

Original Research

Environmental Exposure to Lead Could Enhance Susceptibility to Oxidative Stress in Patients with Pollenosis

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Abstract

The parameters of oxidative stress (MDA, SOD, GPx, H₂O₂, GSH) and metals concentration were measured on a pollen-allergic sample (64 people) and the correlation between parameters were examined. There were no differences in parameters between pollenotics and control (35 people) except for the level of reduced glutathione which was statistically significant lower at pollenotics. There were no differences in the level of metals in blood or hair. The statistically significant correlation between Pb and SOD ($r = 0.74$ $p = 0.000$) at pollenotics suggests that environmental exposure to lead could stimulate the intensity of hay fever symptoms connected with free radicals processes.

Keywords: environmental pollution, oxidative stress, pollen allergy, malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione (GSH), total peroxides, lead (Pb), nickel (Ni)

Introduction

A serious rise in the prevalence of allergic diseases, particularly pollen allergy, prompted us to conduct research on the relationship between environmental factors and pollen allergy. Some authors have pointed out that pollen grains could be a carrier of environmental particles. They have indicated morphological abnormalities of pollen grains in industrial areas [1]. Actually, the character of environmental exposure is changing, particularly in cities where exposure to industrial emissions has decreased but exposure to automobile traffic-generated pollution has increased. Automobile-generated smog is a source of metals, aromatic hydrocarbons and other particles which can act as adjuvant-inducing pollen sensitization. It is also known that these particles can induce oxidative stress. In

our previous work the level of chosen metals in the blood of pollenotics and the oxidative stress markers were examined. It seemed interesting to continue the research in order to get more detailed evaluation of oxidative stress by measurements not only of MDA, GPx or SOD activities but also the peroxides and reduced glutathione levels. The examination of some metal levels (Pb, Ni) in hair, not only in blood; was also performed [2]. The level of metals in hair is a good marker of chronic exposure and the level of reduced glutathione could reflect exposure for environmental aromatic hydrocarbons. The biological consequence of reactive oxygen species interactions depends on metal ions which are able to catalyze important transformations in biological systems (e.g. Fenton's reaction). It has been proved that the effectiveness and direction of biological Haber-Weiss reaction depends not only on iron ions but also on Ca, Ni, Cr, and Mn, which are essential ingredients of environmental pollution [3,4]. They could

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Table 1. The comparison of some parameters at pollenotics (A) and healthy volunteers (K).

Parameter	Group/N	Medium value	Minimum	Maximum	Test U Mann-Whitney p
GPx (U/l)	A/56	6523.5	79.5	22762.8	0.908
	K/33	7551.0	3104.0	37938.1	
SOD (U/ml)	A/64	79.6	18.6	160.7	0.792
	K/35	78.8	17.4	248.6	
MDA (nmol/l)	A/64	9.55	0.9	27.3	0.217
	K/35	8.34	1.7	21.6	
ROS (μ mol/l)	A/30	290.79	21.8	823.6	0.853
	K/19	303.84	49.4	732.4	
GSH (mmol/l)	A/30	1.139	0.8	1.4	0.040
	K/19	1.289	0.8	1.7	

Group: A-pollenotics, K- healthy volunteers; N- number of examined cases; GPx- glutathione peroxydase; GSH- reduced glutathione; MDA-malondialdehyde; ROS- peroxides; SOD- superoxide dismutase; p- probability

catalyze peroxide transformation into hydroxyl radical and oxygen. So the change in metals homeostasis is an important factor in oxidative stress induction, which can be fundamental for many pathological processes. It seems that this kind of mechanism could also be involved into pollen allergy, especially that oxidative stress causes lipid peroxidation, which by damaging the mucosonal membrane helps penetrate allergens.

Experimental Procedure

Research was provided in whole blood or plasma taken from 64 pollenotics being under control of Allergy Clinic, and 35 healthy volunteers. The group of pollenotics consists of 34 women and 30 men. The medium lasting time of disease was 10.29 years (1- 40 years). The studies were approved by the Ethics Committee of the University of Medicine in Wrocław. The patients signed the agreement according to the ethics board. The median age was 28 years (14-57 years). The group of healthy volunteers consists of 24 women and 11 men. The median age was 25.1 years (21-42 years). The research was realized during a period from April to May. Material was prepared and stored in accordance with requirement of the kits.

Malondialdehyde concentration was measured in plasma-EDTA by colorimetric method with tiobarbituric acid according to Wills et al. [5], total peroxides level in plasma-EDTA by OxyStat test from BIOMEDICA at 450 nm [6].

Glutathione was evaluated spectrophotometrically in erythrocytes by BIOXYTECH GSH-400 kit from OXIS [6, 7].

SOD activity [U/ml] was measured in heparinized blood by kinetic method based on inhibition reaction by

McMurray [8], (RANDOKS-RANSOD kit). GPx activity [U/ml] was measured with kinetic method based on the decrease of absorbance at 340 nm, caused by oxidation and reduction of glutathione, according to Paglia and Valentine [9, 10] (RANDOX-RANSEL kit).

