹⁾

Dental technician are exposed to crystalline silica and dust during the production of dental prostheses. Silica dust is used in casting investment materials, divestment of castings, sandblasting of castings, mixing porcelain powders, grinding or polishing dried porcelain material and tasks that involve cleaning dusts, maintaining local exhaust ventilation or dust collection.⁽²⁾ Excessively exposure to crystalline silica, cause potential adverse effects on their health, serious lung disease has which may contribute to their risk of developing silicosis.⁽³⁾ Moreover international agency for research on cancer has been classified crystalline silica as a class I carcinogen.⁽⁴⁾

The presentation and severity of silicosis are influenced by the level and duration of exposure to silica, it is a severe and irreversible respiratory disease, marked by inflammation and scarring of the upper lobes of the lungs.⁽⁵⁾ It is a progressive condition, symptoms started by intense cough, shortness of breath and weakness, it gets worse over time. Other possible symptoms are chest pain, fever, night sweats, and weight loss.⁽⁶⁾ Increased risk of respiratory diseases as silicosis, tuberculosis, chronic obstructive pulmonary disease, chronic bronchitis and lung cancer are associated with Long-term occupational exposure to silica.⁽⁷⁾ Different clinical types of silicosis have been identified which include simple or nodular, silicoproteinoisis (acute silicosis), complicated silicosis and interstitial fibrosis. These structural changes against the silica exposure occur as a consequence for production of fibrogenetic and inflammatory mediators as well as growth

factors, including tumour necrosis factor (TNF- α) and interleukin-8 (IL-8).⁽⁸⁾

Neopterin is pyrazino-pyrimidine compound with 253 D molecular weight, belonging to pteridines class. A pteridine is a group of heterocyclic compounds composed of fused pyrimidine and pyrazine rings.⁽⁹⁾ Neopterin is produced as a result of activation of monocytes, macrophages, and dendritic cells upon stimulation by cytokine interferon gamma produced by T-lymphocytes.⁽¹⁰⁾ Neopterin has a higher stability in body fluids which makes the sample handling and measurement easier compared to other cytokines, increased concentrations of neopterin in body fluids correlate with different infectious diseases.⁽¹¹⁾ Neopterin levels can be used as a sensitive marker of inflammation associated with cell-mediated immunity in various diseases.⁽¹²⁻¹⁵⁾

Cytokines has been associated with various biological procedures such as inflammation, metabolic mechanism, cell growth and proliferation, morphogenesis and fibrosis. Major sources of cytokines in the lung are epithelial cells, endothelial cells, fibroblasts, and inflammatory cells.⁽¹⁶⁾ Tumor necrosis factor alpha (TNF- α) is a pro-inflammatory cytokine, it is involved in systemic inflammation, important in the development, and progression of several diseases including pulmonary fibrosis. The main role of TNF- α is in the regulation of immune cells.⁽¹⁷⁾ Interleukin-8 (IL-8) is a chemokine produced by a variety of cells types including fibroblasts in response to IL-1 and TNF- α . IL-8 is an essential stimulus and chemo-attractant for neutrophils, it has been implicated in a variety of inflammatory diseases.⁽¹⁸⁾

Subjects and Methods

This study was performed on 45 dental technician, 15 smokers dental technicians were classified as (group II) and 15 non smokers dental technicians classified as (group III) from the

prosthodontic department, faculty of dentistry, Alexandria university and 15 subjects as control (Group I), the age of the subjects ranged between 30 to 50 years and the occupational duration of their work in the dental laboratory were ranged from 3 to 15 years. All subjects gave oral informed consent to participate in the study and the study was approved by the local ethical committee of pharos university. A statistical questionnaire was used to investigate the presence of lung or autoimmune diseases and duration of smoking.

Sample collection

The blood specimen collected from 45 dental technician, 5-7 ml of the drawn blood were put in plain tube, left to complete clot formation, the serum was then separated from the clotted blood by centrifugation at 3000 rpm for 15 min., then decanted into clean and sterile plain tubes and stored at (-20) °C.

Estimation of Neopterin

Serum neopterin levels were determined by ELISA kit (Human Neopterin ELISA Kit Elabscience , Catalog No: E-EL-H2421) in ng/ml .

