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The relation between lead exposure and inflammation / endothelial dysfunction

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Abstract

Pb exposure is a well-known risk factor for inflammation including vessels. In our study, we aimed to investigate the relationship between Pb levels of exposed workers and the severity of inflammation/endothelial dysfunction. We analyzed the correlation between blood lead level and the markers of endothelial damage. There was a significant correlation between blood lead levels and vascular cell adhesion molecule-1 (VCAM-1). Elevated levels of blood lead also show correlation with soluble CD40 ligand (sCD40L). The strongest correlation was with sE-selectin. Association between lead exposure and markers of cardiovascular injury may help to predict cardiovascular effects of lead earlier and to protect workers who are exposed to lead.

Keywords: Lead exposure, inflammation, Endothelial Dysfunction, vascular cell adhesion molecule-1 (VCAM-1), soluble CD40 ligand (sCD40L)

Introduction

Cardiovascular diseases are still the main cause of death in developed and many developing countries and the leading cause of cardiovascular diseases is predominantly atherosclerosis. Its main characteristic is that it can remain asymptomatic for many years after the initiation of typical cellular changes [1,2]. The classical risk factors are primary Framingham risk factors including hyperlipidemia, hypertension, smoking, family history of premature coronary diseases, low physical activity and diabetes mellitus [3,4]. There are also modifiable risk factors like hyperthyroidism, hyperuricemia and low-density proteins [5,6].

Atherosclerosis (AS) is a disease of the arterial wall that initiates with inflammation and oxidation, lipid retention and modification [7]. It is characterized by atheroma, fatty streak, foam cells, intimal thickening, and atherosclerotic plaques [8]. Pathological intimal thickening is the most important stage of AS, but histopathological changes that lead to atheroma begin earlier. The initiative step is the accumulation of plasma derived lipids in the intima [9]. Endothelial dysfunction and inflammation seems to be the major facilitators of atherosclerotic diseases. As it has a central role in vascular homeostasis, antithrombotic activity and fibrinolytic properties, endothelium is the most important target in atherosclerotic changes. There are also many environmental factors which accelerate lipid accumulation in arterial intima leading to foam cell formation [10]. Occupational lead exposure is one of the most important etiological environmental factors that causes early atherosclerosis [11,12].

Vascular Cellular Adhesion Molecule-1 (VCAM-1) is an adhesion molecule expressed on activated endothelial cells which is expressed on lymphocytes, monocytes, and eosinophils [13]. Since atherosclerosis demonstrated typical features of inflammation, VCAM-1 has long been accepted as a potential marker of atherosclerosis [14]. The role of activated endothelial cell in the pathogenesis of atherosclerosis is well-known, and soluble forms of sE-selectin / P-selectin are the molecules which demonstrate this activity in peripheral blood [15]. E-selectin is present in Weibel-Palade bodies of endothelial cells, and many studies reveal the excessive secretion in case of inflammation [16,17].

As platelets play a crucial role in the maintenance of homeostasis, they are closely related to inflammatory reactions and vascular remodeling [18]. The main source of CD40 ligands (sCD40L) is platelets and this ligand plays a major role on homeostasis by contributing to platelet activation and clot stability [19,20]. The underlying mechanisms of sCD40L interaction with endothelial cells are decreased thrombomodulin expression and upregulation of inflammatory cytokines [21,22].

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As a natural member of earth crust, lead (Pb) is a chemical element that is easily extracted from its ores and it is toxic heavy metal that accumulates in soft tissues and bones. Pb and Pb alloys are used as a raw material in many products in industry. Pb exposure is a well-known risk factor for inflammation including vessels [23]. Clinical and epidemiological studies demonstrates the adverse impact of low- to moderate-level Pb exposure on adverse health effects [24]. In our study, we investigated the relationship between Pb levels of exposed workers and the severity of inflammation/endothelial dysfunction.

Materials and Methods

Study Population

This is a case-control study. 56 patients who applied to the Occupational Diseases Outpatient Clinics at Ankara Occupational and Environmental Diseases Hospital (between January-2017 and December-2018) with a suspicion of lead intoxication formed the case group. In order to eliminate the confounding effects on inflammation and endothelial dysfunction; subjects who have chronic diseases (diabetes mellitus, atherosclerotic vascular and/or cardiac disease, hypertension, rheumatoid arthritis) and acute inflammatory diseases were excluded from the study. All of the exposed group were male workers who work in vehicle battery production industry. Similarly, all of the control group was formed from male subjects. We constituted the case group who has a blood lead level (BLL) > 10 µg/dL and no clinical problem. All procedures of the study were performed in accordance with the 1964 Helsinki Declaration and its later amendments. The protocol of the study was approved by the Ethical Committee of Yozgat Bozok University (Decision No: 12.10.2016/70).

Measurements

Whole blood and serum samples were taken from each participant. Separation of blood samples for serum was made by centrifugation at 3000 rpm for 10 minutes and added to 2 ml centrifuge tubes for ELISA analyses. Then serum samples were transferred to the Yozgat Bozok University Science and Technology Application and Research Center (BILTEM) in a cold chain environment for all analyses. All samples were stored at -20°C until analysis.

