J Occup Health 2018; 60: 369-375

Journal of Occupational Health

Original

The effect of subacute lead exposure on selected blood inflammatory biomarkers and angiogenetic factors

Anna Machoń-Grecka[1], Michał Dobrakowski[1], Aleksandra Kasperczyk[1], Ewa Birkner[1], Tomasz Pryzwan[1] and Sławomir Kasperczyk[1]

[1]Department of Biochemistry, School of Medicine with the Division of Dentistry, Medical University of Silesia, Zabrze, Poland

Abstract: Objectives: The aim of the study was to examine blood levels of selected pro-inflammatory cytokines, C reactive protein (CRP), and selected factors that influence angiogenesis in workers exposed to lead for a short period of time. **Methods:** The study population consisted of 36 male workers (mean age 41 ± 14 years) exposed to lead for 40 days. **Results:** The mean blood lead level (BLL) was 10.7 ± 7.67 μg/dl at the beginning of the study, and increased to 49.1 ± 14.1 μg/dl at the end of the study period. The levels of macrophage inflammatory protein 1-α (MIP-1α) were significantly higher after the studied exposure to lead compared to the baseline by 71%. Similarly, the values of CRP increased by 35%. Conversely, the values of soluble vascular endothelial growth factor receptor-1 (sVEGFR-1) and fibroblast growth factor-basic (FGF-basic) decreased by 14% and 21%, respectively. After the examined period of lead exposure, analysis of correlations showed positive correlations between vascular endothelial growth factor (VEGF) levels and the levels of interleukin 1β (IL-1β) (R = 0.39), interleukin 6 (IL-6) (R = 0.42), and MIP-1α (R = 0.54). Positive correlations were identified between MIP-1α and FGF-basic (R = 0.38), soluble angiopoietin receptor (sTie-2) (R = 0.41), and sVEGFR-1 (R = 0.47). **Discussion:** Short-term exposure to lead induces the inflammatory response; however, these mechanisms seem to be different from those observed in chronic lead exposure. Subacute exposure to lead may dysregulate angiogenesis via modifications in the levels of angiogenic factors.
(J Occup Health 2018; 60: 369-375)
doi: 10.1539/joh.2017-0307-OA

Received December 2, 2017; Accepted May 7, 2018
Published online in J-STAGE August 17, 2018
Correspondence to: A. Machoń-Grecka, Department of Biochemistry, ul. Jordana 19, 41-808 Zabrze, Poland (e-mail: anna.machon-grecka@med.sum.edu.pl)

Key words: Angiogenesis, Cytokines, Inflammation, Lead, Subacute exposure to lead

Introduction

Occupational lead (Pb) exposure occurs during primary and secondary lead smelting, processing and casting of non-ferrous metals, production and recycling of lead-acid batteries, and scraping and sanding lead paint. Over the past several decades, there has been a remarkable reduction in the environmental sources of lead. A downward trend related to the blood lead levels (BLL) of workers occupationally exposed to Pb has been noted in developed countries, such as the U.S. or the U.K. Nonetheless, a large group of workers still remain exposed to Pb[1]. In Poland in 2004-2005, 3297 employees were working in conditions of exposure to Pb at concentrations higher than the maximum allowable concentration (MAC) in the air (0.050 mg/m^3). In Poland, BLLs higher than 50 μg/dl cause employees to temporarily leave work until BLL normalizes[2].

Exposure to Pb can be detrimental to the nervous, cardiovascular, skeletal, renal, hematopoietic, and immune systems. Workplace Pb exposure has the ability to cause a pro-oxidant/antioxidant imbalance through the direct generation of reactive oxygen species (ROS)[3,4]. Excessive ROS production has been shown to impact the inflammatory response by modulating intracellular signaling pathways[5]. Several studies have looked for a link between oc-

cupational exposure to lead and the levels of pro-inflammatory biomarkers, such as interleukin 1β (IL-1β), interleukin 6 (IL-6), tumor necrosis factor α (TNF-α), and C reactive protein (CRP)[6].