Nickel (Ni) in serum and lead (Pb) in blood heparinized were evaluated spectrophotometrically using electrothermal atomic absorption spectrometry (EAAS). Nickel and lead in hair using the plasma spectrometer with mass detection ICP-MS controlled by computer co-operating with analytical system Ultra Mass 700.

The statistical evaluation of results was done with the parametric Student's t-test for the independent variables with normal factoring (GPx, MDA, Pb, GSH, RFT) and U Mann-Whitney test for variables with abnormal factoring (SOD, Ni). The correlation coefficients also were evaluated.

Results

The MDA level in plasma, evaluated in pollenotics (A) (9.55 nmol/l) in comparison to the control K (8.34 nmol/l), wasn't changed statistically significantly (Table 1).

There also was no significant difference in the amount of peroxides in pollenotics plasma in comparison to the control (290.79 μ mol/l pollenotics; 303.84 μ mol/l control) (Table 1).

The statistically significant difference ($p=0.04$) was noted in the level of reduced glutathione, between pollenotics (A) and control (K). The glutathione concentration was lower in A group (1.139 mmol/l) than in the control (1.289 mmol/l) (Table 1). The medium concentration of reduced glutathione in pollenotics was 17.72% lower than in healthy volunteers.

Table 2. A comparison of metal concentrations in blood pollenotics (A) and healthy volunteers (K).

Parameter	Group / N	Medium value	Minimum	Maximum	Test U Mann-Whitney p
Pb ($\mu\text{g}/100\text{ml}$)	A/64	59.2	18.6	148.9	0.817
	K/35	55.4	27.9	123.7	
Cr ($\mu\text{g}/\text{l}$)	A/34	3.6	1.8	5.0	0.723
	K/16	3.5	2.0	5.2	
Zn ($\mu\text{g}/\text{l}$)	A/34	920.85	673.0	1119.0	0.603
	K/16	909.75	744.0	1092.0	
Ni ($\mu\text{g}/\text{l}$)	A/64	3.6	1.1	43.8	0.371
	K/35	2.7	1.2	6.3	
Mg (mg/dl)	A/34	2.3	2.0	2.6	0.350
	K/16	2.2	1.9	2.5	
Cd ($\mu\text{g}/\text{l}$)	A/34	0.39	1.1	1.0	0.126
	K/16	0.55	0.2	1.7	

Group: A-pollenotics, K-healthy volunteers; N-number of examined cases; Cd-cadmium; Cr- chromium; Mg-magnesium; Ni- nickel; Pb-lead; Zn- zinc; p- probability.

Activity of SOD and GPx enzymes was in the normal range in pollenotics and healthy volunteers. The medium value wasn't significantly different. Pollenotic SOD activity was slightly higher (79.6 U/ml), but not significantly more so than in control (78.8 U/ml) (Table 1). The GPx activity in pollenotics was lower (6523.5 U/l) than in control (7551.0 U/l), but the differences weren't statistically significant (Table 1).

Also, the differences in concentration of nickel in blood of pollenotics and healthy volunteers weren't statistically significant. Slightly higher levels, not significantly, of Pb, Ni, Zn and Cr was observed in pollenotics in comparison to the control. The level of Cd and Mg was similar in both groups (Table 2).

The level of Pb and Ni was also evaluated in hair. The medium value of the concentration of Pb in hair in pollenotics was higher (1.36 ppm) than in control (1.29 ppm), but not statistically significant. Also, the concentration of Ni in hair in pollenotics was higher (1.33 ppm) than in control (1.15 ppm), but also not statistically significant.

The analysis of correlation gives interesting information. The study points at statistically significant correlation between the concentration of Pb in blood and SOD activity at pollenotics ($r=0.7464$; $p=0.000$) (Fig. 1). The correlations between SOD and GPx ($r=0.339$; $p=0.011$) and between Ni and Zn ($r=0.3868$; $p=0.024$) were also found. The significant correlations between 3 main parameters of stress (SOD, GPx and MDA) and the level of Pb counted for the whole examined group (99 person) with the value Pb/SOD ($r=0.604$; $p=0.000$), Pb/GPx ($r=0.389$; $p=0.000$) and Pb/MDA ($p=0.039$) points at important role of Pb in oxidative stress. In the case of MDA the observed correlation with Pb is negative ($r=-0.2080$), which suggests an

additional mechanism connected maybe with direct deactivation of aldehyde by lead.