Estimation of silicon, calcium and zinc levels in Serum

Atomic absorption spectroscopy (AAS) technique was used to determine silica, Ca and Zn levels using the standard curve to convert the optical density into concentration. Atomic absorption spectrometer equipped with a PE AS-72 auto-sampler (Perkin–Elmer Instruments GmbH, Rodgau-Juegesheim, Germany) was used.

Estimation of Enzymes Activity in Serum

The activity of lactate dehydrogenase (LDH) was determined using Abnova colorimetric kit (Catalog Number KA1653)⁽¹⁹⁾, alkaline phosphatase (ALP) activity was determined using a Biovesion colrimetric Kit (Catalog number K412-500), and the activity of Acid phosphatase (ACP) was

determined using syaman kit ⁽²⁰⁾ (cat. No. 10008051).

Analysis of serum cytokines

Analysis of serum cytokines was measured by (Human TNF alpha ELISA eBioscience-Catalog Number: 88-7346) (Human IL-8 ELISA Kit thermo scientific EH2IL8) using sandwich and competitive chemiluminescence immunoassays, as previously described the minimum detectable concentrations in our laboratory were 5 pg/mL for TNF- α , 3 pg/ml for IL-8.

Statistical analysis of the data

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. data presented as mean \pm SE was analyzed by ANOVA to compare between the three studied groups for normally distributed quantitative variables. Significance of the obtained results was judged at the 5% level.

RESULTS

Neopterin concentration

Our result showed that there was a significant increase in the level of Neopterin in smokers and non smokers subject compared with control subject. The result also indicated that there was a significant increase in Neopterin level with increasing exposure time to silica (Table 1).

Concentration of trace elements in serum

Our result showed a significant increase in the levels of silicon ,calcium ,copper and a significant decrease in Zn levels in serum of smokers subject compared with both non smokers and control subjects (Table 2).

concentration of enzymes activities

The result indicated that there was a significant increase in the activity of LDH, ALP and ACP in serum of smoker and non smoker subject compared with control, the highest activity was in smokers dental technician , (Table 3). The enzyme

activity also increased with increasing exposure time.

Concentrations of serum TNF- α and IL-8

The mean serum TNF- α level was significantly higher ($p < 0.001$) in the smokers subjects (33.92 pg/ml) than non smokers (15.37 pg/ml) while control subject was (6.49 pg/ml). The mean serum IL-8 level was significantly higher ($p < 0.001$) in smoker subjects (65.62 pg/ml) vs. controls (29.97 pg/ml). Serum cytokine levels are shown in Table 4.

DISCUSSION

Occupational inhalation of crystalline silica for a long period is known to cause silicosis, often leading to lung fibrosis. It kills thousands of people every year everywhere. Silicosis is clinically detected by either radiological or lung function abnormalities which are a late and irreversible manifestation of disease, markers of early detection for silicosis are essential for predicating susceptibility of persons to disease and development of disease state. Neopterin is a biomarker that have been evaluated to measure effects following exposure to silica. A few reports have been published on dental technician's silicosis as they expose to crystalline silica during their work.

In the current study we found a significant increase in the neopterin level in both smoker and non smoker dental technician compared with control group. This elevation was correlated with duration of work and increase exposure to silica dust. The result with in agreement with Altindag et al., 2003⁽⁷⁾ who reported an increase in serum neopterin levels in 22 crystalline silica-exposed workers and also, with Prakova et al. 2005⁽²¹⁾ who showed an increase in serum neopterin concentrations in 60 patients with silicosis. Neopterin which are produced by monocytes/ macrophages upon stimulation with interferon- γ serves as a marker of activated cell-mediated immune response, it has the ability to induce apoptosis in T

lymphocytes (Wirleitner et al., 2003).⁽²²⁾ Pingle et al., 2008⁽²³⁾ showed that neopterin level used as an indirect marker for oxidative stress, and its levels help in the diagnosis of different diseases specially in occupational diseases.

