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PRACA ORYGINALNA

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Clara Cell protein and myeloperoxidase levels in serum of subjects after exposure to fire smoke

Stężenie białka Clara i mieloperoksydazy w surowicy osób narażonych na dymy pożarowe

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Abstract

Introduction. Fire smoke inhalation is a well-recognized aetiological factor of airway injuries. The objective of this study was evaluation of Clara cell protein (CC16) and myeloperoxidase (MPO) concentrations in serum of patients after exposure to uncontrolled fire smoke.

Methods. The study group consisted of 40 consecutive patients admitted to the Toxicology Unit after exposure to fire smoke. CC16 and MPO concentrations in their serum samples was measured on the day of admission to hospital and rechecked at the 2nd day and on the day of discharge. Patients also underwent routine toxicological diagnostic procedures applied in case of exposures, such as carboxyhaemoglobin (COHb) levels and blood lactate and urinary thiocyanate concentrations. The same diagnostic tests were performed in the control group consisting of 10 healthy subjects not exposed to fire smoke.

Results. The average concentration of CC16 in the serum of subjects exposed to toxic factors was significantly higher at the day of admission in comparison with the respective values recorded on the 2nd day and on the day of discharge. The mean level of CC16 in the serum of the exposed group was also significantly higher than that in the control group. Tests for MPO concentrations in the serum did not reveal any significant changes in patients exposed to fire smoke.

Conclusions. As indicated, acute exposure to smoke induces injury at the alveolar level, which results in a transient increase of CC16 in serum of exposed subjects.

Key words: Clara cell protein, myeloperoxidase, lung toxicity, fire, firefighters

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Streszczenie

Wstęp. Narażenie na dymy pożarowe jest udokumentowaną przyczyną toksycznego uszkodzenia układu oddechowego. Celem badania była ocena stężeń bialka Clara i mieloperoksydazy w surowicy osób narażonych na dymy pożarowe.

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Metody. Grupę badaną stanowiło 40 osób narażonych na dymy pożarowe, które zostały przyjęte na oddział toksykologii. Stężenia białka Clara i mieloperoksydazy oznaczono w dniu przyjęcia do szpitala oraz ponownie w drugim i ostatnim dniu hospitalizacji. U pacjentów wykonano także badania toksykologiczne zlecane przy tego typu narażeniu: stężenie karboksyhemoglobiny i stężenie mleczanów we krwi oraz stężenie rodanków w moczu. Podobny panel badań zlecono u 10 zdrowych osób (grupa kontrolna) nienarażonych na dymy pożarowe.
Wyniki. Stężenie białka Clara było istotnie statystycznie wyższe w surowicy pacjentów narażonych na dymy pożarowe w dniu ich przyjęcia do szpitala w porównaniu z wartością tego parametru ocenianego w drugim i ostatnim dniu hospitalizacji. Stężenie białka Clara było również istotnie wyższe w surowicy osób narażonych w porównaniu z grupą kontrolną. Nie obserwowano istotnych zmian steżenia MPO w surowicy osób eksponowanych na dymy pożarowe.
Wnioski. Narażenie na dymy pożarowe może prowadzić do uszkodzenia pęcherzyków płucnych, a tym samym do wzrostu w surowicy osób narażonych stężenia białka Clara uwalnianego przez te komórki.

Słowa kluczowe: białko Clara, mieloperoksydaza, uszkodzenie płuc, pożar, strażacy

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Introduction

Exposure to the variety of agents emitted during fire accidents may result in acute injuries of airways. The major chemicals released during combustion and pyrolysis processes are: nitrogen and sulphur dioxides, aldehydes, carbon monoxide (CO), and halogenated hydrocarbons [1]. Some of them can exhibit direct toxicity to the respiratory tract. A study evaluating respiratory symptoms in a group of firefighters showed a higher intensity of symptoms after prolonged contact with fire smoke [2]. Such exposure may lead to the development of inflammation of upper and lower airways. Gaughan et al. [3] found that respiratory symptoms or changes in the results of lung function tests observed in subjects involved in firefighting were associated with increased myeloperoxidase (MPO) and eosinophilic cationic protein (ECP) levels in sputum and nasal lavage fluid. These data confirm the important role of neutrophil inflammation and the neutrophil-related enzyme in the pathogenesis of acute upper and lower respiratory symptoms in this group.

