Analysis of the association of plasma lead levels and markers of calcium homeostasis among occupational lead-exposure workers

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ABSTRACT

Background: lead (Pb) is one of the occupational toxicants and is used as a petrol additive to reduce engine spark knock. Pb in the environment may result from natural or industrial sources. In humans, leaded petrol can cause various biological effects depending on the level and duration of the exposure. The aim of this study was to explore the effect of occupational Pb-exposure on calcium homeostasis markers and to verify the possible correlation.
Methods: 100 petrol station workers were selected for the Pb-exposed group, with an equal number of non-exposed subjects chosen as control group. Venous blood samples were collected from both groups and standard procedures were used to measure the biochemical parameters.
Results: the study most relevant results were: the Pb-exposed group showed higher plasma Pb and parathyroid hormone (PTH) levels, and lower levels of total calcium, vitamin D, calcitonin, phosphorus, albumin, and total protein compared to the control group. The results of multivariate general linear model (GLM) analysis showed a significant total variability (all dependent variables) attributable to Pb-exposure (partial $\eta 2 = 0.707$, $p < 0.001$). Between-subjects effects test showed the largest effect of plasma Pb on PTH and vitamin D (partial $\eta 2 = 1.00$, $p < 0.001$).
Conclusion: the results suggest that elevation in the plasma levels of Pb is associated in the Pb-exposed subjects to decreased levels of all studied markers of calcium homeostasis, except PTH, compared to the control group.

Keywords: *lead toxicity, calcium (Ca) homeostasis, petrol additive*

INTRODUCTION

Workers at their workplace may be exposed to a wide range of harmful effects, including chemical substances, physical hazards, and psychological factors that can significantly cause various occupational diseases, including respiratory and musculoskeletal disorders and cancer (1-4). Chemical substances, have also the potential to interfere with the human endocrine system (5,6). Lead (Pb) is one of oldest known occupational chemical hazard; it has become widely distributed and mobilized in the environment because of uncontrolled use. People can be exposed to Pb through the inhalation of Pb-containing vapors, gases, and dust as well as the ingestion Pb-contaminated food (7). Upon exposure, Pb is transported into blood and soft tissues, then into the bone. Pb accumulates mainly in erythrocytes, soft tissues such as kidneys, brain and bone marrow, and mineralized tissues such as teeth and bones (8).

Pb blood concentration has been associated with numerous adverse health consequences and has become a global health concern (8). Unlike calcium (Ca), Pb is not an essential element (has no established biological functions) and does not have a well-known toxicity limit. On the opposite, Ca is an essential element with fundamental roles in a number of important physiological function such as signaling and bone mineralization. Serum Ca level is maintained under tight homeostatic control and influences the activity and function of parathyroid hormone (PTH), vitamin D and phosphate. The close and complex association between Pb and Ca metabolism has been recognized for years. Both elements have similar metabolic characteristics because of their similar biochemical nature as divalent cations. However, compared to Ca, Pb has larger ionic radius, greater electronegativity and irregular charge distribution in the electron cloud which allow it to bind with greater affinity than Ca to the protein binding sites, resulting in impairment of physiological regulatory function of Ca. Biochemically, a number of mechanisms

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have been suggested to explain how Pb can disrupt Ca and phosphorus homeostasis, but the main process is the inhibition of the metabolic activation of vitamin D pathway. This includes the conversion of the inactivated form (25-hydroxychole calciferol) to the activated form (1 25-dihydroxychole calciferol) by 1 α-hydroxylase in the renal tubules, which in turn reduces its absorption from the intestine and renal tubules, eventually causing hypocalcaemia and hypophosphatemia (9,10). Recent studies have shown that even low grade of Pb-exposure is associated with kidney dysfunction (11,12).

Most petrol stations in Iraq use Pb-containing petrol. A study on plasma Pb levels and related biochemical changes can provide important information to facilitate appropriate changes in the working environment of petrol stations. The aim of this study is to assess plasma Pb levels and investigate the effects of Pb exposure on calcium homeostasis markers and total proteins in petrol station workers.

METHODS

Study duration

The duration of the study was 10 months (June 2022-April 2023), while the sample collection took place from June to August 2022.