In the present study, inhalation exposure of dental technician to silica resulted in elevation of silicon levels in serum of both smokers and non smokers dental technician compared with control subjects, this increase is correlated with increase time of exposure. Patients who smoke and have a history of silica exposure increase the risk of lung cancer.⁽²⁴⁾ Both silicosis and lung cancer are believed to result from the strong inflammatory response that silica evokes in the lung as there is a strong evidence that silica causes both silicosis and lung cancer; another suggestive evidence that it causes renal disease; and limited evidence that it causes autoimmune diseases, particularly scleroderma and rheumatoid arthritis.^(25,26) Also, our result revealed that there was a significant increase in serum some of trace elements Ca and Cu with a reduction in the level of Zn in smoker and non smoker dental technician compared with control group. These results in accordance with Tiwari et al., 2004⁽²⁷⁾ who found an increase in serum Cu of workers of quartz stone with a higher level in smokers worker than non-smokers. Cu levels were found to be higher among those having respiratory disorders compared with those free from diseases. Pandey et al., 2012⁽²⁸⁾ demonstrated that Cu plays a very important role in the progress of lung fibrosis which is the primary pathologic change in silicosis and Cu has a direct relationship with ceruloplasmin levels as each ceruloplasmin molecule contains eight Cu atoms, and the Cu level in blood serum is maintained by ceruloplasmin. The decrease in Zn levels is in accordance with Bai et al., 1993⁽²⁹⁾ who showed that zinc (Zn) play a part in the production of collagen and in the

progression of lung fibrosis where pathologic changes in silicosis include collagen tissue in the lungs.

The result of our study indicated that there was a significant increase in the activity of LDH in the serum of dental technician compared to control group with a highest activity in serum of smokers technicians. These results agree with Gulumian *et al.*, 2006⁽³⁰⁾ who found that the increase in LDH activity in workers could be used as indicators of cell damage by crystalline silica. The increase in the levels of LDH may be due to the fact that this enzyme is especially concentrated in the some organs as heart, liver, red blood cells, kidney, muscles, brain and lungs, thus the damage of any of these organs could elevate the activity of LDH in serum. Also, our result demonstrated that there was a significant increase in ALP activity in serum of dental technician, with highest activity in serum of smokers dental technicians (Table 3). ALP levels increased in a proportional way with exposure time (Table 3). The results are in agreement with (Mojiminiyi *et al.*, 2007)⁽³¹⁾ who stated that the ALP activity of cement workers proportionally increased with time exposure to dust. Increasing ALP activity might be due to exposure to heavy metals either by direct inhalation of suspended dust particles in air, dermal contact or indirect ingestion. Some of heavy metals are toxic and might interfere with enzymes system and metabolism of the body (Arogunjo, 2007).⁽³²⁾ The result showed that there was a significant increase in ACP activity, ACP levels also increased with increasing exposure time (Table 3). Jayakumar *et al.*, 2008⁽³³⁾ demonstrated that The increase in the activity of ACP might be due to that it is a hydrolytic lysosomal enzyme released during stress in response to tissue or cell damage.

In the current study our result revealed a significant increase in both pro-inflammatory cytokines TNF- α and IL-8

in smokers and non smokers dental technician compared with control group. These results are in accordance with lee *et al.*, 2010⁽³⁴⁾ who demonstrated an increase in both IL-8 and TNF- α in serum of coal workers' pneumoconiosis. It has been fairly well established that inflammation plays a major role in many of the pulmonary effects associated with cigarette smoking.⁽³⁵⁾ The central role played by inflammation in the pulmonary effects of crystalline silica and cigarette smoke. TNF- α is important in the early onset of inflammation, development, and progression pulmonary fibrosis. TNF- α which can be released by a number of cell types including activated macrophages, monocytes, and polymorphonuclear cells, are initiators of cytokine networks and lead to neutrophil recruitment and chemotaxis. TNF- α has various roles that include synergistic effects in inflammatory and immune responses. It would be responsible for the initiation and perpetuation of the inflammatory reaction observed in the lung of patients with progressive massive fibrosis.⁽³⁶⁾ IL-8 is a member of a structurally similar family of cytokines designated chemokines, which display chemotactic activity for neutrophils. IL-8 has been implicated in a variety of inflammatory diseases which produced in response to pro-inflammatory stimuli. Strieter *et al.*⁽³⁷⁾ reported that the central role of the alveolar macrophage in the elicitation of polymorphonuclear cells into the lung via the production of IL-8.

Castranova and Vallyathan⁽³⁸⁾ proposed that the development and progression of silicosis occurs with four basic mechanisms: a) direct cytotoxicity of silica dust; b) activation of oxidant production by pulmonary phagocytes; c) activation of mediator release from alveolar macrophages and epithelial cells, which leads to recruitment of polymorphonuclear leukocytes and macrophages, resulting in the production of proinflammatory cytokines and reactive

species and in further lung injury and scarring; and d) secretion of growth factors from alveolar macrophages and epithelial cells, stimulating fibroblast proliferation and eventual scarring.