Angiogenesis, the process of new blood vessel formation, is a key component of the pathological processes observed in many disorders, such as cancer, atherosclerosis or cardiovascular disease[7,8]. These disorders are also associated with occupational Pb exposure[9,10]. The impact of Pb on angiogenesis is not completely understood. Several studies suggest a close relationship between ROS and angiogenesis[11]. Furthermore, *in vitro* studies indicate the influence of Pb on growth and tube formation by cultured human umbilical vascular endothelial cells (HUVEC)[12,13]. It has been postulated that Pb promotes inflammatory processes *via* induction of the pro-inflammatory cytokines and modulates angiogenesis in chronically occupational lead exposed workers[14]. However, there are no studies on the relationship between other parameters of the immune response and angiogenic factors during short-term lead exposure. To address this issue, the present study was undertaken to examine blood levels of selected pro-inflammatory cytokines, CRP, and selected angiogenic factors in workers exposed to lead for a short period of time.

Material and Methods

Study population

Each study subject provided written consent to participate in the study. Questionnaire data on age, weight, height, Body Mass Index (BMI), work period, and smoking habits were obtained. The BLL served as a biomarker of lead exposure.

The study population consisted of 36 male workers (mean age 41 ± 14 years) exposed to lead for 40 days. Examined subjects were employed in the lead-zinc manufacturing to perform periodic maintenance of blast furnaces and production lines. Of the participants, 18 workers were occupationally exposed to lead for the first time (BLL 4.16 ± 1.62 μg/d*l*), while the other 18 workers had a history of occupational exposure to lead (BLL 16.95 ± 5.32 μg/d*l*).

The experimental set-up was approved by the Bioethics Committee of the Medical University of Silesia in Katowice (No. KNW/0022/KB1/108/14).

Laboratory procedures

Blood samples were collected from a peripheral vein using tubes (Vacuette; Greiner-Bio, Frickenhausen, Germany) coated with K_3EDTA to obtain whole blood, or plain tubes to obtain serum. Serum was collected using centrifugation (3,000 rpm for 10 min at 4℃), aliquoted, and stored at −80℃ until analysis. Assessments of BLL were performed using graphite furnace atomic absorption spectrometry in an ICE 3400 system.

Serum CRP concentration was determined using the photometric-turbidimetric test for the quantitative determination of human CRP in serum and plasma (Human Gesellschaft fűr Biochemica and Diagnostica mbH, Germany). For this method, the linearity is kept up to 25 mg/d*l* and the lower detection limit is 0.1 mg/d*l*. No prozone phenomenon was observed up to 40 mg/d*l*.

Serum levels of interleukin 1β (IL-1β), interleukin 6 (IL-6), tumor necrosis factor α (TNF-α), and macrophage inflammatory protein 1- α (MIP-1α) were evaluated using a Bio-Plex 200 System (Bio-Rad, Hercules, CA). Levels of angiogenic factors such as soluble angiopoietin receptor (sTIE-2), vascular endothelial growth factor (VEGF), soluble vascular endothelial growth factor receptor-1 (sVEGFR-1), and fibroblast growth factor-basic (FGF-basic) were detected in serum using a Bio-Plex 200 System (Bio-Rad Laboratories Inc., Berkeley, California, USA) according to the manufacturer's instructions. The results were expressed as pg/m*l*.

Statistical analysis

All statistical analyses were performed using the Statistica 9.1 PL software program (StatSoft Polska, Warsaw, Poland). Data are reported as mean ± SD or as median and IQR. Shapiro-Wilk's test was used to verify normality, and Levene's test was used to verify homogeneity of variances. Statistical comparisons were made using a t-test, a t-test with separate variance estimates, or the Mann-Whitney U-test. Dependent variables were analyzed using the matched-pairs tests: Student's t-test and Wilcoxon's test. The Spearman non-parametric correlation was calculated. A p-value < 0.05 was considered statistically significant.

Results

The epidemiologic data are presented in Table 1 and 2. CRP levels and concentrations of lead in the blood are presented in Table 3. The mean BLL was 10.7 ± 7.67 μg/d*l* at the beginning of the study, and increased to 49.1 ± 14.1 μg/d*l* by the end of the study period. At the same time, the levels of CRP increased by 35% (Table 3).

The levels of MIP-1α were significantly higher after Pb exposure (71% higher than baseline), while the levels of IL-1β, IL-6 and TNF-α did not change. Simultaneously, the values of sVEGFR-1 and FGF-basic decreased by 14% and 21%, respectively. Levels of other angiogenic factors (sTie-2 and VEGF) did not change due to Pb exposure (Table 4).