1 ml whole blood samples (each sample), 5 ml nitric acid and 5 ml ultrapure water added to the Teflon tubes and digested by Microwave Digestion System (Milestone, Ethos model). Pb levels of the digested samples in the microwave oven were determined by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) system (Thermo Scientific ICAP Qc, USA).

ELISA system (BMG LABTECH ClarioStar) used for analysis sE-selectin, VCAM-1 and sCD40L in the serum samples. Specific ELISA kits used for preparation samples in the microplates for analyses (SunRed). 5-point calibration were generate for each curves and microplates were read at 450 nm. Linear regression was used for sE-selectin, Vcam-1 and sCD40L levels.

Statistical Analysis

In order to make the statistical evaluation, we used SPSS 22.0 software for occupational sociodemographic and laboratory/toxicology data. Data distribution was determined with Kolmogorov-Smirnow test and the statistical significance level was accepted as 0.05. The difference between the averages of two independent variables was evaluated with t-test; the difference between more than two variables was evaluated with ANOVA (analysis of variance) and additionally with Pearson Correlation analysis. The averages are given with the standard deviations.

Results

Our study group was consisted of 104 participants. 56 (53.8%) of them formed the case group and 48 (46.2%) of them were control group.

Hemoglobin levels, WBC counts, creatinine, AST and ALT levels did not show any statistically significant difference between the two groups. However, mean blood lead levels were 21.69 µg/dL in the case group and 2.43 µg/dL in the control group, and the difference was statistically significant ($p<0.001$). The VCAM-1, SCD40L and sE-selectin levels were also, statistically significantly higher in the case group ($p<0.05$). The comparison of laboratory values between the groups is given in Table 1.

Table 1. The mean values of continuous variables of case and control groups

N=104	Control / Case	N	Mean	Std. Deviation	p
WBC (kµ/L)	Controls	56	7.56	1.87	0.097
	Cases	48	8.18	1.93	
HGB (g/dL)	Controls	56	15.42	1.50	0.077
	Cases	48	14.90	1.44	
HCT (%)	Controls	56	45.82	3.61	0.296
	Cases	48	45.05	3.88	
PLT (kµ/L)	Controls	56	234.41	50.19	0.775
	Cases	48	237.56	62.07	
ALT (U/L)	Controls	56	27.09	16.62	0.218
	Cases	48	23.46	12.58	
AST (U/L)	Controls	56	20.70	4.77	0.064
	Cases	48	19.00	4.38	
Creatinine (mg/dL)	Controls	56	0.83	0.08	0.101
	Cases	48	0.81	0.08513	
BLL (µg/dL)	Controls	56	2.43	2.22	<0.001**
	Cases	48	21.69	7.96	
VCAM-1 (ng/ml)	Controls	56	7.80	4.39	0.024*
	Cases	48	10.20	5.95	
sCD40L (ng/ml)	Controls	56	6.65	4.19	0.003**
	Cases	48	11.29	9.50	
sE-selectin (ng/ml)	Controls	56	35.06	18.98	<0.001**
	Cases	48	55.66	13.53	

WBC: White blood count; HGB: hemoglobin; HCT: hematocrit; PLT: platelet; ALT: alanine transaminase; AST: aspartate transaminase; BLL: blood lead level; VCAM: vascular cell adhesion molecule-1; sCD40L: soluble CD40 ligand

$p > 0.05$, statistically non-significant; * $p < 0.05$, ** $p < 0.01$

We analyzed the correlation between blood lead level and the markers of endothelial damage. There was a significant correlation between blood lead levels and VCAM-1 (r=0.275, p<0.01). Elevated levels of blood lead also show correlation with sCD40L (r=0.393, p<0.01). The strongest correlation was with sE-selectin (r=0.541, p<0.01). Table 2 and figures 1, 2, 3 demonstrate these associations.

Table 2. Pearson Correlation Coefficients for Pb, VCAM, sCD40L, sE-selectin

Blood Lead	r	1	0,275*	0,393*	0,541*
VCAM-1	r		1	0,337*	0,252*
sCD40L	r			1	0,360*
sE-selectin	r				1

