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Possible changes of New-Generation inflammation markers with occupational lead exposure

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Abstract: Objectives: Occupational lead (Pb) exposure is still an important health problem in the world. Long-term Pb exposure causes several adverse effects. The aim of this study was to investigate the changes of inflammation markers with chronic Pb exposure by analyzing neopterin levels and kynurenine (Kyn) to tryptophan (Trp) ratio that reflects indolamine 2,3-dioxygenase activity and to compare with healthy volunteers' parameters. **Methods:** Blood lead levels (BLLs) were analyzed by atomic absorption spectrometry. Urinary neopterin and serum Kyn and Trp levels were analyzed by high-performance liquid chromatography. **Results:** According to our results, mean BLL of the 29 workers was 20.4±9.6 μg/dl. Urinary neopterin levels, serum Kyn levels, and Kyn/Trp of Pb workers (188±52 μmol/mol creatinine, 2.70±0.66 μM, and 43.19±10.38 μmol/mmol, respectively) were significantly higher than controls (144±35 μmol/mol creatinine, 2.08±0.34 μM, and 32.24±7.69 μmol/mmol, respectively). Pb-exposed workers were divided into further three groups according to their BLLs: as 10-19 μg/dl (*n*=18), 20-29 μg/dl (*n*=8), and 30-49 μg/dl (*n*=3). Neopterin levels of the workers with BLL of 30-49 μg/dl were significantly higher than those of BLL with 10-29 μg/dl, while Trp levels decreased. Kyn/Trp of workers with BLL of 30-49 μg/dl were elevated significantly compared with the workers with BLL<30 μg/dl. In addition to neopterin, Kyn and Kyn/Trp levels were positively influenced by Pb exposure. **Conclusions:** Increased level of inflammation markers confirms the adverse effects of Pb even low BLLs, and we suggest that monitoring BLLs with inflammation markers could help to prevent serious occupational health problems.
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Key words: IDO activity, Immune system, Inflammation, Lead, Neopterin, Occupational exposure

Introduction

One of the oldest known metals, lead (Pb) was mainly used in the production of batteries and in sheathing electric cables as well as pigments in paint and ceramic and as insecticides[1)]. Since Pb is being used in various areas, Pb industry requires a large volume of annual production, which means that people are exposed to Pb from the environment and daily used products and the number of workers that were exposed to Pb in manufacturing facilities is also increasing with each day[2)]. Most of the Pb used by industry comes primarily from mined ores and secondarily from recycled scrap metal or batteries. Many of the anthropogenic sources of Pb, like Pb in gasoline, have been eliminated because of Pb's persistence, bioaccumulative nature, and toxicity. Nevertheless, as a result of human activity, environmental levels of Pb have increased more than 1000-fold over the past three centuries[3)].

Lead has a complex kinetic nature in the body. Occupational exposure to Pb mainly occurs by inhaling air that contains Pb particles or fume. Pb absorption through lungs is relatively efficient and depends on the form, particle size, and concentration[4)]. Blood Pb is mainly represented by erythrocyte Pb levels. Erythrocyte Pb refers to

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Table 1. Characteristics of the study groups

Characteristics	Controls	Workers
Number of groups	29	29
Age (min-max)	35.4±9.2 (18-53)	37.5±7.1 (24-53)
Working years	–	7.5±6.7 (0.8-23)
Smoking (Y/N)	19/29	17/29

both short-term exposed lead and the released fraction of accumulated lead[5]. After chronic exposure, the majority of Pb in the body is found in bone and only about 1% of Pb is found in the blood. Current evidence indicates that the accumulation and elimination phases of blood Pb kinetics are not symmetrical; elimination is slower than accumulation as a result of the gradual release of bone Pb stores to blood[6].

