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Original Contribution

THE POTENTIAL ROLE OF NEOPTERIN AS A BIOMARKER FOR SILICOSIS

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ABSTRACT

Human macrophages and dendritic cells produce Neopterin derivates after stimulation with interferon-γ and serves as a marker of activated cell-mediated immune response. Aim: Assessment of serum neopterin levels in patients with different X-ray chances about silicosis. Methods: The serum neopterin concentrations were measured using ELISA in 60 patients with silicosis, according to conventional X-ray observation (ILO, 2002) and 16 healthy donors. Results: The serum neopterin levels (2,74±1.12 ng/ml) were significantly higher ($p<0.0005$) in comparison with the control group (1,56±0,39 ng/ml), without significant differences between serum neopterin levels in the three groups (First group-2,53±0,99 ng/ml; Second group-2,66±1,42 ng/ml and Third group-3.07±1,08 ng/ml). Conclusions: The increased serum neopterin concentration could be used as a marker for silicosis.

Key Words: silicosis, neopterin

INTRODUCTION

Pneumoconioses are professional pulmonary diseases caused by inhaling of non-organic dust particles The most common pneumoconiose, particularly for the countries in Asia, Africa, South America and Eastern Europe, is silicosis. According to Petrova, (2004), the number of workers threatened with silicosis in Bulgaria for the period 1985-2000 varies between 11 238 and 24 011, and the number of people suffering from silicosis for the same period – between 4 566 and 5 472 (1).

The main cause of the development of silicosis is the free crystalline silicon dioxide. The key role of macrophages in the pathogenesis of the disease has been shown in experimental studies. Mediators (cytokines and hemokines) released from the activated macrophages lead to persistent inflammatory infiltration and pulmonary fibrosis (2, 3, 4). The participation of macrophages as a basic component of the T-cell immune response leads to stimulation of the fibroblasts and collagen formation. This fact explains the late forms of silicosis, diagnosed after a differently long dust-free period (5, 6). The activated macrophages are a source of superoxide radicals and cytokines (IL-1, TNF-α, neopterin, leucotriens), which affect the T-helper cells (7, 8, 9, 10, 11). The participation of cell-mediated immune reactions is linked to the release of proinflammatory cytokines and γ-interferon from the T-lymphocytes (**Figure 1**).

Neopterine is regarded as an early biomarker of the cellular immune response. It is a low-molecular-mass compound belonging to the class of pteridines and a metabolite of guanosine triphosphate, which is produced by the activated macrophages and dendritic cells after stimulation with γ-interferon (7, 8, 12). An international working group acknowledges the fact that the levels of oxidative stress, serum neopterin and TNF-α can be used as markers of the effect of exposition to silica (13).

The purpose of this study is to determine the levels of neopterin in silicosis patients with a different type of X-ray and morphological alterations, described according to the International Classification of

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Radiographs of Pneumoconioses by the International Labour Organisation – ILO, 2002.

MATERIALS AND METHODS

Levels of neopterin were examined in serum of 60 silicosis patients aged 38 to 76 (63.90±9.57) and of a control group of 16 healthy individuals aged 46 to 80 (55.62±10.65).

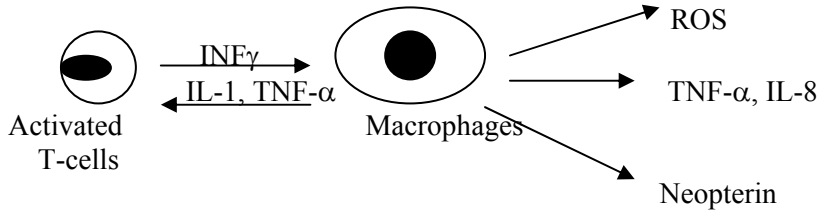

Figure 1. *Releasing of cytokines, reactive oxygen species (ROS) and neopterin by the activated macrophages*

The silicosis patients were divided into three groups according to the characteristics of the radio-morphological alterations (ILO, 2002). In the first group of 21 people the X-ray pictures showed a prevalence of unevenly spread striped shadows (s, t, u) and single oval shadows sized up to 1,5 mm (p). The second group included 23 patients with X-ray alterations composed mainly of oval shadows with size to 1,5 mm (p), from 1,5 to 3 mm (q) and above 3 mm (r). The third group comprised individuals with X-ray evidence for progressive massive fibrosis of type A and B. The control group consisted of 16 clinically healthy individuals not exposed to dust.