The role of CC16 as a protein marker of severity of airway injuries after exposure to irritants is well documented [4]. This protein, known also under other names like uteroglobine in rabbits, is produced in humans mainly by Clara cells localised in terminal bronchioles; however, some studies have shown that this protein may be synthesised by non-ciliated cells along the tracheobronchial epithelium [5] and in the urogenital tract, particularly in the prostate [5]. Although this protein may be secreted by the male urogenital tract, the lung seems to be the predominant, if not the exclusive source of serum CC16. The CC16 plays both an immunosuppressive and anti-inflammatory role in the lung. It also prevents degradation of lung surfactant phospholipids and inhibits production of interferon-g [6]. CC16 is also thought to participate in the detoxication of some substances deposited in the respiratory tract [6].

The question addressed by the present study was whether the extent of acute respiratory injury caused by acute exposure to fire smoke corresponds with alterations in serum CC16 and MPO levels.

Material and methods

Subjects and exposure

Forty consecutive patients from a large town, immediately after accidental exposure to fire smoke, were admitted to the Toxicology Unit between January and December 2009. The main source of exposure was uncontrolled fire in dwelling house or flat, resulting from careless handling of stoves, cigarettes, or charcoal grills. Patients spent at least 15–20 minutes in the atmosphere containing a mixture of harmful gases and fumes produced by the combustion and pyrolysis of some plastics, wood, fabric, and other substrates. The average age in the group of patients exposed to harmful fumes and gases released during fire smoke was 49.75 ± 16.48 years. Sixteen of them (40%) were smokers at the time of study or in the past.

The control group consisted of 10 healthy individuals.

The Regional Bioethical Committee approved the study protocol and all the participants submitted their written consent prior to the study.

Clinical symptoms

Each patient was examined for the presence and intensity of the following symptoms; cough, wheeze, sputum production, and shortness of breath on exertion, as well as ocular, nasal, and pharyngeal symptoms. Additionally, the following symptoms and signs of poisoning with carbon

monoxide were analysed: headache, dizziness, chest pain, syncope, nausea and vomiting, confusion or agitation, stupor or coma, and seizures (data not shown).

Laboratory tests

Carboxyhaemoglobin (COHb) levels, arterial blood gases (ABG), Full Blood Count (FBC), urinary thiocyanate levels, and serum lactate concentrations in serum were evaluated on admission to the hospital. Haemoglobin levels, White Blood Cell and Red Blood Cell (WBC and RBC) counts, lactate, pH, pO_2, pCO_2, BE, and thiocyanate levels were used for further analysis.

Clara cell protein determination

CC16 concentration in serum was measured using a latex immunoassay [7] with the specific rabbit antibody against CC16 protein 1 (Dako A/S, Denmark). The serum samples were pre-treated by heating for 30 minutes at 56°C and addition of polyethylene glycol 600 (16%, v/v 1/1) and trichloroacetic acid (10%, v/v, 1/40) to eliminate possible interference (complement, chylomicrons). The samples were centrifuged after sedimentation for 10 minutes at 200 g and CC16 was determined in the supernatant.

Myeloperoxidase determination

MPO in serum was measured using commercially available enzyme-linked immunosorbent assay (ELISA) [Quantikine human MPO immunoassay; R@D Systems, Minneapolis, MN].

Principle of the assay

The assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for MPO was precoated into a microplate. Standards and samples are pipetted into the wells, and any MPO present was bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for MPO was added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution was added to the wells and colour developed in proportion to the amount of MPO bound in the initial step. The colour development was stopped and the intensity of the colour was measured.