Significant differences between the subgroup of workers exposed to lead before the study and those without such exposure are presented in Table 5. There were no significant differences between the subgroups of younger and older workers, smokers and non-smokers, and work-

Table 1. Epidemiologic data for study subjects

	Mean	SD
	n=36	
Lead exposure duration (days)	40	3
Work period (years)	15	12
Age (years)	41	14
Height (cm)	176	6.7
Weight (kg)	79.7	12.6
BMI (kg/m^2)	25.9	3.7
Smokers (%)	69.4	-

Table 2. Epidemiologic data for the subgroups of workers with and without a history of occupational lead exposure.

	N-HOE		HOE	
	Mean	SD	Mean	SD
	n=18		n=18	
Work period (years)	13.3	12.9	17.6	11.6
Age (years)	37.7	14.2	44.6	12.4
Height (cm)	174.2	6.1	176.5	7
Weight (kg)	77	12.9	81.4	12.2
BMI (kg/m^2)	25.3	3.8	26.1	3.7

N-HOE: no history of lead exposure, HOE: workers with a history of lead exposure

ers with higher and lower BMIs (data not shown).

At baseline, there were no correlations between the levels of pro-inflammatory cytokines and angiogenic factors (data not shown). After the examined period of Pb exposure, analysis of correlations showed positive correlations between VEGF level and the levels of IL-1β (R = 0.39), IL-6 (R = 0.42), and MIP-1α (R = 0.54). Positive correlations between MIP-1α and FGF-basic (R = 0.38), sTie-2 (R = 0.41), and sVEGFR-1 (R = 0.47) were also shown (Table 6).

The analysis of correlations did not show any correlations between the BLL and the levels of pro-inflammatory cytokines and angiogenic factors (data not shown).

Discussion

Epidemiologic and experimental studies have demonstrated that exposure to lead increases the risk of hypertension[15], and other research has provided a strong evidence that Pb exposure is a risk factor for ischemic heart disease and the occurrence of functional disturbances within the heart and blood vessels[16]. Among the explanations for these disorders is the impact of lead on the inflammatory response and angiogenesis[4].

In vitro studies have demonstrated dose-dependent immunomodulatory effects of Pb[17,18], and human studies have found that occupational lead exposure is associated with altered levels of certain pro-inflammatory cytokines, although the majority of studies focus only on chronic lead exposure. Valentino et al.[6] reported higher plasma TNF-α in workers chronically exposed to Pb (BLL = 9.1-46.0 μg/d*l*) compared to non-exposed workers. However, the level of IL-6 did not change in this study. Similar results were obtained in lead recycling plant workers (BLL =30.7 μg/d*l*), who exhibited significantly higher TNF-α than controls[19]. Our previous study on chronically Pb exposed workers (BLL=37.0 μg/d*l*) demonstrated not only higher levels of TNF-α, but also higher levels of IL-6 and IL-1β[14].

In this study, BLL increased significantly from 7 μg/d*l* to 49.1 μg/d*l* over 40 days. However, this remarkable increase was not associated with any significant alterations in IL-1β, IL-6, or TNF-α levels. However, at the same time we found a significant increase of the serum CRP level (35%). These results indicate that the CRP elevation observed during short-term lead exposure is likely caused by mechanisms associated with other pro-inflammatory factors. The results of the National Health and Nutrition Examination Survey (NHANES), based on a population of more than 9000 people environmentally exposed to Pb, demonstrated that the odds of having elevated CRP levels was 77% greater in a group with BLLs ≥ 30.9 (μg/*l*) than in a group with BLLs < 11.6 (μg/*l*)[1]. Similarly, it has been shown that the mean values of CRP were significantly higher in workers with occupational lead exposure compared to control subjects[20,21].

Elevated levels of CRP in lead exposed populations may have many clinical implications. CRP is a pro-inflammatory factor which powerfully predicts future cardiovascular disorders[22]. Previous *in vitro* studies have detected the effects of CRP on chemokine and adhesion molecule levels. Montecucco et al.[23] demonstrated a CRP-induced increase in the concentration of MIP-1α in human adherent monocytes. In addition, an *in vitro* investigation on human peripheral blood mononuclear cells (PBMC) showed that lead exposure results in a dose-dependent increase of MIP-1α production by PBMCs[24]. MIP-1α is a small protein belonging to the C-C subfamily of chemokines, inducible proteins that exhibit a variety of pro-inflammatory activities. MIP-1α has been proposed to induce production of IL-1β, IL-6 and TNF-α[25]; however, this effect was not observed in this study. Our data suggest a possible early effect of lead on pro-inflammatory markers, which may be related to the ability of MIP-1α to recruit pro-inflammatory cells, especially lymphocytes and monocytes[26].

