



Original Article

Assessment of Serum Magnesium Fractions in Workers Exposed to Pb from Pb-Battery Plant

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ABSTRACT

Background: Most of studies assessed the effect of Pb-exposure on serum total magnesium (tMg). The hypomagnesium condition depended on protein concentration in the sample and influence of lifestyle factors. This study assessed the effect of Pb- exposure on serum tMg, corrected Mg (cMg), ionized Mg (iMg), percentage of iMg from tMg, and percentage of iMg from cMg with contemplation of lifestyle factors.

Study design: Case control study.

Methods: The serum magnesium fractions were assessed in 176 male Pb-exposed workers in the year 2015 at Tamil nadu in India and 80 control subjects with no occupational exposure of Pb. The serum tMg and albumin concentrations were estimated using diagnostic kit methods. Blood lead levels (BLLs) were estimated using atomic absorption spectrophotometer method. The fraction of cMg and iMg were calculated from serum tMg and albumin concentration among individual subjects.

Results: The BLLs was significantly ($P < 0.001$) increased in the study group as compared to control. Serum tMg, cMg, iMg, % of iMg from tMg and % of iMg from cMg concentrations were not significantly decreased in the study group as compared to control. Pb-exposure was significantly associated with abnormal frequency distribution of serum iMg ($P = 0.048$) and % of iMg from tMg ($P = 0.016$). Smoking habit was significantly associated with cMg ($P = 0.039$) and % of iMg from cMg concentration ($P = 0.018$). The alcohol consumption was significantly ($P = 0.049$) associated with cMg.

Conclusion: The Pb-exposure and lifestyle factor such as smoking and alcohol consumption were associated with alteration of serum magnesium fractions.

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Introduction

Magnesium (Mg) is second most abundant intracellular cation in the body. The highest percentage of body Mg is found in bones followed by muscle and soft tissue¹. It is a divalent cation and plays critical role in calcium and potassium transport, cell signaling, energy metabolism, gene stability, DNA repair, and replication². Mg exists in blood in three forms such as ionized fraction (iMg), which comprises about 55% of serum tMg, the Mg bound to protein, particularly to albumin about 20% and Mg complexed to anions about 25%. These three fractions are in equilibrium with each other³. The serum fractions of tMg and iMg were closely related in hypermagnesaemia and poorly related in hypomagnesaemia, which depends on protein concentration⁴. The measurement of serum tMg overestimates the incidence of hypomagnesaemia when hypoalbuminemia is present⁵. Chronic alcoholics found reduced levels of serum tMg and iMg concentration than in the control⁶. The deficiency of serum iMg was associated with acute migraines⁷. Serum iMg, quotient Mg (iMg/tMg) and bound-Mg concentration were noted reduced levels in acute myocardial infarction patients and serum tMg was not altered⁸. Significantly decreased levels of iMg and bound-Mg (tMg-iMg) were reported in hyperthyroid patients⁹.

About 3% increase of serum tMg concentration was reported in short-term occupational Pb-exposure¹⁰. The elevation of tMg caused by the increased release of Mg from the tissue due to its displacement from binding sites by Pb ions. Significantly decreased serum tMg and thiamine concentration was presented in Nigerian population exposed to Pb through their occupation and the low BLLs can enhance Pb absorption and also potentiate Pb-neurotoxicity in the presence of decreased serum tMg¹¹. Significantly decreased of erythrocyte Mg was reported in workers, who had BLLs $>20 \mu\text{g/dL}$ ¹². Moreover, decreased serum tMg was reported in occupational Pb- exposure¹³. In Pb- poisoning cases a significant drop of plasma Mg and without alteration of erythrocyte and urinary-Mg was reported¹⁴. Moreover, severe hypomagnesaemia was reported with persistent urinary loss of Mg in Pb-poisoning case¹⁵. Rats under subchronic Pb- intoxication were noted significantly reduced Mg in hard tissue and this reduction was due to competitive antagonism between Pb and Ca and Mg¹⁶. Rat intoxicated with ethanol plus Pb presented significantly decreased serum tMg, so it was concluded that the Pb-exposed human subject abusing alcohol might be vulnerable to accumulation of Pb in organs of the body and deficiency of bio-elements was associated with health injury¹⁷.

In some studies, hypomagnesium was also associated with oxidative stress, pro-inflammatory state, endothelial dysfunction, platelet aggregation, insulin resistance¹⁸, obesity¹⁹, acute exacerbation of COPD²⁰, cardiovascular disease²¹, diabetes²², coronary artery disease²³, lipid profiles²⁴ and smoking²⁵. Mg deficiency was associated with reduction of serum sphingomyelin with elevations of lipid profiles (Cholesterol, LDL-C, VLDL-C and triglycerides) and oxidative stress, characterized by reduction in glutathione (GSH) and activation of e-NOS and n-NOS^{26,27}.