Epidemiological and experimental studies indicate that chronic Pb exposure resulting in blood lead levels (BLLs) as low as 10 μg/d*l* in children and adults is associated with a wide range of adverse effects[7]. For instance, increased Pb exposure leads to impaired heme biosynthesis and hence reduction of hemoglobin levels and cause serious anemia. Effect on heme synthesis triggers the changes in the renal and neurological systems[1]. The other main target for chronic Pb toxicity is the nervous system. Long-term exposure can result in impaired cognitive functions[3]. Moreover, it has been classified as probable carcinogenic to humans (Group 2A) by the International Agency for Research on Cancer[8]. Carcinogenicity mechanism of Pb has not been clarified yet, but it is well known that inflammation is a critical component of cancer progression[9]. However, data on the causal relationship between Pb exposure and inflammation are inadequate and conflicting.

An inflammation and immune system activation marker, neopterin, is mainly synthesized by activated macrophages and shows the degree and prognosis of various diseases[10]. Neopterin is also useful in early diagnosis of immune alterations due to occupational exposures[11-15]. In parallel to neopterin, tryptophan degradation by the immunosuppressive enzyme indoleamine-2,3-dioxygenase (IDO) can be altered by immune modulation and inflammation[16].

The aim of this study was to investigate the changes of inflammation markers with chronic Pb exposure by analyzing urinary neopterin levels and serum kynurenine (Kyn) to tryptophan (Trp) ratio, which reflects IDO activity, and compare with healthy volunteers' parameters.

Materials and Methods

Study groups and sample collection

Blood and urine samples taken from 29 male Pb-exposed workers (aged between 24 and 53 years), who were occupied in the national small arm ammunition company and admitted to Ankara Occupational Diseases Hospital for routine control. The working durations ranged from 1 to 23 years. As their controls, a total number of 29 healthy male volunteers without occupational Pb exposure (aged between 18 and 53 years) recruited for the study. The demographics of the participants were shown in Table 1. The principles of the Ethical Committee accordance to the Helsinki Declaration were followed during the entire study (document #HEK12/10-9). None of the participants had any systemic disease and medication treatment.

Sample collection was done early in the morning. Neopterin and creatinine levels were determined in urine samples. Urinary neopterin levels were expressed as micromole per mole creatinine. After centrifugation of blood specimens at 3,500 rpm for 15 min at room temperature, sera were separated to measure Trp and Kyn levels. All samples were stored at −20℃ until analysis and kept from direct light exposure. The kynurenine-to-tryptophan ratio was calculated to estimate the degree of tryptophan conversion and expressed as micromole per millimole (μmol/mmol).

Alanine aminotransferase and aspartate aminotransferase activities and white blood cell, red blood cell, platelet count, hemoglobin, and hematocrit levels were also determined as routine measurements in the hospital biochemistry laboratory.

Blood lead levels

BLLs were only measured for the worker group during their routine control. To measure BLL, 1 m*l* blood sample was transferred in microwave test tubes and 5 m*l* nitric acid following 5 m*l* ultrapure water solution was added to the samples. The blends were kept in the tubes approximately half an hour. Digestion procedure for blood samples were carried out using 1,600 W Cem Mars Xpress microwave system at 210℃ for 10 min and were kept 5 min more in the oven just after the procedure was completed. Every sample was transferred from microwave test tubes to polypropylene flasks and then filled with distilled water up to 20 m*l* totally. All of the samples were kept at +4℃ in a refrigerator. The blood Pb levels of the workers were determined by Graphite Atomic absorption spectrometer equipped with Zeeman background correction system (AAS, Varian, SpectrAA-240, Australia), using a graphite tube atomizer at 283.3±0.5 nm wavelength, and using the carrier gas argon. The results of BLL analyses were given as μg/d*l*.