Study population

The aim of the study was explained to the participants in details after obtaining the informed consent from each subject prior to the study. Additionally, the study was approved by the scientific and medical ethics committee of the College of Pharmacy at Mustansiriyah University. During the study, the Declaration of Helsinki dated 1975, as amended in 2013, was respected and applied. Subject selection was based on a pre-proposed questionnaire, which included details of socioeconomic status, medical history (including vaccinations, medications, prior illness), lifestyles (smoking and alcohol intake), and occupational exposure to Pb (working hours per day and years of exposure). Subjects who smoked more than 5 cigarettes per day and consumed at least 3 glasses of alcoholic drinks per week in the past 12 months were categorized as smokers and alcohol consumers, respectively, in both Pb-exposed and control groups. Subjects with diabetes mellitus, hypertension, a history of major illness, recent surgery, chronic diseases affecting vitamin D status or those taking medications causing vitamin D deficiency were excluded from the study. Subjects before and during the study exposed to sunlight five times a week for about 20 min per day (i.e. 100 min each week): this exposure is considered adequate for sufficient vitamin D production by the skin. A total of 200 subjects have been enrolled in the study: 100 in the Pb-exposed group and 100 in the control group.

Pb-exposed group

The subjects in this group were petrol station workers, with age ranging from 33 to 38 years and Pb exposure durations ranging from 7 to 10 years, with a daily exposure of 7 hours.

Control group

The subjects in this group were selected from the general population with no history of occupational exposure to Pb at the workplace. The age of subjects in this group ranged from 32 to 37 years, and their socioeconomic status was similar to that of the Pb-exposed group.

Sample collection

Blood samples from Pb-exposed subjects and control subjects were coded upon collection to prevent potential bias. Approximately 8 mL of venous blood was collected from each subject. 3 mL was collected in EDTA vacuum tubes for the determination of plasma Pb level. The remaining 5 mL was transferred into a plain tube and centrifugated at 2,500 rpm for 10 min to extract serum. The isolated serum was used to measure biochemical parameters including serum Ca, phosphorous, 25OH-Vitamin D, calcitonin, PTH, total proteins and albumin. All samples were frozen at - 40 oC until analysis.

Plasma lead determination

The plasma Pb level was determined using graphite furnace atomic absorption spectrophotometer according to the method described by Parson et al. (13).

Biochemical parameters

Serum total Ca, phosphorus, albumin, and total protein were measured using a fully-automatic analyzer, Cobas C111 analyzer (Roche Diagnostics, USA). Serum vitamin D, calcitonin, and PTH were measured using a Cobas e411 analyzer (Roche Diagnostics, USA).

Statistical analysis

Sample size for the study was calculated using G power 3.1.9.4 software. The calculation indicated that a sample size of 100 for each group in a case-control study with an alpha error of 0.05, provides 96.9% power with an effect size of 0.5.

The data were analyzed using Statistical Package for Social Sciences (SPSS 22). To assess the differences between controls and exposed subjects, Student's t-test was utilized. Additionally, differences in age, body mass index (BMI), smoking and alcohol consumption were evaluated using Chi-square test. Results were considered significant when the probability (P-value) was less than 0.05. Mean values and their corresponding standard deviations (SD) or frequency were used to express the results.

Multivariate general linear model (GLM) analysis (two-way MANOVA) was employed with the Ca homeostasis markers as dependent variables, Pb-exposure/non exposure, smoking status, and Pb-exposure/non-exposure x smoking status (interaction

term) as explanatory variables, and Pillai's value are reported for the analysis. Consequently, we used tests for between-subjects effects to assess which dependent variables were significantly associated with the significant explanatory variables. Before two-way MANOVA, we checked the assumption for MANOVA using Kolmogrov-Smirnov test for normality, Mahalanobis distance test for outliers in the data, Leven's test for homoscedasticity, and Pearson Correlation for multicolinearity.

When checking the assumptions of the two-way MANOVA test, we found that the data showed non-normally distribution (based on a Kolmogrov-Smirnov test, p-value <0.001 for all dependent variables). However, MANOVA test can be performed even when data violate the assumption of normality as MANOVA is considered to be a robust method (14). Furthermore, Mahalanobis distance test suggested two outliers in the data. As MANOVA is highly sensitive to outliers, the two significant outliers were excluded. Due to non-normal distribution of the data, Leven's test was applied to check for homoscedasticity of the covariance matrices; dependent variables suggested variance heterogeneity (p-value <0.001 for all dependent variables). Although, if sample sizes are equal, heterogeneity is not an issue as Tabachnick et al. reported about the violation of this assumption (15). The correlation coefficients between each two variables in dependent variables (total Ca, PTH, vitamin D, calcitonin, phosphorus, albumin, total protein) were less than 0.8, indicating no multicollinearity.