Studies on short-term Pb-exposure showed an increased serum t-Mg. The chronic and Pb-poisoning case showed decreased concentration of serum tMg. The hypomagnesium condition was dependent on protein concentration⁴. Incidence of hypomagnesium overestimates the serum tMg measurement when hypoalbuminemia is present⁵. Serum tMg measurement does not reflect the biologically active Mg²⁺ fraction. Serum iMg measurement provides better discrimination in normal and abnormal patients²⁸. Decreased level of serum tMg was also associated with lifestyle factors such as obesity, smoking, alcohol consumption, hypertension and diabetes.

The present study has chosen the serum magnesium fractions such as tMg, iMg, cMg, % of iMg from tMg and % of iMg from cMg to assess the effect of Pb-exposure and lifestyle factors among workers from Pb- battery plant.

Methods

This case-control study, we enrolled 176 male Pb-battery manufacturing workers in the year 2015 at Tamil nadu in India and considered them as study group and 80 healthy subjects with no occupational exposure to Pb considered them as a control group. Serum Mg fractions were compared between study and controls.

The institutional Ethical Committee (IEC) approved the study with letter no.142/6/dated 3-12-2014. Subjects were informed about the study and consent was obtained before their participation in the study.

Using the mean difference of serum tMg reported in occupational Pb-exposure and controls the sample size was calculated¹³. Total sample size obtained for this study was 198 with 119 study and 79 control samples. Sample size was calculated using openEpi info, version 3 with input data of confidence interval (CI) 85%, power 80%, allocation ratio 1.5 and difference between means is 0.1. The subjects with risk of cardiovascular disease, thyroid dysfunction, and diabetes were excluded.

Blood lead

The blood lead levels (BLLs) used as an indicator of Pb-exposure. The BLLs was measured using the method of Barman et al²⁹. In this method, two ml of whole blood sample was digested by a microwave digestion system (ETHOS-D, Italy) with 2 mL of nitric acid (HNO₃) and 0.2 mL of hydrogen peroxide (H₂O₂). The digested samples were made up to 5 mL using triple distilled water and centrifuged. The BLL was measured by an atomic absorption spectrophotometer (GBC-Avanta, Australia). Twenty µg/dL of the standard solution were prepared from the lead standard solution and added to the lowest concentration of the sample. The analysis found 100% recovery with % of relative standard deviation at <0.5 for three

replicates. The frequency distribution of BLLs among study and control groups were done by using OSHA standard³⁰.

Body mass index and Blood pressure

BMI was calculated by using subjective weight (kg) and height (m) and expressed as kg/m². The frequency distribution of BMI and blood pressure (SBP & DBP) among study and control group did by using WHO classification³¹ and JNC 7th report³² respectively.

Serum magnesium

Serum total magnesium was determined using colorimetric and end point method. The diagnostic kit was manufactured by Linear Chemical SL, Joaquim Costa 18.2 planta, 08390 Montag, Barcelona, Spain. In this method, the specific binding of Calmagite, a metallochromic indicator and magnesium at alkaline pH with the resulting shift in the absorption wavelength at 520 nm. Intensity of the color formed is proportional to the concentration of Mg in the sample. Magnesium concentration in the samples was expressed as mg/dL. The detection limit of method is 0.01 mg/dL and linearity is up to 10 mg/dL. Corrected magnesium (cMg) and ionized magnesium (iMg) was calculated using serum tMg and albumin concentration with formulas:

$$(1) \text{ cMg (mg/dL) } = \text{Total magnesium} - 0.707 \times (\text{albumin} - 3.4)$$

$$(2) \text{ iMg (mg/dL) } = [0.9 + (0.55 \times \text{total magnesium}) - (0.3 \times \text{albumin})]$$

Statistical analysis

All the data were analyzed SPSS version 20. The data was presented in mean and standard deviation and proportion. Independent *t*-test was used to find out the differences in age and serum Mg fractions between study and controls. Chi-square test was applied to show differences in BMI, SBP, DBP, BLLs, smoking, alcohol consumption and serum Mg fractions between study and controls. Spearman's correlation coefficient test was used to find out the association between BLLs and serum Mg fraction in study and control. The probability of less than 0.05 is considered as significant.

Serum albumin

The serum albumin concentration was measured by using Prietest clinical chemistry reagents³³. This diagnostic kit was manufactured by Robnik (India) private limited, industrial area, Mahape, Navi Mumbai, India. In this approach, albumin in a buffered solution reacts with the anionic Bromocresol green dye and gives a green color measured at 628 nm. The intensity of green color was directly proportional to concentration of albumin present in the sample. The results were expressed as g/dL of sample.

Serum magnesium fraction

Percentage of iMg from tMg and percentage of iMg from cMg were calculated using the values of tMg, iMg and cMg.