Determination of tryptophan degradation and kynurenine concentrations

Serum Trp and Kyn measurements were performed by high-performance liquid chromatography (HPLC, HP Agilent 1100) using reversed-phase C18 columns. Protein

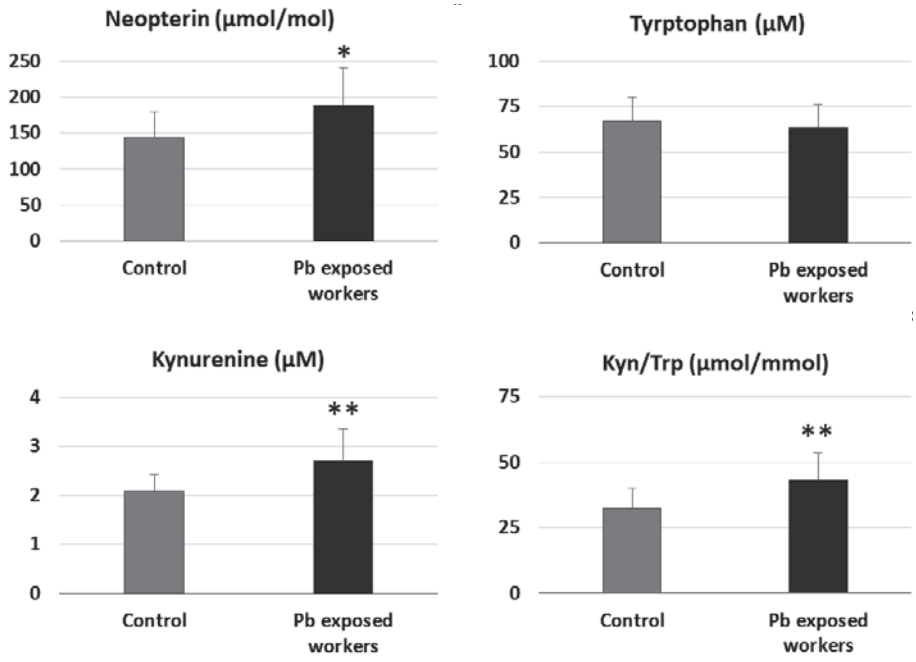

Fig. 1. Comparison of inflammation markers of Pb-exposed workers and controls; neopterin, tryptophan, kynurenine levels, and Kyn/Trp ratio. $^{*}p<0.01$ vs. control. $^{**}p<0.001$ vs. control.

was precipitated by perchloric acid. Trp concentration was monitored by detection of its natural fluorescence (285 nm excitation and 365 nm emission wavelength), and Kyn was measured using ultraviolet (UV) absorption at 360 nm. The Kyn/Trp was calculated to assess IDO activity[17].

Measurement of neopterin levels

Neopterin concentrations in urine samples were measured using an HPLC (HP Agilent1100). Neopterin was quantified using a fluorescence detector (λex: 353 nm and λem: 438 nm). The neopterin levels were calculated as micromoles of neopterin per mole of creatinine. Creatinine concentrations were determined simultaneously using an UV detector at the wavelength of 235 nm[18].

Statistical analysis

Results were expressed as mean±standard deviation (mean±SD). The differences among groups were evaluated with Kruskal-Wallis analysis of variance. As the data did not show normal distribution, nonparametric analysis was used. The comparison between two independent groups was done using the Mann-Whitney *U*-test. Correlations of the parameters were analyzed using Spearman's nonparametric correlation test. *p*-value <0.05 was considered statistically significant.

Results

As shown in Table 1, the age of control and Pb workers were homogeneous, and there was not any difference between the workers and controls. In both groups, the smoking rate was similar. Smoking did not have any influence on measured parameters.

Comparison of neopterin, tryptophan, kynurenine levels, and Kyn/Trp of the Pb-exposed workers and control were given in Fig. 1. Urinary neopterin levels of Pb workers were significantly higher (188.32±52.03 µmol/mol creatinine) compared with controls (144.17±35.39 µmol/mol creatinine; $p<0.01$). The average tryptophan levels of Pb-exposed workers were slightly lower than control but not significant. Serum kynurenine levels of workers were significantly increased (2.70±0.66 µM) compared with controls (2.08±0.34 µM), and Kyn/Trp was also significantly higher (43.19±10.38 µmol/mmol) than control (32.24±7.69 µmol/mmol; $p<0.001$).

