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ORIGINAL ARTICLE

Effect of Pb- exposure on Serum Calcium and Phosphorus Components among Pb- Battery Manufacturing Workers

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KEYWORDS

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ABSTRACT: Most of the studies assessed the effect of Pb-exposure on serum total calcium and phosphorus. This study assessed the effect of Pb- exposure on serum total calcium (TCa^{2+}) and phosphorus (P) components such as corrected calcium (CCa^{2+}), ionized calcium (ICa^{2+}), percentage of $ICa^{2+}$from $TCa^{2+,}$ percentage of $ICa^{2+}$from $CCa^{2+,}$ ratios of TCa^{2+}: P, CCa^{2+}: P, ICa^{2+}: P and product of Ca^{2+} and P in workers of different category of Pb-exposure. This study enrolled 176 male Pb-exposed workers and categorized into two groups based on blood lead levels (BLLs) recommended by BEI-ACGIH. The low Pb-exposure group is 86 workers with their BLLs is ≤ 30 µg/dL and high Pb-exposure is 90 workers with their BLLs is > 30 µg/dL). 80 healthy workers with no occupational exposure to Pb were included them as control for the comparison. The serum calcium, phosphorus and albumin concentrations were done by using diagnostic kit methods. BLLs were estimated by using the flame atomic absorption spectrometric method. The serum TCa^{2+}, CCa^{2+} and ICa^{2+} were significantly decreased in low and high Pb-exposure groups as compared to control. The ratios of TCa^{2+}: P, CCa^{2+}: P, ICa^{2+}: P was significantly decreased in the high Pb-exposure as compared to low Pb–exposure and controls. Product of TCa^{2+}X P was significantly increased in the high Pb-exposure group as compared to low Pb- exposure. A negative association was noted between BLLs and serum $TCa^{2+,}$ $ICa^{2+}$and CCa^{2+} in a high Pb- exposure group. The components of serum calcium and phosphorus were significantly altered in Pb- exposed workers.

INTRODUCTION

Calcium phosphates (CaPs) are a main source of bone and teeth and these components are used to repair the damaged bones [1]).Pb-exposure causes the dissociation of calcium in the bone [2]. In animals study found reduced bronchial Pb accumulation to increase of water borne Ca^{2+} concentration [3].Calcium circulates in the blood plasma in two main states i.e., non-diffusible protein bound calcium and diffusible free calcium fraction. The latter one is sub-divided into two fractions,i.e.,complexed Ca^{2+} and ionized Ca^{2+} which is a physiological active form [4].The change of plasma albumin concentration influence the total Ca^{2+},ionized Ca^{2+} and corrected Ca^{2+}. The parameter of serum ICa^{2+} and $CCa^{2+}$are used as better indicators for calcium homeostasis as compared to TCa^{2+}. A decrease of serum ICa^{2+} stimulates a release of parathyroid hormone, which maintains calcium homeostasis by increasing bone mineral dissolution with releasing calcium and phosphorus, increasing of renal reabsorption of calcium and excretion of phosphorus and enhancing the gastrointestinal absorption of both calcium and phosphorus [5].The Pb-exposure was independently associated with calcitropic hormones and calcitriol activities [6].

Dongre et al. [7] noted decreased levels of serum TCa^{2+}, ICa^{2+} and phosphorous (P) with duration of Pb-exposure.

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Babu et al. [8] assessed the serum TCa^{2+} and P in control (working other than Pb-industries), lowPb-exposure (workers wearing PPE) and high Pb-exposure (not wearing PPE) and found significantly decreased levels of serum TCa^{2+} in high Pb-exposure group as compared to low Pb-exposure group. Short term Pb-exposure presented increased levels of serum TCa^{2+}, Mg and Zn by 3%, 3% and 8%, respectively [9]. Anetor et al. [10] presented decreased levels of serum TCa^{2+}, ICa^{2+} and slightly increased levels of serum phosphorus in Pb-exposed workers. Blood lead was associated with hypertension and development of preeclampsia with low calcium levels [11-12].A recent study reported significantly decreased levels of serum TCa^{2+} and ICa^{2+}levels in jewellery workers [13].All these studies were indicating that the Pb-exposure affected the mineral metabolism by inhibiting 1-α-hydroxylase enzyme in renal tubules, which leads to decrease of calcitriol synthesis resulting in impaired Ca^{2+} absorption in a gastrointestinal tract and renal tubules.