*. p<0.01

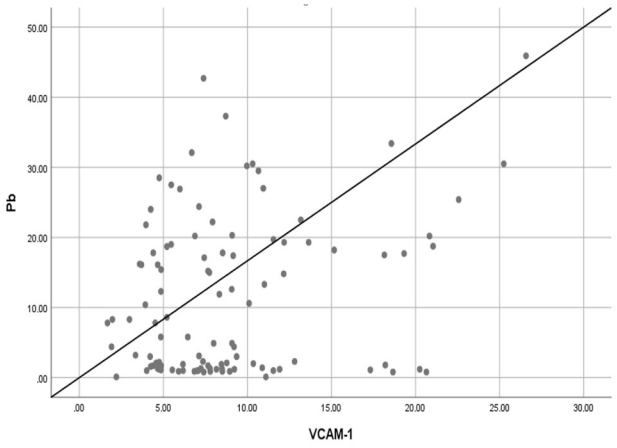

Figure 1. Relation between Pb and VCAM-1

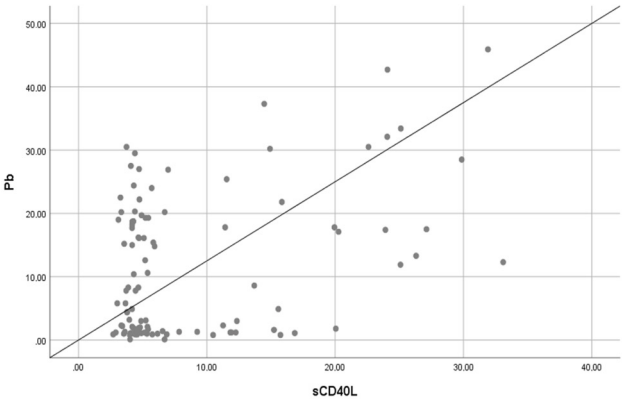

Figure 2. Relation between Pb and sCD40L

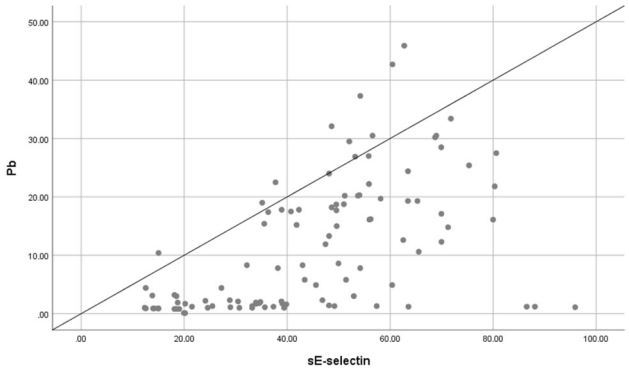

Figure 3. Relation between Pb and sE-selectin

Discussion

It has been revealed by many studies that inflammation plays a crucial role in the relationship between lead exposure and vascular pathologies including atherosclerosis. Determination of inflammation at cellular level is essential in establishing the hazardous effects of lead and similar toxic xenobiotic exposures at an early stage. In order to become a new biomarker candidate, inflammation and other functional biomarkers have been numerously researched in many studies for this purpose. A 2018 meta-analysis and systematic review showed a relationship between lead exposure and cardiovascular disease [25]. In this publication, many studies concerning the relationship between lead exposure and inflammation were reviewed and it has been demonstrated that the increase in cytokine and acute phase reactants such as CRP, haptoglobin and ceruloplasmin show parallelism. Another 2018 review has presented studies that support the strong relationship between lead exposure, inflammation and cytokines. The cellular and humoral immune responses are affected by lead via numerous inflammatory markers [26].

In our study, we compared VCAM, sCD40L and sE-selectin levels of lead exposed workers with non-exposed controls. We found that high lead levels were associated with high levels of some inflammatory biomarkers.

Correlation analysis showed that increased levels of blood lead was associated with higher levels of VCAM-1. This result was consistent with a previous study of our research group. In the study the VCAM-1 levels showed association with increased blood lead levels [25]. VCAM-1 was shown to be a marker for atherosclerosis [8] and lead intoxication is related to cardiovascular disease [26]. Therefore, this result may indicate that lead exposure may be closely related to its cardiotoxic effects via VCAM-1.

Our analysis showed that higher blood lead levels were associated with higher sCD40L expression. Many studies suggest sCD40L may be a marker for cardiovascular disease [13-15], but a cohort study did not find any correlation between sCD40L plasma levels and all-cause mortality, as well as cardiovascular mortality at one-year follow-up. But on the other hand, they emphasized short term associations in some subgroups [27]. In our study, our results about sCD40L demonstrated a statistically significant relationship between lead exposure and levels of this biomarker and therefore there may be a specific association in a lead exposure situation. This should be investigated in more detail with studies which has larger participation.

In our study, another finding is about the association between sE-selectin and blood lead levels. sE-selectin is shown to be a blood marker for vascular injury and platelet activation [9-11]. The association in our study may show that sE-selectin may also be a marker for cardiovascular disease associated with lead exposure.

As a result, we investigated both the relationship between lead exposure and inflammation, and whether these biomarkers could

be clinically candidates for early warning about the initiation of atherosclerosis, by comparing the levels of biomarkers and lead exposure severity. According to our results, this correlation supports a valuable clinical approach if validated more strongly by larger studies.

Conclusion

Our study shows an association between lead exposure and three important markers of cardiovascular injury. This may help us to predict cardiovascular effects of lead earlier and to protect workers who are exposed to lead.

Conflict of interest

The authors declare that they have no competing interest.

Financial Disclosure

All authors declare no financial support.

Ethical approval

The protocol of the study was approved by the Ethical Committee of Yozgat Bozok University (Decision No:10.2016/70).

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