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Blood morphology and the levels of selected cytokines related to hematopoiesis in occupational short-term exposure to lead

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ABSTRACT

The aim of the study was to investigate the influence of a short-term exposure to lead on the blood morphology and the levels of selected cytokines related to hematopoiesis in occupationally exposed workers.
The study population included 37 males occupationally exposed to lead for 36 to 44 days. Their blood lead level raised from 10.7 ± 7.67 μg/dl at baseline to the level of 49.1 ± 14.1 μg/dl at the end of the study.
The level of hemoglobin and values of MCH and MCHC were decreased due to a short-term exposure to lead by 2%, 2%, and 1%, respectively. The counts of WBC, LYM, and MXD increased significantly by 5%, 7%, and 35%. Similarly, the count of PLT increased by 7%, while PDW, MPV, and P-LCR decreased by 6%, 3%, and 9%, respectively. The levels of IL-7, G-CSF, HGF, PDGF AB/BB, SCF, and PECAM-1, decreased significantly by 30%, 33%, 8%, 30%, 25%, and 20%, respectively.
A short-term occupational exposure to lead results in a decreased hemoglobin level and increased counts of WBC and PLT. Changes in counts and proportions of different types of leukocytes and decreased values of PLT indices, such as PDW, MPV, and P-LCR, due to the subacute lead-exposure may be associated with lead-induced decreased levels of cytokines related to hematopoiesis, including SCF, G-CSF, IL-7, and PDGF.

1. Introduction

High density, softness, low melting point, resistance to corrosion, and opacity to gamma and X radiation are the properties of lead which have caused it to be widely used in various industries. Consequently, lead remains the main pollutant despite its well documented toxicity to humans. Biological effects of lead action depend on the level and duration of exposure (Ogawa et al., 2008). Developed countries have introduced systematic measures for primary and secondary prevention for lead poisoning. Due to the improvement of the workplace environment and the preventive measures for lead workers, the classical clinical forms of lead poisoning are quite unlikely to occur in these countries (Di Lorenzo et al., 2006; Ogawa et al., 2008). In Poland, current regulations allow blood lead levels in lead exposed workers to be up to 50 μg/dl before an employer is required to remove the individual from lead exposure (Trzcinka-Ochocka et al., 2006). However, adverse health effects of lead on many tissues and organs, including hematopoietic, nervous, renal, and cardiovascular systems can be detected at concentrations of lead in the blood lower than 10 μg/dl (Heo et al., 1996; Jang et al., 2011).

Inhalation is the primary route of occupational exposure to lead (Barman et al., 2014). After absorption, lead distributes into three major compartments in the body: blood, soft tissues, and bones. Blood lead accounts for about 90% of total body lead burden after acute exposure. >95% of blood lead is accumulated in erythrocytes disturbing their function. Anemia is a well-known toxic effect of lead action (Jang et al., 2011). A decrease in the hematocrit or hemoglobin level due to lead exposure may be caused by increased erythrophagocytosis, hemolysis and splenic sequestration of red blood cells or by impaired erythropoiesis. It is well-established that lead impairs the biosynthesis of heme by inhibiting δ-aminolevulinic acid dehydratase (ALAD) and ferrochelatase (Jang et al., 2011; Kasten-Jolly et al., 2010). In addition, lead has been shown to have a direct negative effect on alpha and beta globin synthesis (Kasten-Jolly et al., 2010).

Lead influences not only the count and function of red blood cells but also platelet and leukocyte indices through interferences with the immune response. Intoxication with lead may lead to both immunosuppression or immunopotentiation (Barman et al., 2014; Heo et al., 1996). Lead may impact receptors, receptor-associated tyrosine kinases,

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G proteins, and several enzymes involved in cell signaling. As a result, important cellular functions, such as differentiation and proliferation, may be affected due to the lead action. In addition, many authors postulate that lead modulates the immune system function by differential effects on T-helper (Th) cells. Experimental studies indicated that lead was able to suppress the proliferation of Th1 cells and activate responses mediated by Th2 cells (Jorissen et al., 2013). All of these mentioned lead-mediated immunotoxicities may induce changes in the blood morphology associated with impaired hematopoiesis. However, data on this topic are inadequate, especially concerning acute or subacute lead poisoning. Therefore, the present study was designed to assess the influence of a short-term exposure to lead on the blood morphology and the levels of selected cytokines related to hematopoiesis in occupationally exposed workers.

2. Material and methods

2.1. Study population

The study population included 37 males occupationally exposed to lead for 36 to 44 days. Their mean age was 41.2 ± 13.54 years. Blood lead level (PbB) served as an exposure marker. Examined subjects were employed in the lead-zinc works to perform periodic maintenance of blast furnaces and production lines. In the exposed population, 18 workers were occupationally exposed to lead for the first time, while 19 workers had a history of occupational exposure to this xenobiotic. Workers were exposed to high doses of lead because they did not adhere to the occupational safety and health requirements and did not properly use the personal protective equipment, such as clothing, anti-dust masks, and goggles.