Each subject was given a questionnaire, which included information about age, occupational history, health status and bad habits like smoking.

Serum neopterin levels were determined by ELISA, kit DRG Diagnostics, Germany in ng/ml.

Basic Statistics and Table for Windows were used for statistical evaluation of the results.

RESULTS

Table 1 shows some characteristics of the studied silicosis patients and the control group. All of the silicosis patients have anamnestic data of dyspnoea, increasing at physical effort, dry cough without expectoration and physical examination data of different levels of bronchial obstruction. Second clinical degree of arterial hypertension is the only accompanying disease, which is treated with ACE inhibitors and/or Ca channel blockers. Individuals with autoimmune diseases, such as rheumatoid arthritis, lupus erythematodes disseminatus, sclerodermia, diabetes mellitus, Graves' disease, etc. are excluded.

Table 1. *Some characteristics of the studied patients with silicosis and the control group*

Groups	***Age (years)***	***Exposition (years)***	***Latent period (years)***	***Serum neopterin ng/ml***
First group n=21	59,19±10,05	16,5±8,68	9,17±4,82	2,53±0.99* (p<0.001)
Second group n=23	61,91±8,42	16,86±7,47	8,62±7,76	2,66±1,42* (p<0.006)
Third group n=16	72,25±3,83** (p<0.02)	12,31±7,67	8,50±6,40	3,07±1.08* (p<0.0001)
Control group n=16	55,62±10,65	-	-	1,56±0.39

* - *significant difference from the control group (p<0.05)*
** - *significant difference from other groups (p<0.05)*

The results of the studied serum neopterin are shown in **Figure 2**. It was found that serum neopterin levels (2.74±1.12 ng/ml) in silicosis patients were significantly higher than in

controls (1.56±0.39 ng/ml, $p<0.05$). There was no significant difference in serum neopterin levels between the groups with different X-ray and morphological characteristics of silicosis: in the first group – 2.53±0.99 ng/mL, in the second group – 2.66±1.42 ng/mL and in the third group – 3.07±1.08 ng/mL ($p>0.05$). The average group level of neopterin in all three groups of silicosis patients was significantly higher than in the control group (**Figure 3**).

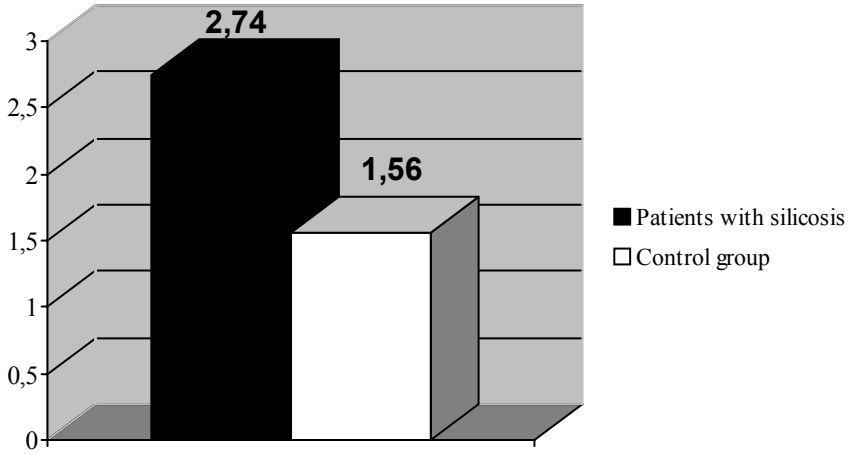

Figure 2. *Serum neopterin levels in silicosis patients and in controls*

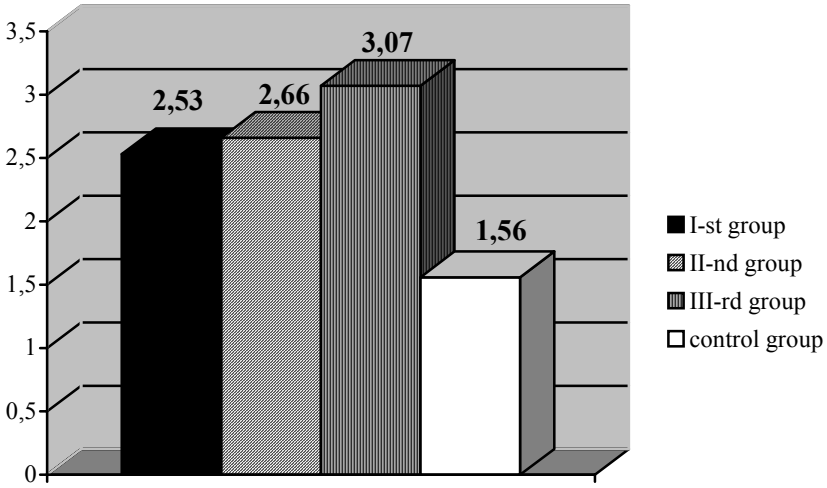

Figure 3. *Mean group levels of neopterin in silicosis patients with different X-ray alterations according to ILO, 2002.*