Mean BLL of the 29 workers was found to be 20.4±9.6 µg/d*l* (11.2-45 µg/d*l*). As shown in Table 2, Pb-exposed workers were further divided into three groups according to their BLL as 10-19 µg/d*l* ($n=18$), 20-29 µg/d*l* ($n=8$), and 30-49 µg/d*l* ($n=3$). The average age of Pb-exposed workers with BLL <30 µg/d*l* was less than the workers with BLL of 10-19 µg/d*l*. Neopterin levels of the workers with BLL of 30-49 µg/d*l* were significantly higher ($p<0.05$) than those of BLL with 10-29 µg/d*l*, while tryptophan levels decreased ($p<0.05$). Kyn/Trp of workers with BLL of 30-49 µg/d*l* was elevated significantly compared the workers with BLL<30 µg/d*l*.

Correlations between the parameters were analyzed, and it was found that neopterin, kynurenine, and Kyn/Trp

Table 2. Measured parameters of Pb exposed workers.

Parameter	Blood Lead Level Groups		
	10-19 μg/d*l* (n=18)	20-29 μg/d*l* (n=8)	30-49 μg/d*l* (n=3)
BLL	14.64±2.42	24.29±1.82 **	43.80±3.04 **,S
Neopterin	164.69±38.74	187.59±38.31	268.08±19.26 *
Tryptophan	65.39±10.59	63.67±17.49	53.16±6.07 *
Kynurenine	2.57±0.46	2.69±0.71	3.44±1.20
Kyn/Trp	39.76±7.06	42.85±5.95	63.55±14.44 **,S
WBC	6.90±1.63	6.75±1.66	6.53±1.86
RBC	5.11±0.45	4.98±0.49	5.14±0.84
HGB	15.55±1.20	15.76±1.74	15.33±2.22
HCT	45.04±3.11	45.48±4.72	43.77±6.76
PLT	200.61±39.07	208.13±33.86	197.67±48.76
ALT	25.69±11.56	18.00±4.08 *	24.33±11.02
AST	24.28±7.02	20.13±3.60	19.33±5.51
Creatinine	0.73±0.17	0.71±0.14	0.97±0.32

Data are given as mean±SD. Units are neopterin (nmol/*l*), tryptophan (μM), kynurenine (μM), Kyn/Trp (μmol/mmol), alanine aminotransferase (ALT), aspartate aminotransferase (AST) activities and white blood cell (WBC), red blood cell (RBC), haemoglobin (HGB), hematocrit (HCT), platelet (PLT)
*p<0.05 vs 10-19 μg/d*l* **p<0.01 vs 10-19 μg/d*l* Sp<0.05 20-29 μg/d*l*

Table 3. Correlations between the parameters

	Correlation coefficiency			
	Trp	Kyn	Kyn/Trp	Pb
Neop	–0.561 0.24*	0.168 0.535	0.663 0.009**	0.742 0.001**
Trp		0.468 0.012**	–0.372 0.051	–0.289 0.136
Kyn			0.642 0.000**	0.385 0.43*
Kyn/Trp				0.667 0.000**

*. Correlation is significant at the 0.05 level
**. Correlation is significant at the 0.01 level

levels were positively influenced by Pb exposure. A strong positive correlation was also detected between Kyn/Trp and neopterin levels (Table 3). Scattergram between Pb exposure and neopterin or Kyn/Trp was shown in Fig. 2.

Discussion

Lead is a ubiquitous element that has a wide industrial use. Hence, people exposed to Pb from the environment and daily used products each day[2]. Studies from Turkey have reported BLLs of healthy subjects as <3 μg/d*l*[19,20]. Epidemiological and experimental studies indicate that the individuals with elevated BLLs (≥10 μg/d*l*) are at risk for serious long-term effects on their health[21]. In our results, the mean BLL of the 29 workers was found to be higher than ≥10 μg/d*l*. Three of workers' BLLs were higher than the limit, 30 μg/d*l*, recommended as a biological exposure index for Pb in blood according to the American Conference of Governmental Industrial Hygienists[3]. These levels were also seen to be higher than the maximum permissible blood lead concentrations in the USA that dropped from 35 μg/d*l* in 1975 to 25 μg/d*l* in 1985 and to 10 μg/d*l* in 1991[22]. According to Occupational Safety and Health Administration report, chronic exposures leading to BLLs above 20 μg/d*l* can cause subclinical effects on cognitive functions as well as adverse effects on sperm/semen quality and delayed conception[23].