Similar to the pro- and anti-inflammatory balance, the angiogenic balance plays an important role in various

Table 3. Blood lead level (BLL) and C reactive protein level (CRP) before and after subacute exposure to lead.

	Before exposure		After exposure		Relative change %	*p*
	Mean	SD	Mean	SD		
BLL (μg/d*l*)	10.7	7.68	49.1	14.2	359%	<0.001
CRP (mg/*l*)	7.17	4.37	10.95	5.77	34%	0.035

p-value: comparison between values obtained before and after exposure to lead using Student's t-test or Wilcoxon's test.

Table 4. The levels of pro-inflammatory cytokines and angiogenic factors before and after subacute exposure to lead.

Pro-inflammatory cytokines	Before exposure		After exposure		Relative change %	*p*
	Median	IQR	Median	IQR		
IL-1β (pg/m*l*)	0.25	0.13	0.25	0.17	0%	0.632
IL-6 (pg/m*l*)	2.07	3.25	2.34	2.74	13%	0.925
TNF-α (pg/m*l*)	5.31	6.77	4.46	5.96	−16%	0.157
MIP-1α (pg/m*l*)	1.04	0.84	1.77	2.16	71%	0.013*
Angiogenic factors						
sTIE-2 (pg/m*l*)	18455	8183	18326	9491	−1%	0.246
VEGF (pg/m*l*)	72.20	66.40	63.53	65.94	−12%	0.850
sVEGFR-1 (pg/m*l*)	6997	3530	6015	3995	−14%	0.048*
FGF-basic (pg/m*l*)	461	164	367	127	−21%	0.017*

IQR: interquartile range, IL-1β: interleukin 1β, IL-6: interleukin 6, TNF-α: tumor necrosis factor α, MIP-1α: macrophage inflammatory protein 1-α, sTIE2: soluble angiopoietin receptor, VEGF: vascular endothelial growth factor, sVEGFR-1: soluble vascular endothelial growth factor receptor-1, FGF-basic: fibroblast growth factor-basic
p-value: comparison between values obtained after and before exposure to lead using the Mann-Whitney U test.
* Statistically significant ($p < 0.05$).

pathological and physiological processes[28]. It has been shown that inorganic elements may influence the regulation of angiogenesis and vascular function[29]. Thus, it is essential to establish the possible ability of lead to maintain or alter angiogenesis. There are only a few *in vitro* studies showing changes in angiogenetic factors due to the toxic action of lead. In cultured vascular smooth-muscle cells, lead had a stimulatory effect on angiogenesis[30]. Another study demonstrated that lead inhibits tube formation by cultured human vascular endothelial cells in a dose- and time-dependent manner[12]. These discrepancies appear to be a result of different study protocols.

Inflammation may promote angiogenesis in a number of ways. Many pro-inflammatory cytokines, such as TNF-α, IL-6, and IL-1β, may have also angiogenic activity[27]. Moreover, an *in vitro* study has shown that MIP-1α promotes VEGF expression and angiogenesis in human osteosarcoma cells by down-regulating miR-374b expression via JNK, ERK, and p38 signaling pathways[31]. Our results reveal that MIP-1α levels positively correlate with the levels of VEGF, FGF-basic, sTie-2, and sVEGFR, while the levels of IL-1β and IL-6 correlated with the level of VEGF. Similar correlations between the levels of pro-inflammatory cytokines and angiogenic factors were not observed prior to lead exposure. These results indicate that short-term lead exposure triggers mechanisms that link angiogenesis and inflammation.