Results

The characteristics of study and control groups are presented in Table 1. Variables of BMI, blood pressure (SBP & DBP), smoking and alcohol consumption of study group was suitable matched with control. BLLs in the study group were significantly ($P < 0.001$) increased as compared to control.

Table1: Characteristic of the study and control groups

Variables	Study group(n=176)	Control group(n=80)	P value
Body mass index(Kg/m ²)			1.000
Normal (18.5-24.9)	77	35	
Overweight/Obese(≥25)	99	45	
Systolic blood pressure (mmHg)			0.463
<140	150	65	
>140	26	15	
Diastolic blood pressure (mmHg)			0.823
<90	158	71	
>90	18	09	
Blood lead levels (µg/dL)			0.001
<40	127	80	
>40	49	00	
Smoking			0.747
Yes	038	19	
No	138	61	
Alcohol consumption			0.500
Yes	95	39	
No	81	41	

The levels of serum magnesium fraction such as serum tMg, cMg, iMg, % of iMg from tMg and % of iMg from cMg are reported in Table 2. The levels of serum Mg fractions (serum tMg, cMg, iMg, % of iMg from tMg and % of iMg from cMg) were not significantly decreased in the study group as compared to controls.

The normal and abnormal frequency distribution of serum Mg fraction was done using 5th percentile of control group. The abnormal frequency of serum iMg (*P*=0.048 is equal to one-tailed) and % of iMg from tMg (*P*=0.016 is equal to two-tailed) levels were significantly decreased in the study group as compared to controls (Table 3).

Smoking habit significantly decreased the levels of cMg and % of iMg from cMg. Alcohol consumption was significantly decreased cMg. The other lifestyle factors such as BMI, SBP, and DBP among these subjects did not influence the serum magnesium fraction (Table 4).

Table4: Serum magnesium fraction in Pb-exposure and lifestyle factors

Variables	n	tMg (mg/dL)		iMg (mg/dL)		cMg (mg/dL)		% of iMg from tMg		% of iMg from cMg	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Body mass index (Kg/m ²)											
Normal(18.5-24.9)	112	2.32	0.49	0.95	0.32	1.10	0.83	39.59	9.91	48.81	5.97
Overweight/Obese(≥25)	144	2.34	0.48	0.95	0.33	1.14	0.91	39.86	8.87	49.44	9.67
Systolic blood pressure (mm Hg)											
<140	215	2.33	0.47	0.95	0.31	1.08	0.85	40.12	9.11	48.65	7.71
>140	41	2.35	0.54	0.91	0.37	1.32	0.96	37.81	10.08	51.99	10.48
Diastolic blood pressure (mm Hg)											
<90	229	2.33	0.49	0.95	0.33	1.10	0.87	39.91	9.22	48.80	7.65
>90	27	2.35	0.42	0.93	0.32	1.30	0.95	38.40	9.95	52.38	12.12
Blood lead levels (µg/dL)											
<40	207	2.33	0.48	0.96	0.32	1.13	0.88	39.91	9.46	49.18	9.07
>40	49	2.24	0.50	0.89	0.32	1.10	0.85	39.07	8.59	49.17	3.30
Smoking											
Yes	57	2.33	0.49	0.98	0.34	0.91	0.82	41.21	8.78	46.91	9.78
No	199	2.33	0.48	0.94	0.32	1.18	0.88	39.33	9.41	49.83	7.71
Alcohol consumption											
Yes	134	2.33	0.46	0.97	0.30	1.03	0.80	40.80	10.30	49.05	9.90
No	122	2.33	0.51	0.93	0.35	1.24	0.90	38.60	8.16	49.33	6.06

Discussion

The present study assessed the effects of Pb- exposure on serum magnesium fractions in workers exposed to Pb from Pb-battery plant. The measurement of BLLs was used as body burden of Pb-exposure. The Pb- exposure among study and

Table 2: Serum magnesium fractions of study and control groups

Variables	Study group(n=176)		Control group(n=80)		P value
	Mean	SD	Mean	SD	
Serum total magnesium(mg/dL)	2.33	0.50	2.34	0.40	0.800
Serum ionized magnesium(mg/dL)	0.94	0.35	0.96	0.26	0.743
Serum corrected magnesium(mg/dL)	1.10	0.87	1.20	0.87	0.500
% of Ionized Mg from total Mg	39.42	10.55	40.47	5.60	0.301
% of ionized Mg from corrected Mg	48.87	9.88	49.86	2.10	0.377

Table 3: Normal and abnormal frequencies distribution of serum magnesium fraction of study and control group