Statistical analysis

Results are expressed as mean values ± SD

Results of physical examination and toxicological and biochemical tests on the 1st day of hospitalization in the group of subjects exposed to toxic substances and the control group were compared using the Mann-Whitney test.

The results of tests for CC16 and MPO performed on the 1st and 2nd days and on the day of discharge in the group of subjects exposed to fire were compared with those obtained in the control group using Tamhane's test .

Pearson's correlation coefficient was applied to evaluate the relationship between CC16 concentration and arterial blood gas analysis in the group of subjects exposed to fire.

A p value < 0.05 was considered as significant.

Results

Twenty-eight patients (70%) complained of at least one pathologic symptom associated with the exposure. The most frequently observed problems on admission in this group included symptoms of lower airways, reported by 21 patients (52.5% of the studied cohort). These included dyspnoea, which was noted in 21 cases (52.5%), and cough in 12 cases (30%). Nasal or pharyngeal symptoms were recorded in 14 (35%) cases and symptoms associated with conjunctivitis in 9 (22.5%) cases (Table 1).

The average level of COHb in the studied group constituted 6.77 ± 6.37% of total haemoglobin, whereas in the control group it was 1.63 ± 0.57%, respectively. The mean concentrations of thiocyanates and lactates in the group of hospitalized patients were 5.84 ± 7.12 mg/l and 2.05 ± 2.84 mmol/l, respectively (Table 2).

COHb levels and thiocyanate concentrations were significantly higher in the group of patients exposed to toxic gases and fumes compared to the controls ($p < 0.05$) (Table 2).

The highest concentration of CC16 in the examined group, 18.61 ± 11.84 mg/l, was noted on the admission day (the 1st day of hospitalization). It tended to decrease gradually to 16.70 ± 10.58 mg/l on the second day of hospitalization and subsequently 13.41 ± 8.27 mg/l on the day of discharge (Figure 3).

The average concentration of CC16 in the control group was 10.67 ± 2.08 mg/l, and was significantly lower ($p < 0.05$) than the concentration recorded on the first day of hospitalization in the serum of patients exposed to toxic substances. Likewise, the concentration of CC16 in the serum of 28 symptomatic subjects from the exposed group was significantly higher ($p < 0.05$) than in the controls (Figure 3).

Mean serum MPO levels in the examined group did not significantly change throughout their stay at the hospital. Mean serum MPO levels in the

Table 1. Symptoms reported by subjects exposed to toxic substances released during uncontrolled fire

Tabela 1. Objawy zgłaszane przez osoby narażone na substancje toksyczne uwalniane podczas pożaru

Symptoms/*Objawy*	**Number/% of the group *Liczba/% badanej grupy* Total group/cała grupa n = 40 (100%)**
Symptomatic subjects (positive for at least one symptom associated with exposure) *Osoby zgłaszające objawy (co najmniej jeden objaw związany z narażeniem)*	28 (70%)
Ocular symptoms/*objawy oczne*	9 (22.5%)
Upper airways (nasal or pharyngeal) symptoms/*górne drogi oddechowe (objawy ze strony nosa lub gardła)*	14 (35%)
Lower airways symptoms/total/*objawy ze strony dolnych dróg oddechowych/wszystkie zgłaszne*	21 (52.5%)
Dyspnoea/*duszność*/Cough/*kaszel*	12 (30%)

n — number of subjects/*liczba osób*

Table 2. Results of physical examination and toxicological and biochemical tests at rest (the 1st day of hospitalization) in the group of subjects exposed to toxic substances released during uncontrolled fire and the control group

Tabela 2. Wyniki badania przedmiotowego, badań toksykologicznych i biochemicznych w grupie osób narażonych na substancje toksyczne uwalniane w trakcie pożaru w pierwszym dniu hospitalizacji oraz w grupie kontrolnej