Discussion of Results

Oxidative stress is defined like a status of higher production of reactive oxygen species, especially free radi-

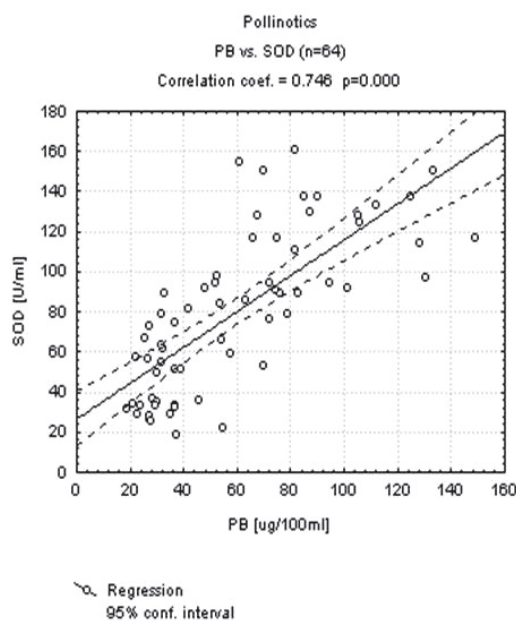


Fig. 1. The correlation between concentration of Pb and SOD activity at pollenotics.

icals. Free radicals are very reactive species and could damage naturally occurred molecules. One of the oxidative stress evaluation methods is based on the measurement of products formed during free radical transformation of natural substances, e.g. malondialdehyde as lipid's peroxidation product. Lipid peroxidation is initiated by detaching a hydrogen atom from a polyunsaturated fatty acid molecule. This process is induced easily by hydroxyl, alkyl or peroxide radicals but superoxide anion isn't able to do it. Oxidative stress generally causes the stimulation of an antioxidative barrier, especially main enzymes, like superoxide dismutase, which catalyze the dismutation reaction of superoxide anion radical to hydroperoxide and water. Glutathione peroxidase also plays an important role in catalyzing the reduction of hydroperoxide to water in the presence of glutathione. The level of hydroperoxide formation is an important oxidative stress indicator [11].

All these parameters (MDA, SOD, GPx, H_2O_2) did not show significant differences between pollenotics and control. There is only a statistically significant decrease of reduced glutathione amount in pollenotics. The thiol groups of endogenous molecules and glutathione are very sensitive on oxidative stress and the level of glutathione could be decreased as a consequence of oxidative stress. Glutathione plays the role of red-ox buffer in the body and is an important antioxidant. Its thiol group easily reacts with free radicals, particularly the hydroxide radical. Also metals, especially lead and nickel, could decrease the level of reduced glutathione by blocking its sulphhydryl group. We did not, however, find the statistically important differences in concentration of metals (Pb, Ni, Cr, Mg, Zn, Cd) in blood pollenotics in comparison with the control; the statistically significant correlation between concentration of Pb in blood and SOD activity was observed ($r = 0.74$ $p = 0.000$) as in the previous study. Many reports indicate the important role of Pb in oxidative stress generation. The examination of people exposed to lead in industry shows a positive correlation between concentrations of Pb, MDA levels and SOD activity [12]. Ding and Co. have shown that Pb increases hydroxyl radical generation [13] and others have proven that chronic lead intoxication causes oxidative stress in rat brains [14]. Also in our study, correlation between concentrations of Pb in blood and every basic parameters of oxidative stress was noted. The significant positive correlation between Pb and SOD in pollenotics, compared with the lack of correlation in control, could indicate on additional effect of lead in pollenotics. It seems that environmental lead could stimulate the intensity of hay fever symptoms connected with the free radicals process.

In the presented study we also evaluate peroxides concentration in blood of pollenotics but no significant differences have been observed between pollenotics and control. It confirms the hypothesis about less important role of peroxides in oxidative stress in pollenotics, which is also expressed with lower GPx activity. Peroxide is produced in the two-electron reduction of oxygen or one-electron reduction of peroxide anion radical. Peroxide

anion radical could produce hydroxyl radical in reactions catalyzed by metals and it seems that this is a pathway more favorable at pollenotics and a reason for the slight increase in lipid peroxidation measured as MDA levels.

Until now, there have not been reports on oxidative stress at pollenotics. Recently Matés et al. published the results of the first study on antioxidant enzymes, including TBARS level determination in allergy [15]. Research was provided on erythrocytes and mononuclear cells. Increased activity of SOD, GPx and TBARS concentrations in erythrocytes, was observed. In this study there is nothing about metals' influence and the correlation between metals and oxidative stress parameters. Although there are papers indicating a relationship between industrialization and atopy, little is known about the allergenic influence of environmental factors [16]. Fernvik in their own research on smog and pollen influence on cytokine, IgE and bronchial hyperresponsiveness (BHR) production in mice show the strongest immunological answer is exposed on both pollen and some traffic particulate matter fractions (TMP).

Conclusion

1. A statistically significant correlation between concentrations of Pb in blood pollenotics and SOD activity has been observed.
2. The lack of correlation between Pb and SOD in control group and statistically significant correlation in pollenotics suggests a synergistic influence of lead and atopic allergic reaction on oxidative stress.
3. Among various reactive oxygen species, peroxides seem to play a less significant role than other ROS in oxidative reaction at pollenotics.
4. Statistically significant differences in reduced glutathione levels in pollenotics and control group are observed.

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