According to management guideline for high Pb exposure, single BLL is not an indicator by itself to predict long-term effects, but BLL is still used to evaluate the severity of exposure to Pb and make a decision on treatment regimen[24,25]. In the monograph, risks for Pb-exposed adults were divided into two as short-term risks associated with exposure lasting less than 1 year and long-term risks that occurred with Pb exposure more than 1 year[25]. In our study, except for one worker that has been working for 9 months, the other 28 workers exposed to Pb more

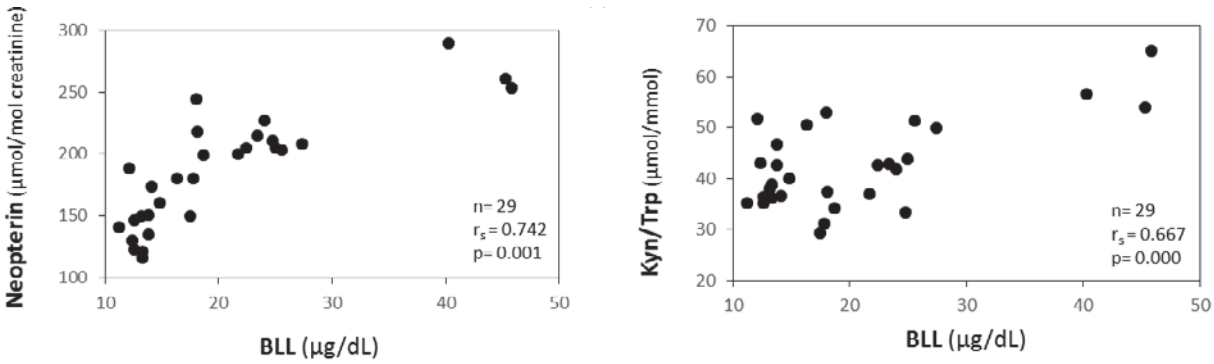

Fig. 2. Scattergram between Pb exposure and neopterin levels or Kyn/Trp.

than 1 year. Hence, they can be evaluated in the long-term risk category. In the monograph, long-term risks of BLL even higher than 5 µg/d*l* were defined as possible hypertension and kidney dysfunction and risks such as subclinical neurocognitive deficits reported to be increased with BLL higher than 10 µg/d*l*[25]. Although Pb-exposed workers were not suffering from any organ deficiency, the increase of Trp degradation with increased BLL may contribute to the possible neurological outcomes as further metabolites of Kyn pathway are associated with neurological disorders[26].

The BLL of 19 workers was between 10 and 19 µg/d*l*, which is the range recommended to monitor BLL and to decrease exposure in the guideline. Eight of the workers' BLL were between 20 and 29 µg/d*l* that is the range recommended to remove from exposure if repeat BLL measured in 4 weeks remains more than 20 µg/d*l* (or if first BLL ≥30 µg/d*l*) and also to do annual Pb medical exam. Only three of the workers' BLL were in the range between 30-49 µg/d*l*, which requires prompt medical examination based on the guideline[24,25]. These workers were also shown to have the highest levels of inflammation markers, significantly increased neopterin levels, and IDO activity. Therefore, these workers may need any chelation therapy, which is not normally necessarily in these BLL ranges according to the guideline.