Cut-off value	Study (n=176)	Control (n=80)	P value
Serum total magnesium(mg/dL)			0.378
<1.5	08	06	
≥1.5	168	74	
Serum ionized magnesium(mg/dL)			0.048
<0.5	22	4	
≥0.5	154	76	
Serum corrected magnesium (mg/dL)			0.099
<0.16	19	6	
≥0.16	157	74	
% of Ionized Mg from total Mg			0.016
<30	25	03	
≥30	151	77	
% of Ionized Mg from corrected Mg			0.230
<47	18	06	
≥47	158	74	

The serum magnesium fractions such as tMg, iMg cMg and % of iMg from tMg were negatively associated with BLLs in the study group. In the control group, the levels of tMg and iMg was negatively associated with BLLs and the levels of cMg, % of iMg from tMg and % of iMg from cMg was positively associated with BLLs (Table 5).

control groups was assessed by using the OSHA regulation. 72.8% study group workers had BLLs < 40 µg/dL and 27.2% workers had BLLs >40µg/dL. In case of control group, 100% of workers had BLLs < 40 µg/dL. The magnesium deficiency was associated with hypertension, arrhythmia, arterial calcification, atherosclerosis, heart failure and an increased

risk for thrombosis. The magnesium deficiency is a principal driver of cardiovascular disease and public health crisis²¹.

Table 5: Spearman correlation coefficient(r) between blood lead levels and serum magnesium fractions in study and control group

Variables	Study group	Control group
Blood lead levels (µg/dL)	1.000	1.000
Serum total magnesium(mg/dL)	-0.036	-0.026
Serum ionized magnesium(mg/dL)	-0.066	-0.022
Serum corrected magnesium (mg/dL)	-0.084	0.025
% of Ionized Mg from total Mg	-0.091	0.153
% of Ionized Mg from corrected Mg	0.091	0.110

A study, related to short-term Pb-exposure showed an increased serum tMg¹⁰. The chronic Pb-exposure and Pb-poisoning cases showed decreased serum tMg concentration¹¹⁻¹⁵. During the present study, we noticed decreased serum tMg concentration in the study group as compared to control. The condition of hypomagnesium was dependent on protein concentration in sample⁴. The incidence of hypomagnesaemia overestimates through the measurement of serum tMg when hypoalbuminemia is reported⁵. The determination of serum tMg does not reflect the biologically active Mg²⁺ fraction. A reduced level of serum tMg was related to lifestyle factors such as obesity¹⁹, smoking²², alcoholism³⁴, high blood pressure³⁵ and diabetes²². The present study has chosen the serum magnesium fractions such as tMg, iMg, cMg, % of iMg from tMg and % of iMg from cMg to assess the effect of Pb-exposure and lifestyle factors among workers from Pb- battery plant. The parameters of iMg, cMg, % of iMg from tMg and % of iMg from cMg were obtained from serum tMg and serum albumin concentration. During the present study, we noted decreased levels of serum magnesium fractions (tMg, cMg, iMg, % of iMg from tMg and % of iMg from cMg) in the study group as compared to controls. Significantly altered abnormal frequencies of serum iMg and % of iMg from tMg levels were noted in the study group. The serum magnesium fractions such as tMg, iMg, cMg and % of iMg from tMg were negatively associated with BLLs in the study group. In control group, the levels of tMg and iMg was negatively associated with BLLs and the levels of cMg, % of iMg from tMg and % of iMg from cMg was positively associated with BLLs in controls.

Some studies reported decreased serum tMg concentration in smokers^{21,36}. Niemela et al³⁷ reported decreased serum iMg in smokers. During the present study, we assessed the influence of smoking habit on serum Mg fractions and found significantly decreased levels of cMg and % of iMg from cMg. Among smokers, the levels of serum tMg, iMg and % of iMg from tMg were not altered. Smoking does not affect the serum mineral levels including magnesium³⁸. Significantly reduced serum tMg and iMg concentration was reported in chronic alcoholics⁹. In the present study, alcohol consumption was noted significantly decreased in cMg levels. The alcohol consumption pattern among these subjects does not influence serum magnesium fraction because these subjects were not chronic alcoholics.

Conclusion

The Pb-exposure and lifestyle factors such as smoking and alcohol consumption were associated with alteration of serum magnesium fractions. BMI, DBP, and SBP did not influence serum Mg fractions.

Conflict of interest statement

The authors declare that there is no conflict of interests.

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Highlights

- The present study assessed the serum magnesium fractions: corrected Mg (cMg), ionized Mg (iMg), % of iMg from tMg and % of iMg from cMg in study and controls with contemplation of lifestyle factors.
- The levels of serum tMg, cMg, iMg, % of iMg from tMg and % of iMg from cMg were decreased in study group as compared to controls.
- The serum tMg, iMg, cMg and % of iMg from tMg were negatively associated with blood lead levels.
- Smoking habit was significantly associated with decreased serum cMg and % of iMg from cMg.
- Biologically active forms of serum iMg and % of iMg from tMg were significantly decreased in Pb-exposure.

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