Parameter analysed Analizowany parametr	**Subjects exposed to fire smoke *Osoby narażone na dymy pożarowe* n = 40 Mean ± SD *Średnia ± SD***	**Control group *Grupa kontrolna* n = 10 Mean ± SD *Średnia ± SD***	**P value***
Heart rate/*częstość pracy serca*	90.10 ± 16.32	82.70 ± 11.93	0.138
Systolic blood pressure/*skurczowe ciśnienie tętnicze* [mm Hg]	130.63 ± 27.20	119.00 ± 9.36	0.022*
Diastolic blood pressure/*rozkurczowe ciśnienie tętnicze* [mm Hg]			
COHbg%/*karboksyhemoglobina* [g%]	6.77 ± 6.37	1.63 ± 0.57	0.001*
Thiocyanates/*rodanki* [mg/l]	5.84 ± 7.12	0.00	0.001*
Lactates/*mleczany mmol/l* [0,5–2,2 N]	2.05 ± 2.84	1.55 ± 0.46	0.858
pH [7.35–7.45]	7.38 ± 0.07	7.41 ± 0.02	0.102
PO_2 [80.0–100.0 mm Hg]	72.94 ± 3.95	61.53 ± 22.33	0.264
pCO_2 [35.0–45.0 mmHg]	36.51 ± 8.99	37.97 ± 3.62	0.113
BE [mmol/l]	–2.89 ± 2.29	–0.96 ± 1.39	0.002*
Hgb [12–17g/dl]	14.0 ± 1.51	15.81 ± 1.04	0.000*
WBC [4.0–10.0 × 10^3/ml]	9.05 ± 2.72	8.26 ± 2.08	0.574
RBC [3.9–5.5 × 10^3/ml]	4.35 ± 0.51	5.18 ± 0.36	0.000*

n — number of subjects/*liczba osób*
*significant < 0.05 compared to control

hospitalized patients did not differ significantly from those found in the serum of the controls (Figure 4).

The study revealed a strong correlation between concentrations of CC16 on the first day of hospitalization and levels on the 2nd day and the day of discharge (Table 3).

Concentrations of CC16 in the serum of subjects exposed to toxic agents measured on the 2nd day showed positive correlation with levels on the 1st day and also with partial pressure of carbon dioxide (pCO_2) checked on the 1st day (Table 3).

Concentrations of CC16 measured on the day of discharge in the studied group displayed positive correlation with such parameters from the 1st day as CC16 concentration and pCO_2 and also with CC16 concentration on the 2nd day (Table 3).