Each study subject provided a written consent to a study. Questionnaire data on age, weight, height, medical history, and smoking were obtained. To analyze confounding factors, the exposed population was additively divided into pairs of subgroups based on history of occupational exposure to lead before the study, smoking habits and a median of age and BMI. 5% and 3% of workers were diagnosed with hypertension and coronary artery disease, respectively, while none of them were diagnosed with diabetes. The experimental set-up has been approved by the Bioethics Committee of the Medical University of Silesia in Katowice No. KNW/0022/KB1/108/14.

2.2. Laboratory procedures

2.2.1. Blood collection. Blood of all examined workers was drawn at the beginning of the study for the first time and after a period of a short-term exposure to lead for the second time. Samples of blood obtained from each study subject were collected from the cubital vein using vacuum tubes (Vacuette®; Greiner-Bio, Frickenhausen, Germany) that contained K3EDTA to obtain whole blood or plain tubes to obtain serum. Blood samples were frozen and stored at −20 °C until tested.

2.2.2. Determination of lead concentration. The assessments of the PbB were performed by graphite furnace atomic absorption spectrometry using an ICE 3400 instrument (Thermo Fisher Scientific Waltham, MA, USA). The laboratory met the requirements of proficiency tests (Lead and Multielement Proficiency – CDC in Atlanta). The ClinCal® Whole Blood Calibrator and ClinCal® Serum Calibrator (Recipe, Germany) were used for calibration of the instrument and control materials. ClinCheck Whole Blood Control Levels I, II, and III, and ClinCheck Serum Control Levels I and II were used for quality control.

2.2.3. Peripheral blood morphology. The analyzer Sysmex K-4500 was used to determine parameters of the blood morphology: red blood cells (RBC) count, hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin mass (MCH), mean corpuscular hemoglobin concentration (MCHC), red blood cell distribution width - coefficient of variation (RDW-CV), red blood cell distribution width- standard deviation (RDW-SD), white blood cells (WBC) count, counts and percentages of lymphocytes (LYM, LYM%) and granulocytes (GRA, GRA%), mixed cell count (MXD) and percentage (MXD%), platelets (PLT) count, platelet distribution width (PDW), platelet large cell ratio (P-LCR), and mean platelet volume (MPV).

2.2.4. Determination of cytokines. The levels of interleukin 7 (IL-7), interleukin 9 (IL-9), granulocyte colony stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), hepatocyte growth factor (HGF), stem cell factor (SCF), platelet-derived growth factor (PDGF), and platelet endothelial cell adhesion molecule 1 (PECAM-1) were detected in serum using a Bio-Plex 200 System (Bio-Rad Laboratories Inc., USA). The Bio-Plex system is based on three core elements. The first core element is a technology that uses fluorescently dyed magnetic microspheres (beads), each with a distinct color code to permit discrimination of individual tests within a multiplex suspension and allows the simultaneous detection of diverse analyte molecules in a single well of a 96–well microplate. Moreover, the magnetic beads allow for magnetic separation during the washing steps. The second core element is a dedicated flow cytometer with two lasers (a 532 nm Nd-Yag laser used to excite phycoerythrin in the assay and a 635 nm solid state laser used to excite the dyes inside the beads to determine their "color" or "region") and associated optics to measure the different molecules bound to the surface of the beads. The third core element is a higher-speed digital signal processor that efficiently manages the fluorescent output.

The principle of these bead-based assays is similar to capture sandwich immunoassays. The samples and standards were incubated with the coupled beads (antibodies directed against the desired cytokines were covalently coupled to internally dyed beads) in the wells of 96-well plates and washed. Next, the biotinylated detection antibodies specific for different cytokine epitopes were added. After incubation and washing, streptavidin (phycoerythrin solution) was added to bind biotinylated detection antibodies on the beads. Next, the suspensions of washed beads were analyzed using the Bio-Plex System. Software presented data as both median fluorescence intensity as well as concentration (pg/ml).

2.2.5. Statistical analysis. The statistical analysis was performed using the Statistica 9.1 PL software program. The statistical analyses included the means and standard deviations of the data. Shapiro-Wilk's test was used to verify normality, and Levene's test was used to verify the homogeneity of variances. Statistical comparisons were made using the ***t***-test, ***t***-test with separate variance estimates, the Mann-Whitney ***U*** test or the Chi squared test. Dependent variables were analyzed using Student's ***t***-test and Wilcoxon's test. The Spearman non-parametric correlation was calculated. A value of $p < 0.05$ was considered to be significant.

3. Results

Epidemiologic data and concentrations of lead in the blood are presented in Table 1. The mean PbB at the beginning of the study was 10.7 ± 7.67 μg/dl and increased to the level of 49.1 ± 14.1 μg/dl at the end of the study period (Table 1).

Table 1
Epidemiological data and blood lead levels (PbB) in the study population.

	Mean	SD
Lead exposure duration (days)	40	3.2
Age (years)	41.2	13.54
BMI (kg/m^2)	25.75	3.71
Percentage of smokers (%)	69%	–
Percentage of workers diagnosed with HT	5%	–
Percentage of workers diagnosed with CAD	3%	–
$PbB_{\text{before exposure}}$ (μg/dl)	10.7	7.67
$PbB_{\text{after exposure}}$ (μg/dl)	49.1	14.1

BMI – body mass index, HT – hypertension, CAD – coronary artery disease.