RESULTS

The results are presented in Tables 1–4. Table 1 displays demographic details, including age, BMI, daily exposure to Pb, exposure to Pb per year, the number of smokers and number of subjects who consume alcohol among studied participants. In Table 2, it is evident that the mean plasma Pb and PTH levels were higher in Pb-exposed workers compared to control group, and these differences were found to be statistically significant (p <0.001). Conversely, the levels of total calcium, vitamin D, calcitonin, phosphorus, albumin and total protein in Pb-exposed worker were lower than those in control group, and the differences were also significant (p <0.001).

Table 1
Demographic details of the study subjects (n=200)

Demographic factors	Pb-Exposed (n=100) Mean (SD) /frequency (percentage)	Control (n=100) Mean (SD) /frequency (percentage)	p
Age (year)	35.5 (1.50)	35.20 (1.47)	0.1567
BMI (Kg/m^2)	26.04 (0.24)	26.00 (0.22)	0.2206
Daily exposure to Pb (hour)	7	-	-
Exposure to Pb (year)	8.58 (0.76)	-	-
Smoking:			0.1489
Yes	45 (45%)	35 (35%)	
No	55 (55%)	65 (65%)	
Alcohol-Consumption:			
Yes	52 (52%)	27 (27%)	0.0002
No	48 (48%)	73 (73%)	

Table 2
Comparison of plasma lead (Pb) levels, serum total calcium, PTH, vitamin D, calcitonin, phosphorus, albumin and total protein levels between Pb-exposed subjects and control subjects

Parameters	Reference interval	Pb-Exposed (n=100) Mean (SD)	Control (n=100) Mean (SD)	p
Lead (µg/L)	0-145.8	514.0 (95.8)	118.2 (17.4)	<0.001
Total Calcium (mmol/L)	2.2-2.59	2.13 (0.13)	2.39 (0.05)	<0.001
PTH (ng/L)	10-65	61.41 (8.70)	45.35 (4.97)	<0.001
Vitamin D (µg/L)	>20	21.77 (6.23)	32.64 (6.80)	<0.001
Calcitonin (ng/L)	0-11.8	13.20 (1.18)	15.93 (1.69)	<0.001
Phosphorus (mmol/L)	0.8-1.5	1.08 (0.1)	1.26 (0.07)	<0.001
Albumin (g/L)	35.0-50.0	38.1 (1.9)	41.2 (1.8)	<0.001
Total protein (g/L)	60.0-80.0	68.3 (1.2)	70.8 (1.8)	<0.001

Table 3
Comparison of plasma lead (Pb) levels, serum total calcium, PTH, vitamin D, calcitonin, phosphorus, albumin and total protein levels between smokers versus non-smokers and alcohol consumers versus non-alcohol consumers in Pb-exposed subjects

Parameters	Non-smokers (n=55) Mean (SD)	Smokers (n=45) Mean (SD)	p	Non-alcohol consumers (n=48) Mean (SD)	Alcohol consumers (n=52) Mean (SD)	p
Pb (µg/L)	486.2 (80.8)	536.8 (101.6)	0.007	490.9 (88.1)	539.0 (98.3)	0.012
Total Calcium (mmol/L)	2.16 (0.14)	2.11 (0.11)	0.083	2.14 (0.11)	2.12 (0.12)	0.387
PTH (ng/L)	58.02 (7.87)	64.17 (8.43)	0.001	58.86 (7.49)	64.16 (9.15)	0.002
Vit. D (µg/L)	23.58 (6.34)	20.28 (5.78)	0.009	21.82 (5.74)	21.70 (6.78)	0.925
Calcitonin (ng/L)	13.22 (1.15)	13.17 (1.22)	0.826	13.21 (1.08)	13.17 (1.29)	0.868
Phosphorus (mmol/L)	1.06 (0.05)	1.1 (0.13)	0.049	1.07 (0.05)	1.1 (0.14)	0.059
Albumin (g/L)	38.2 (1.5)	38.0 (2.1)	0.469	38.0 (1.5)	38.1 (2.1)	0.814
Total protein (g/L)	68.1 (1.0)	68.4 (1.3)	0.156	68.2 (1.1)	68.4 (1.3)	0.358

Table 3 shows the results of Pb and biomarkers related to Ca homeostasis according to smoking and alcohol consumption. A significant increase of Pb, PTH and phosphorus levels and a decrease of vitamin D in smokers compared to non-smokers were observed. Among alcohol consuming subjects, Pb and PTH levels were significantly higher in alcohol consumers than in non-alcohol consumers.