Recent experimental studies suggest that Pb may modulate angiogenesis via induced interleukin 8 (IL-8) gene expression[29] through the mitogen activated protein kinase (MAPK) pathway and activator protein 1 (AP-1)[13]. Furthermore, IL-8 is also one of the pro-inflammatory cytokines[8]. Dobrakowski et al.[32] showed that the level of IL-8 was significantly increased by 34% compared to baseline after a short-term exposure to lead. This observation also supports the hypothesis that lead exposure may indirectly affect angiogenesis. In the present study, we reported that the serum levels of sVEGFR-1 and FGF-basic were significantly decreased due to short-term lead exposure. The soluble form of VEGFR-1 retains its high-affinity binding to VEGF, and is likely to negatively regulate VEGF availability by sequestering the ligand and by forming inactive heterodimers with membrane-bound VEGF receptors[28]. In light of this, our results suggest that

Table 5. The comparison between the subgroups of workers with and without a history of occupational exposure to lead

	N-HOE n=18		HOE n=18		*p*	Relative change %
	Median	IQR	Median	IQR		
a: BLL (μg/d*l*)	3.70	2.60	17.10	9.30	0.000	362%
b: BLL (μg/d*l*)	45.52	6.20	48.60	22.00	0.438	8%
D: BLL (μg/d*l*)	39.70	6.90	30.90	26.40	0.028	−22%
a: MIP-1α (pg/m*l*)	1.08	0.70	0.90	1.20	0.825	−19%
b: MIP-1α (pg/m*l*)	0.80	1.54	2.70	2.07	0.002	245%
D: MIP-1α (pg/m*l*)	0.03	1.28	1.20	2.70	0.011	4800%
a: VEGF (pg/m*l*)	67.89	60.54	75.80	74.70	0.899	12%
b: VEGF (pg/m*l*)	50.07	39.93	82.20	70.40	0.020	64%
D: VEGF (pg/m*l*)	−15.27	67.56	14.20	58.05	0.041	−193%
a: FGF-basic (pg/m*l*)	0.77	0.24	0.70	0.17	0.658	−14%
b: FGF-basic (pg/m*l*)	0.60	0.32	0.80	2.80	0.002	32%
D: FGF-basic (pg/m*l*)	−0.16	0.34	0.10	2.12	0.001	−153%

N-HOE: no history of lead exposure, HOE: workers with a history of lead exposure, a: levels at baseline, b: levels at the end of the study period, D: median of differences between the (a) and (b) values. *p*-value: comparison between N-HOE and HOE subgroups using the Mann-Whitney U test. *p*-values < 0.05 were considered statistically significant.

Table 6. Correlations between levels of pro-inflammatory cytokines and angiogenic factors after subacute exposure to lead.

Angiogenic factors	VEGF	FGF-basic	sTie-2	sVEGFR-1
Pro-inflammatory cytokines				
IL-1β	0.39	NS	NS	NS
IL-6	0.42	NS	NS	NS
TNF-α	NS	NS	NS	NS
MIP-1α	0.54	0.38	0.41	0.47

Spearman's rank correlation was used to test correlations between angiogenic factors (columns) and pro-inflammatory cytokines (rows). R-values are shown for the statistically significant correlations ($p < 0.05$). NS: not significant

lead simultaneously dysregulates angiogenesis-triggering pro- and antiangiogenic mechanisms. It is possible that Pb may have impact on sVEGFR-1 transcription via interactions with GATA proteins[33]. These proteins are transcription factors that bind GATA DNA elements through Cys_4 structural zinc-binding domains, which are the potential targets for lead[34]. The influence of lead on FGF-basic is also documented in the previous studies. Kaji et al.[35] have shown that lead at 0.5 and 5.0 μM concentrations significantly reduced the ability of FGF-basic to stimulate vascular endothelial cells. These changes may be caused by direct lead-induced inhibition of the production and/or the activity of FGF-basic[36]. Furthermore, lead-induced alterations in heparan sulfate proteoglycans at the vascular endothelial cell surface[38] may lead to lower bioavailability of FGF-basic[39].

Earlier studies indicate that the impact of lead on angiogenic factors depends on the duration of exposure rather than BLL. We showed that a subgroup of workers with a history of occupational exposure to lead consistently had increased FGF-basic and VEGF levels compared to baseline, while the opposite results were observed in the subgroup of workers without a history of occupational exposure to lead. This observation suggests that Pb may modulate angiogenic factors levels through different mechanisms, depending on the duration of exposure.

The results of this study should be evaluated in the context of its limitations. The possible confounding role of inorganic elements such us other metal ions was not taken into consideration and is probably the major limitation of this study. Moreover, it is possible that the study was underpowered to detect significant differences in

measured parameters.

Conclusions

Short-term exposure to lead induces inflammatory responses, but the mechanisms seem to be different from those observed in chronic lead exposure. Additionally, subacute exposure to lead may dysregulate angiogenesis via modification of the levels of angiogenic factors.