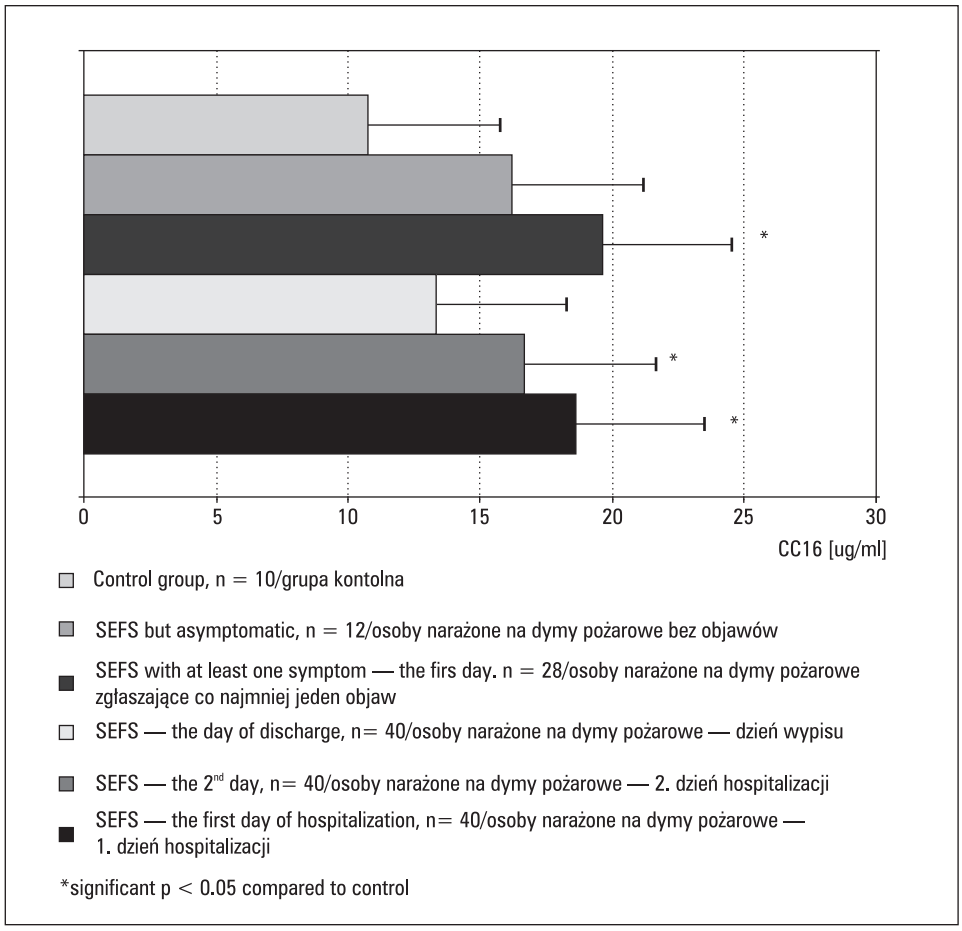

Figure 3. The concentration of CC16 in the serum of subjects exposed to fire smoke (SEFS) and in the controls

Rycina 3. Stężenie CC16 w surowicy osób narażonych na dymy pożarowe oraz w grupie kontrolnej

Discussion

A number of studies have dealt with the search for diagnostic tools measuring non-invasively the extent of lung inflammation during recent decades [8, 9]. Such works evaluated the integrity of the pulmonary epithelium from increased serum concentration of proteins synthesized and physiologically detected mainly within the respiratory tract, e.g. CC16 [10]. This protein, secreted by the respiratory epithelium, increasingly appears to attenuate the inflammatory response within the respiratory tract [11]. This is produced mainly by Clara cells, which are localized mainly in the proximal or central portion of the pulmonary acinus, at the junction between the conducting airways and the exchange area [12]. CC16 inhibits both monocyte and polymorphonuclear neutrophil chemotaxis and phagocytosis *in vitro* [12–14]. The determination of CC16 in serum is a sensitive marker for detection of early changes in the lung/ /epithelial barrier permeability and/or evaluation of the integrity of Clara cells that constitute a particular target for many pneumotoxicans, as has been shown in various clinical and laboratory studies [15].

The highest observed concentration of CC 16 in our study was detected on the admission day in the group of subjects exposed to fire, and it tended to decrease gradually during the hospital stay. The average concentration of CC16 in the control group was significantly lower than that measured on the first day of hospitalization both in the investigated group and in 28 symptomatic subjects. The study additionally revealed a strong correlation between concentrations of CC16 on the first day of hospitalization and its levels on the 2nd day and the day of discharge. Concentrations of CC16 in the serum of subjects exposed to toxic agents, measured on the 2nd day, showed positive correlation with levels checked on the 1st day and pCO_2 on the arterial blood gases test also measured on the 1st day.

The increased concentration of CC16 in our study directly after the exposure to fire smoke may be explained by increased airways permeability, which resulted from exposure to the toxic agents