The results of multivariate GLM analysis (two-way MANOVA) with the Ca homeostasis markers as dependent variables and Pb-exposure/non exposure, smoking status, and Pb-exposure/non exposure x smoking status (interaction term) as explanatory variables are presented in Table 4. The analysis showed a strong effect of Pb-exposure/non exposure on all measured biomarkers and partial η2 represents the proportion of total variability (all dependent variables) attributable to Pb-exposure/non exposure (partial η2 = 0.707, p <0.001). Smoking status has a small and statistically significant effect on all measured biomarkers (partial η2 = 0.129, p <0.008). Pb-exposure/non exposure x smoking status (interaction term) has a small and statistically significant effect on all measured biomarkers (partial η2 = 0.218, p = <0.001).

To explore which biomarkers were affected by the Pb-exposure/non exposure, smoking status, and Pb-exposure/non exposure x smoking status (interaction term), the between-subjects effects are presented in the second part of the table. The results showed that all biomarkers were affected significantly by Pb-exposure/non exposure and the largest effects was on PTH and vitamin D (partial η2 = 1.00, p<0.001); calcitonin was affected significantly by the smoking status (partial η2 = 0.044, p=0.016); calcitonin (partial η2 = 0.485, p<0.001) and phosphorus (partial η2 = 0.361, p<0.001) were affected significantly by Pb-exposure/non exposure x smoking status (interaction term).

DISCUSSION

A number of biochemical alterations related to organ dysfunction have been attributed to the occupational toxicant Pb. At the same time, endocrine disorder has also been associated with this pervasive toxicant (16), for example, its role in calcium homeostasis and phosphorus metabolism via its impairment of vitamin D metabolism (17-19). It is not clear whether the decrease in serum phosphorus level is due to impaired absorption of phosphorus or to the increase of renal wasting of the element. The increase of plasma Pb level and the decrease of total Ca and phosphorus levels have been reported as evidence of endocrine dysfunction in people occupationally exposed to Pb (19). The results of our study on Ca levels are in agreement with other studies on the topic (10,19) and the inverse correlation of total Ca and vitamin D with Pb levels is one of the main findings in Pb-exposed group in agreement with other studies (20). The finding has been explained by the disruption of the renal hydroxylation of vitamin D due to high blood Pb levels, since Pb inhibits the synthesis of calcitriol resulting thus in a decrease in Ca absorption in the intestine and its reabsorption in renal tubules (19), and in an increase of serum PTH. Actually, has been reported that around 41% of Pb-exposed subjects show high PTH levels (21); the authors of the study postulate that PTH secretion was significantly stimulated in Pb-exposed workers to maintain the normal serum Ca level in those subjects. The present study is also in agreement with the results reported in the meta-analysis by Kuldip et al. (22) where the inverse relationship between chronic Pb exposure and Ca homeostasis markers is explicit. Chronic occupational exposure to Pb can also increase serum alkaline phosphatase (23), probably because this enzyme hydrolyses phosphate esters and releases

Table 4
Results of multivariate GLM analysis (two-way MANOVA) and the between-subjects effects with the studied biomarkers (total calcium, PTH, vitamin D, calcitonin, phosphorus, albumin, total protein) as dependent variables while Pb-exposure/non exposure, smoking status, and Pb-exposure/non exposure x smoking status (interaction term) as explanatory variables in Pb-exposed and normal control subjects

Tests	Dependent Variables	Explanatory Variables	F-Ratio	Df	p	Partial η2
Multivariate	All Biomarkers (total calcium, PTH, vitamin D, calcitonin, phosphorus, albumin, total protein)	Pb-exposure/non exposure	7.29	780.00	<0.001	0.707
		Smoking	3.08	125.00	0.008	0.129
		Pb-exposure/non exposure x Smoking	1.57	780.00	<0.001	0.218
Between-subjects effects	Total calcium	Pb-exposure/non exposure	68.00	43	<0.001	0.957
		Smoking	0.19	1	0.658	0.002
		Pb-exposure/non exposure x Smoking	0.30	23	0.999	0.052
	PTH	Pb-exposure/non exposure	15232139.25	43	<0.001	1.00
		Smoking	0.00	1	1.00	<0.001
		Pb-exposure/non exposure x Smoking	0.37	23	0.996	0.062
	Vitamin D	Pb-exposure/non exposure	9515232.26	43	<0.001	1.00
		Smoking	0.00	1	1.00	<0.001
		Pb-exposure/non exposure x Smoking	0.37	23	0.996	0.062
	Calcitonin	Pb-exposure/non exposure	49.45	43	<0.001	0.942
		Smoking	5.99	1	0.016	0.044
		Pb-exposure/non exposure x Smoking	5.33	23	<0.001	0.485
	Phosphorus	Pb-exposure/non exposure	20.68	43	<0.001	0.872
		Smoking	2.68	1	0.104	0.020
		Pb-exposure/non exposure x Smoking	3.19	23	<0.001	0.361
	Albumin	Pb-exposure/non exposure	4.46	43	<0.001	0.596
		Smoking	1.03	1	0.310	0.008
		Pb-exposure/non exposure x Smoking	1.07	23	0.377	0.160
	Total protein	Pb-exposure/non exposure	4.55	43	<0.001	0.601
		Smoking	3.36	1	0.069	0.025
		Pb-exposure/non exposure x Smoking	1.03	23	0.432	0.154