DISCUSSIOIN

Inhalation of free crystalline silica (SiO_2) causes lesions in the lungs of exposed individuals as well as in the lungs of experimentally exposed animals. The threshold limit value for the fine (respirable) fraction of SiO_2 is 0.07 mg/m^3. Dust particles sized from 0.5 to 5-7μm possess an outstanding fibrogenic effect because they reach the alveoli and are consumed by the alveolar macrophages and trigger mainly cellular immune response.

Watcher et al. (1989) find out that neopterin is eliminated by the urinary tract and its levels can be used to determine the cellular immune activity with no typical clinical symptoms present (10). The increased serum neopterin level in the silicosis patients compared to the control group, noticed by us, confirms the role of the cellular immune response and the persisting macrophage activation in the pathogenesis of the disease (**Figure 1**).

Widely known is the combination of

the disease with rheumatoid arthritis (Caplan's syndrome), systematic lupus erythematodes diseminatus, progressive system sclerosis and pemfigoid bullosus (14, 15). Mahmud et al (2005) showed in their study that serum levels of tumour necrosis factor receptor II (p75) and of neopterin are more sensitive markers for active lupus erythematodes disseminatus than tumour necrosis factor-alpha, C3 and C4 (16). The study of neopterin in patients with transplants, infectious, inflammatory and malignant diseases shows the important role of the cellular immunity in those diseases (17). Prior et al. (1988) found increased levels of urinary neopterin in 69.4% of the examined sarcoidosis patients (18). The absence of systematic autoimmune diseases in our studied patients confirms the statement that increased neopterin levels are exclusively connected to the participation of cell-mediated reactions in the pathogenesis of silicosis.

Altindag et al (2003) found increased urinary and serum neopterin levels in silica-exposed workers compared to the control group (19). The authors did not specify if there were individuals with X-ray and morphological signs of silicosis or with autoimmune diseases among the studied subjects. Measuring high neopterin levels in silica-exposed workers, as well as in silicosis patients poses the question about the potential role of neopterin as a biomarker for proving silicosis.

Werner et al. (1987) suggested a referent value of serum neopterin for children under 18 years – 0.9-3.4 ng/ml, and for adults – under 2.5 ng/ml (11). During the conducted study it was found that in 53.3% of the silicosis patients the neopterin levels were above the mentioned referent values, the greatest percent being in group III – 81,2%, followed by group I – 52.3% and group II – 34,8%. The significantly higher neopterin levels in the three groups with silicosis compared to the control group levels confirm the possibility of using neopterin as a biomarker for the disease.

There was determined a tendency of increasing of the serum neopterin parallel to the increase of fibrosis alterations in the pulmonary parenchyma (**Fig. 3**). The highest level was in group III, where massive fibrosis of type A and B was formed. Second was the neopterin level in group II, where X-ray alterations in the shape of nodular shadows sized p, q and r prevailed. The lowest neopterin levels were in group I, consisting of silicosis patients with unevenly spread shadows s, t and u (ILO, 2002). The lack of significant difference between neopterin levels in the three groups of silicosis patients indicates that the macrophageal activation is constant, while the observed X-ray and morphological changes in the lungs are probably due to the additive effect of the activated cellular immune response. No evidence of connection between serum neopterin levels and the age of the studied individuals was found. This result does not correspond with that of Frick et al. (2004), who, while studying 43 healthy individuals (21 female and 22 male) discovered an increase in neopterin and homocystein levels with age (20) and related the results to immune activation of the T-cells and the macrophageal system.

CONCLUSION

The increased neopterin levels in silicosis patients confirm the possibility of its use as an early biomarker of cellular immune response activity and the participation of macrophage in the pathogenesis of the disease. The concentration of serum neopterin could be used in the diagnostic criteria of the disease, together with the typical radio-morphological changes.

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