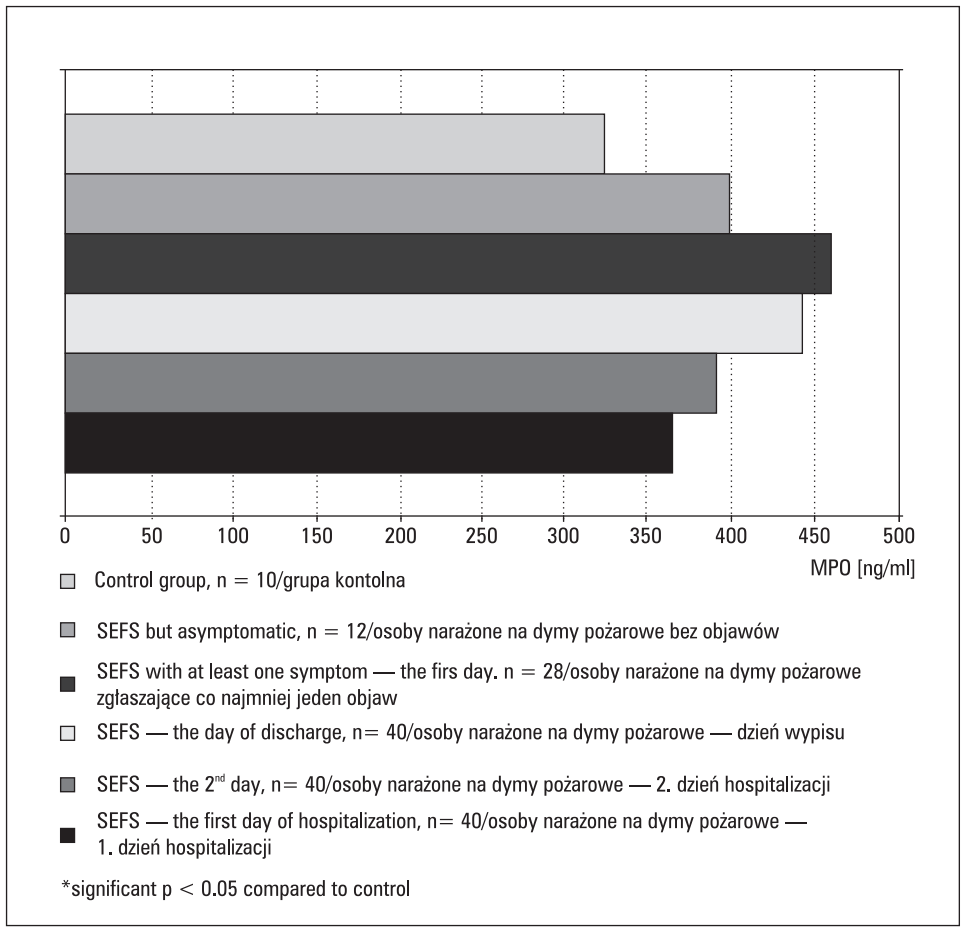

Figure 4. The concentration of myeloperoxidase in the serum of subjects exposed to fire smoke (SEFS) and in the controls

Rycina 4. Stężenie MPO w surowicy osób narażonych na dymy pożarowe oraz w grupie kontrolnej

Table 3. Correlation between CC 16 and pO_2, pCO_2 indicators in the subjects exposed to toxic substances released during uncontrolled fire

Tabela 3. Zależność pomiędzy CC16 a pO_2, pCO_2 w grupie osób narażonych na substancje toksyczne uwalniane w trakcie pożaru

Analysed parameter *Analizowany parametr*	**CC6 mg/l 1st day** *Pierwszy dzień*	**CC16 mg/l 2nd day** *Drugi dzień*	**CC16 mg/l day of discharge** *Dzień wypisu*	**PO_2 [mm Hg]**	**PCO_2 [mm Hg]**
CC6 mg/l 1st day	1.000	$R_S = 0.742$ $p = 0.000^*$	$R_S = 0.471$ $p = 0.002^*$	$R_S = –0.017$ $p = 0.919$	$R_S = 0.157$ $p = 0.334$
CC16 mg/l 2nd day	$R_S = 0.742$ $p = 0.000^*$	1.000	$R_S = 0.698$ $p = 0000^*$	$R_S = –0.216$ $p = 0.181$	$R_S = 0.335$ $p = 0.035^*$
CC16 mg/l day of discharge	$R_S = 0.471$ $p = 0.002^*$	$R_S = 0.698$ $p = 0.000^*$	1.000	$R_S = -0.062$ $p = 0.153$	$R_S = 0.329$ $p = 0.038^*$
PO_2 [mm Hg]	$R_S = –0.017$ $p = 0.919$	$R_S = –0.216$ $p = 0.181$	$R_S = –0.062$ $p = 0.702$	1.000	$R_S = –0.298$ $p = 0.062$
PCO_2 [mm Hg]	$R_S = 0.157$ $p = 0.334$	$R_S = 0.335$ $p = 0.035^*$	$R_S = 0.329$ $p = 0.038^*$	$R_S = –0.298$ $p = 0.062$	1.000