Acknowledgments: This work was supported by the Medical University of Silesia.

Conflicts of interest: None declared.

References

1) Prokopowicz A, Sobczak A, Szuła-Chraplewska M, et al. Effect of occupational exposure to lead on new risk factors for cardiovascular diseases. Occupational and Environmental Medicine 2017; 74(5): 366-373.
2) Jakubowski J. Lead and its inorganic compounds, other than lead arsenate and exposure limits (OELs). Podstawy i Metody Oceny Środowiska Pracy 2014; 2(80): 111-144.
3) Kasperczyk A, Słowińska-Łożyńska L, Dobrakowski M, et al. The effect of lead-induced oxidative stress on blood viscosity and rheological properties of erythrocytes in lead exposed humans. Clinical Hemorheology and Microcirculation 2014; 56(3): 187-195.
4) Chang WCh, Chang ChCh, Wang YS, et al. Involvement of the epidermal growth factor receptor in Pb^{2+}-induced activation of cPLA2/COX-2 genes and PGE2 production in vascular smooth muscle cells. Toxicology 2011; 279(1-3): 45-53.
5) Chou YH, Woon PY, Huang WCh, et al. Divalent lead cations induce cyclooxygenase-2 gene expression by epidermal growth factor receptor/nuclear factor-kappa B signaling in A 431carcinoma cells. Toxicology Letters 2011; 203(2): 147-153.
6) Valentino M, Rapisarda V, Santarelli L, et al. Effect of lead on levels of some immunoregulatory cytokines in occupationally exposed workers. Human and Experimental Toxicology 2007; 26(7): 551-556.
7) Khurana R, Simons M, Martin JF, et al. Role of Angiogenesis in Cardiovascular Disease. Circulation 2005; 112(12): 1813-1824.
8) Radomska-Leśniewska DM, Hevelke A, Skopiński P, et al. Reactive oxygen species and synthetic antioxidants as angiogenesis modulators: Clinical implications. Pharmacological Reports 2016; 68(2): 462-471.
9) Vaziri ND. Mechanisms of lead-induced hypertension and cardiovascular disease. American Journal of Physiology - Heart and Circulatory Physiology 2008; 295: H454-H465.
10) Poręba R, Gać P, Poręba M, et al. Environmental and occupational exposure to lead as a potential risk factor for cardiovascular disease. Environmental Toxicology and Pharmacology 2011; 31(2): 267-277.
11) Ushio-Fukai M, Nakamura Y. Reactive oxygen species and angiogenesis: NADPH oxidase as target for cancer therapy. Cancer Letters 2008; 266(1): 37-52.
12) Ueda D, Kishimoto T, Dekio S, et al. Inhibitory effect of lead on tube formation by cultured human vascular endothelial cells. Human Cell 1997; 10(4): 283-291.
13) Lin YCh, Wei PL, Tsai YT, et al. Pb^{2+} Induced IL-8 Gene Expression by Extracellular Signal-Regulated Kinases and the Transcription Factor, Activator Protein 1, in Human Gastric Carcinoma Cells. Environmental Toxicology 2015; 30(3): 315-322.
14) Machoń-Grecka A, Dobrakowski M, Boroń M, et al. The influence of occupational chronic lead exposure on the levels of selected pro-inflammatory cytokines and angiogenetic factors. Human & Experimental Toxicology 2017; 36(5): 467-473.
15) Nawrot TS, Thijs L, Den Hond EM, Roels HA, Staessen JA. An epidemiological re-appraisal of the association between blood pressure and blood lead: a meta-analysis. J Hum Hypertens 2002; 16(2): 123-131.
16) Navas-Acien A, Guallar E, Silbergeld EK, et al. Lead exposure and cardiovascular disease-a systematic review. Environmental Health Perspectives 2007; 115(3): 472-482.
17) Villanueva MBG, Koizumi S, Jonai H. Cytokine Production by Human Peripheral Blood Mononuclear Cells after Exposure to Heavy Metals. Journal of Health Science 2000; 46(5): 358-362.
18) Honda A, Tsuji K, Matsuda Y, et al. Effects of air pollution-related heavy metals on the viability and inflammatory responses of human airway epithelial cells. International Journal of Toxicology 2015; 34(2): 195-203.
19) Di Lorenzo L, Vacca A, Corfiati M, et al. Evaluation of Tumor Necrosis *Factor-alpha* and Granulocyte Colony-Stimulating Factor Serum levels in lead-exposed smoker workers. International Journal of Immunopathology and Pharmacology 2007; 20(2): 239-247.
20) Anetor JI, Adeniyi FA. Decreased immune status in Nigerian workers occupationally exposed to lead. African Journal of Medicine and Medical Sciences 1998; 27(3-4): 169-172.
21) Khan DA, Qayyum S, Saleem S, et al. Lead-induced oxidative stress adversely affects health of the occupational workers. Toxicology and Industrial Health 2008; 24(9): 611-618.
22) Montecucco F, Burger F, Pelli G, et al. Statins inhibit C-reactive protein-induced chemokine secretion, ICAM-1 upregulation and chemotaxis in adherent human monocytes. Rheumatology (Oxford) 2009; 48(3): 233-242.
23) Montecucco F, Steffens S, Burger F, et al. C-reactive protein (CRP) induces chemokine secretion via CD11b/ICAM-1 interaction in human adherent monocytes. Journal of Leukocyte Biology 2008; 84(4): 1109-1119.
24) Gillis BS, Arbieva Z, Gavin IM. Analysis of lead toxicity in human cells. BMC Genomics 2012; 13: 344. (doi: 10.1186/1471-2164-13-344).
25) Cook DN. The role of MIP-1α in inflammation and hematopoiesis. Journal of Leukocyte Biology 1996; 59(1): 61-66.
26) Maurer M, von Stebut E. Macrophage inflammatory protein-1.