Df, degrees of freedom

inorganic phosphate (24). The positive correlation between plasma Pb and phosphorus levels may be due to the increased activity of alkaline phosphatase.

Low level of total proteins in serum usually co-occurs with low albumin level (25). In the present study, the significantly decrease in serum albumin and total proteins among Pb-exposed workers when compared with control group agrees with the results of previous studies (26,27). Thus, determination of total proteins and albumin can be helpful to identify the dysfunction in the liver organ in Pb-exposed workers.

Mean serum calcitonin and vitamin D levels were decreased in Pb-exposed workers compared to non-exposed subjects. Calcitonin stimulates the synthesis of active form of vitamin D, which in turn, reduces the synthesis of calcitonin (28). It is however fundamental to investigate further on the relationship between calcitonin and vitamin D.

Previous studies (10,29), demonstrated that the blood Pb levels were substantially higher in smokers and alcohol drinkers than in non-smokers and non-alcohol drinkers in Pb-exposed workers. The same results were obtained in the present study; furthermore, we observed that a large percentage of smokers Pb-exposed subjects show low total calcium (41%) and high PTH (40%) Among the effects produced by smoking, besides the influence on plasma Pb, we can mention the lowering of vitamin D level, observed in this study and in previous literature (30) and the increase of PTH level. This mechanism is not completely understood, but increase in PTH level is expected as a compensatory effect caused by the reduction of the level of vitamin D (31). Indeed, an alteration of vitamin D-PTH axis has been detected in smokers (32). A final association is the one related to plasma Pb and serum PTH elevation in Pb-exposed subjects which were also alcohol consumers.

The analysis of a single blood measurement of Pb is not an ideal biomarker for chronic Pb exposure, because Pb can disrupt numerous cellular functions in bone cells both directly and indirectly. Furthermore, due to the limitations of blood lead levels as a dependent variable, we included smoking as a factor in our analysis of associations between lead exposure and biomarkers of Ca homeostasis. To elucidate the possible mechanism by which Pb and/or smoking may influence the levels of Ca homeostasis biomarkers, we conducted MANOVA analysis in Pb-exposed and control subjects. The result indicated that Pb-exposure/non exposure, smoking, and Pb-exposure/non exposure x smoking status (interaction term) could impact biochemical functions within the body. Notably, we found an important decrease in the proportion in the total variability of all dependent variables attributed to plasma Pb after adjustment for smoking. This finding suggests that the effects of smoking may be partially linked to enzymatic and hormonal activity/production involved in Ca homeostasis, alongside Pb role as a possible endocrine disruptor.

The findings of this study may require further comprehensive studies conducted in *vivo* and in *vitro*, as well as longitudinal assessment at multiple time points involving subjects with occupational Pb exposure. This will help us to expand our understanding of the role of Pb in Ca homeostasis biomarkers.

Among the limitations of the study, we can mention that only male subjects have been enrolled and that, due to financial constraints, the effects of Pb exposure on hematological parameters, have not been investigated.

CONCLUSION

Occupational exposure to Pb is commonly associated with wide-ranging of different health problems. This study found that Pb exposure significantly decreased serum total Ca, vitamin D, calcitonin, phosphorus, albumin, and total proteins but increased serum PTH in exposed subjects. A regular assessment of Ca homeostasis markers should be performed to observe the toxic effects of Pb. Therefore, for the early detection of Ca homeostasis disturbance in occupational Pb exposure, it is suggested to investigate total calcium, PTH, vitamin D, calcitonin, phosphorus, albumin and total proteins as biochemical indicators of exposure to Pb. The overall results indicated that people working in petrol stations are at risk to absorb an excessive amount of Pb and are thus exposed to Pb toxicity which adversely affects calcium homeostasis markers.

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