*significant $p < 0.05$ compared to control

emitted during the fire. Bernard et al. [16] recorded a transient increase of serum CC16 in the samples from firefighters after 20 minutes of smoke inhalation. What is important, this change was found in the absence of any functional signs of lung impairment. Other authors also found serum CC16 to be highly sensitive to lung epithelium injury in a study performed on cyclists exposed to ambient O_3 [17].

Similarly, an acute exposure of mice and rats to O_3 produced a transient dose-dependent elevation of CC16 in serum, which also paralleled the elevation of albumin in bronchoalveolar lavage [11].

Mean serum MPO concentrations in our study did not significantly change in the group of subjects exposed to fire smoke during their stay in the hospital and also did not differ significantly from the concentrations measured in the serum of the controls. The highest concentration of MPO was observed in the serum of 28 hospitalized symptomatic patients at admission; however, these concentrations did not differ significantly to those measured subsequently throughout their hospital stay. These data indicate that inhalation of fire smoke may be responsible for the development of an inflammatory response, which is characterized by neutrophil recruitment to the lower respiratory tract and release of MPO. This glycoprotein synthesised by neutrophils and monocytes catalyses the hydrogen peroxide-dependent formation of reactive species [18] and therefore plays an important role in pulmonary inflammation. Conversely to our data, Gaughan at al. [3] found a rise in MPO concentrations in sputum and nasal lavage fluid of firefighters showing respiratory symptoms.

The most frequent complaints on admission in the group of subjects exposed to fire included lower airway symptoms such as dyspnoea and cough. Gaughan et al. [3] described a relationship between respiratory symptoms resulting from exposure to toxic substances emitted during the fire in a group of firefighters with a decrease in forced expiratory volume in one second (FEV_1).

The irritants released during the fire may induce increased airway resistance, by nerve stimulation and by release of histamine, with consequent respiratory symptoms, e.g. coughing, wheezing, shortness of breath, and changes in pulmonary function [19, 20].

In our study we concentrated on the pathophysiological effects of irritant gases; therefore, toxicity of carbon monoxide and cyanides was not the main subject of the study. Moreover, we did not observe a predominant pattern of cyanide or carbon monoxide toxicity in the examined group. These two gases are released during uncontrolled fire in varying concentrations depending on the substrates undergoing combustion, and they may contribute to the overall toxicity of fire smoke [1]. The group of patients exposed to toxic gases and fumes showed significantly higher COHb levels and thiocyanate concentrations on admission compared with the control group. COHb formation is a well-recognized effect of exposure to carbon monoxide [21]. Increased levels of this form of haemoglobin indicate that the possibility of carbon monoxide poisoning should always be considered after exposure to fire smoke, and COHb levels should be routinely checked in such cases. On the other hand, relatively low levels of COHb and no clinical signs of carbon monoxide poisoning may suggest that in our group this kind of exposure did not play an important role.

A similar question should be raised about the contribution of cyanide toxicity. Direct measurement of cyanide concentration in blood is not feasible; therefore, determination of urinary thiocyanates (cyanide metabolites) has become a useful diagnostic tool in this kind of poisoning [22]. Statistically significant differences in thiocyanate levels between the studied group and the controls point to obvious exposure to those toxins.

Conclusions

Acute exposure to smoke results in an increase of CC16 in serum of exposed subjects. Serum CC16 seems to be an important marker for detection of acute airway injuries caused by smoke.

Conflict of interest

Authors declare no conflict of interest.

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