The International Journal of Biochemistry & Cell Biology 2004; 36(10): 1882-1886.

27) Huang SP, Wu MS, Shun CT, et al. Interleukin-6 increases vascular endothelial growth factor and angiogenesis in gastric carcinoma. Journal of Biomedical Science 2004; 11(4): 517-527.

28) Duda DG. Molecular Biomarkers of Response to Antiangiogenic Therapy for Cancer. ISRN Cell Biology 2012; 2012: 587259. (doi: 10.5402/2012/587259).

29) Saghiri MA, Orangi J, Asatourian A, et al. Functional role of inorganic trace elements in angiogenesis part III: (Ti, Li, Ce, As, Hg, Va, Nb and Pb). Critical Reviews in Oncology/Hematology 2016; 98: 290-301.

30) Fujiwara Y, Kaji T, Yamamoto Ch, et al. Stimulatory effect of lead on the proliferation of cultured vascular smooth-muscle cells. Toxicology 1995; 98(1-3): 105-110.

31) Liao Y-Y, Tsai H-Ch, Chou P-Y, et al. CCL3 promotes angiogenesis by dysregulation of miR-374b/ VEGF-A axis in human osteosarcoma cells. Oncotarget 2016; 7(4): 4310-4325.

32) Dobrakowski M, Kasperczyk A, Pawlas N, et al. Association between subchronic and chronic lead exposure and levels of antioxidants and chemokines. Int Arch Occup Environ Health 2016; 89(7): 1077-1085.

33) Coma S, Allard-Ratick M, Akino T, et al. GATA2 and Lmo2 control angiogenesis and lymphangiogenesis via direct transcriptional regulation of neuropilin-2. Angiogenesis 2013; 16 (4): 939-952.

34) Ghering AB, Jenkins LM, Schenck BL, et al. Spectroscopic and functional determination of the interaction of Pb2+ with GATA proteins. Journal of the American Chemical Society 2005; 127(11): 3751-3759.

35) Kaji T, Suzuki M, Yamamoto C, et al. Severe damage of cultured vascular endothelial cell monolayer after simultaneous exposure to cadmium and lead. Archives of Environmental Contamination and Toxicology 1995; 28(2): 168-172.

36) Kaji T, Yamamoto C, Sakamoto M. Effect of lead on the glycosaminoglycans metabolism of bovine aortic endothelial cells in culture. Toxicology 1991; 68(3): 249-257.

37) Kaji T, Ohkawara S, Nakajima M, et al. Lead-induced alteration of heparan sulfate proteoglycans in cultured vascular endothelial cells. Toxicology 1997; 118(1): 1-10.

38) Yayon A, Klagsbrun M, Esko JD, et al. Cell surface, heparin-like molecules are required for binding of basic Fibroblast Growth Factor to its high affinity receptor. Cell 1991; 64(4): 841-848.