In our study, the inflammation marker, Kyn/Trp, was significantly higher, and urine neopterin levels of Pb-exposed workers were relatively higher compared with control. As it is well known, neopterin is mainly synthesized by activated macrophages and reflects endogenous release of interferon-gamma (IFN-γ). IDO induction has been correlated with the activation of GTP-cyclohydrolase I, the key enzyme in neopterin biosynthesis[27]. It seems that Pb exposure may trigger to neopterin production and correlate with IFN-γ-induced IDO in these workers[16]. National Toxicology Program Monograph on potential health effects from low-level exposures to Pb highlighted the evidence of many adverse health effects at BLLs even below 10 µg/d*l*. However, in this monograph, data on monocyte/macrophage function were reviewed as inadequate[7]. In our study, we demonstrated that BLLs of workers were correlated with inflammation markers neopterin and Kyn/Trp. Based on these results, BLLs over 10 µg/d*l* may be suggested to lead to increased macrophage activity mainly induced by IFN-γ. This assumption is supported in another study showing that TNF-α level was significantly influenced by low-to-medium doses of Pb exposure, and serum TNF-α levels were correlated with BLL[28]. In contrast, the treatment of macrophages with Pb was reported to result in dysregulation of the production of proinfammatory cytokine, TNF-α[29]. Likewise, García-Lestón et al.[30] suggest that occupational exposure to Pb may deregulate important immune functions, which may contribute to development of several immunological pathologies. In that study, Pb-exposed workers showed significant decreases in TNF-α. Moreover, plasma neopterin levels of Pb-exposed workers were not significantly different compared with control. This would be considered as plasma neopterin may be unable to reflect the immune response well enough as urine neopterin. Moreover, significant decrease of the Kyn/Trp in the Pb-exposed group may be associated with the exposure duration to Pb, which is stated as around 20 years. Exposure time may affect the inflammation pathway and so the changes in immune response. In our study, the mean time of exposure was found to be 7.5±6.7 years, and the only three workers exceeded the time of 20 years.

Kim et al.[31] conducted a cohort study evaluating the association of BLL with mortality of inorganic Pb-exposed workers, and the result was consistent with the studies showing that BLL was associated with major depression and other psychological disorders. Authors especially draw attention to the increased suicide of males with ≥20 µg/d*l* BLLs and considered that this might be caused by major depression that might be associated with higher lead exposure. Correlatively, in a long-term animal study, it has been reported that long-term exposure to Pb can induce stress-like response by decreasing brain serotonin levels. The results interpreted as Pb induced impairment

in serotonin metabolism by reduced Trp concentration[32]. Tryptophan is a precursor of serotonin. Therefore, decreased bioavailability of tryptophan was suggested to affect serotoninergic neurotransmission and might play a synergistic role in the induction of depressive symptoms[33]. In our study, lower tryptophan levels of workers compared with control demonstrate that this reduction in the serotonin precursor tryptophan might be associated with higher BLL.

Tryptophan is also degraded to kynurenine, which is tightly controlled by the immune system. Dysregulations of the kynurenine pathway are associated with neurodegenerative and other neurological disorders, as well as psychiatric diseases such as depression and schizophrenia by influencing brain function with changing *N*-methyl-D-aspartate receipt or activity[34]. According to our results, serum kynurenine levels of workers were significantly higher than control. Due to the fact that increased serum kynurenine levels directly affect kynurenine pathway in the brain[34], neurological symptoms related to Pb exposure may be associated with increased serum kynurenine levels via immune stimulation. Increased serum kynurenine levels have also been studied and reported of the risk groups for impaired neurological functions such as geriatric population and cancer patients[35,36]. Hence, higher BLL may also contribute to neurological problems via immune-related Trp degradation.

Conclusion

Although the toxic effects of Pb have been known for centuries, overexposure to Pb should be monitored to prevent serious occupational health problems in the world. Besides continued efforts to reduce lead exposures both within and outside the workplace, epidemiological studies continue to provide evidence of health effects even at BLLs below 10 μg/d*l*. In our experiment, increased inflammation markers confirm this view. The routine monitoring of BLLs, immune modulation markers, and inflammation markers would help early diagnosis of possible disorders.

Conflicts of interest: The authors declare that there are no conflicts of interest.

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