Conclusion

Silicosis is a permanent pulmonary disease caused by inhalation of dust containing free crystalline silica, it progress even when exposure to causal factor stops. Occupational exposure of silica leads to the increase in enzymes activities and production of inflammatory and fibrotic mediators. TNF- α play important role in inflammation by controlling other cytokines like IL-8.

Neopterin, a biochemical parameters can be used as exposure biomarkers to silica dust, providing a better way for early diagnosis of this deadly disease.

Improvement in hygiene, techniques such as wet drilling, efficient ventilation systems (artificial, natural, local and exhaust ventilation) and usage of personal protective devices can prevent silicosis to some extent. Further future studies are recommended to overcome the current study's limitation of relatively small sample size.

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Table (1): Comparison between the three studied groups according to Neopterin in each duration of work

Neopterin (ng/ml)	Control (n=15)	Non Smokers (n=15)	Smokers (n=15)	p
3 – 5 years	2.95 ± 0.96	7.43 ± 0.86 ^a	11.58 ± 0.87 ^{ab}	<0.001 [*]
6 – 10 years	4.90 ± 0.95	8.98 ± 0.68 ^a	13.62 ± 1.18 ^{ab}	<0.001 [*]
>10 years	4.88 ± 0.61	13.14 ± 0.98 ^a	24.25 ± 3.36 ^{ab}	<0.001 [*]
Total cases	4.24 ± 1.23	9.85 ± 2.62 ^a	16.48 ± 6.08 ^{ab}	<0.001 [*]

p: p value for F test (ANOVA) for comparing between the different studied group

*: Statistically significant at $p \leq 0.05$ a: Significant with Control ,b: Significant with Non Smokers

Table (2): Comparison between the three studied groups according to trace elements

	Control (n=15)	Non Smokers (n=15)	Smokers (n=15)	p
Silicon ($\mu\text{mol/L}$)	10.64 ± 9.35	23.25 ± 4.13 ^a	35.60 ± 9.05 ^{ab}	<0.001 [*]
Calcium (mg/dl)	9.77 ± 0.69	14.39 ± 1.03 ^a	18.75 ± 2.94 ^{ab}	<0.001 [*]
Zn ($\mu\text{g/dl}$)	87.22 ± 13.94	59.90 ± 5.36 ^a	48.62 ± 5.87 ^{ab}	<0.001 [*]
Cu ($\mu\text{g/dl}$)	101.64 ± 9.35	163.46 ± 7.57 ^a	177.48 ± 8.39 ^{ab}	<0.001 [*]

p: p value for F test (ANOVA) for comparing between the different studied group

*: Statistically significant at $p \leq 0.05$, a: Significant with Control , b: Significant with Non Smoker.

Table (3): Comparison between the three studied groups according to enzymes activities

	Control (n=15)	Non Smokers (n=15)	Smokers (n=15)	p
LDH (IU/L)	40.08 ± 2.78	60.96 ± 9.92 ^a	69.35 ± 14.21 ^{ab}	<0.001 [*]
ALP (U/L)	47.71 ± 11.14	163.90 ± 7.71 ^a	176.60 ± 9.38 ^{ab}	<0.001 [*]
ACP (U/L)	2.25 ± 0.28	12.07 ± 3.19 ^a	15.17 ± 1.98 ^{ab}	<0.001 [*]

p: p value for F test (ANOVA) for comparing between the different studied group

*: Statistically significant at $p \leq 0.05$ a: Significant with Control, b: Significant with Non Smokers

Table (4): Comparison between the three studied groups according to cytokines concentration

	Control (n=15)	Non Smokers (n=15)	Smokers (n=15)	p
IL-8 (pg/ml)	29.97 ± 1.51	54.15 ± 16.14 ^a	65.62 ± 17.66 ^{ab}	<0.001 [*]
TNF-α (pg/ml)	6.49 ± 0.97	15.37 ± 2.95 ^a	33.92 ± 9.0 ^{ab}	<0.001 [*]

p: p value for F test (ANOVA) for comparing between the different studied group

*: Statistically significant at $p \leq 0.05$

a: Significant with Control, b: Significant with Non Smokers.