The low calcium and phosphorus (Ca: P) ratio diet interfere homeostasis of calcium with increased bone resorption as indicated by higher PTH and urinary-Ca^{2+}levels [14]. High intake of phosphorus relative to calcium intake is associated with a low Ca: P ratio, which has adverse health effects of arterial calcification and bone loss [15]. Rats fed with Ca: P ratio diet of 1:0.5 (dietary P restriction) suppressed the increased PTH, osteocalcin and urinary deoxypyridinoline and increased Ca^{2+}absorption [16]. Reduction of Ca^{2+} and Mg^{2+} content in animal hard tissue was noted in Pb-poisoning cases [17]. In animal study, reported higher Ca: P ratio was indicated higher blood Pb content. The authors concluded that this interaction was found rapid loss of Pb from the skeleton, which was accompanied by higher blood lead content [18].The high threshold of serum calcium and phosphorus (Ca X P) product causes ectopic calcification [19].

Most of the occupational Pb-exposure studies were reported in the serum total Ca^{2+}, Ionized Ca^{2+} and phosphorus. The components of serum Ca^{2+} and phosphorus i.e., corrected Ca^{2+} (CCa^{2+}), % of ICa^{2+} from TCa^{2+}, % of ICa^{2+} from CCa^{2+}, TCa^{2+}: P ratio, CCa^{2+}: P ratio, ICa^{2+}: P ratio and product of Ca^{2+} and P were not assessed in Pb- exposed workers [7-10].This study assessed the effect of Pb- exposure on serum total calcium (TCa^{2+}) and phosphorus (P) components such as corrected calcium (CCa^{2+}), ionized calcium (ICa^{2+}), percentage of ICa^{2+} from $TCa^{2+,}$ percentage of ICa^{2+} from $CCa^{2+,}$ ratios of TCa^{2+}: P, CCa^{2+}: P, ICa^{2+}: P and product of Ca^{2+} and P in workers of different category of Pb-exposure.

MATERIALS AND METHODS

The present study is a case-control survey. This study enrolled 176 male Pb- battery manufacturing workers and considers them as study group and 80 healthy subjects with no occupational exposure to Pb considered them as a control group. The study group was further categorized into two groups based on BLLs recommended by Biological Exposure Index – American Conference Industrial Hygienist (BEI-ACGIH). The first group consist of 86 workers and their BLLs is <30 µg/dL and termed as low Pb-exposure and the second group consists of 90 workers and their BLLs is >30 µg/dL and consider as high Pb-exposure group. The components of serum calcium and phosphorus were compared the study group (low and high-exposure) with control. The ethical committee of the institution has approved the study. The subjects were informed about the study and consent was obtained before their participation in the study.

Blood lead

The blood lead levels (BLLs) used as an indicator of Pb-exposure. The BLLs was measured by using the method of Barman et al [(20).] In this method, two millilitres of whole blood sample was digested by a microwave digestion system (ETHOS-D, Italy) with 2 mL of nitric acid (HNO_3) and 0.2 mL of hydrogen peroxide (H_2O_2). The digested samples were made up to 5 mL using triple distilled water and centrifuged. Blood lead was analyzed by flame atomic absorption spectrometry (GBC-Avanta, Australia).

Body mass index

BMI was calculated by using subjective weight (Kg) and height (m) and expressed as Kg/m^2.

Serum Calcium

The concentration of calcium in serum of the study and control subjects was determined using the cresolphthalein complex method of Moorhead and Briggs' [21]. In this method, calcium in an alkaline medium reacts with o-cresolphthalien complexone to form an intense red color chromophore, which absorbs light at 580 nm. The intensity of color development is directly proportional to the concentration in the sample. The concentration of Ca^{2+} in the samples was expressed as mg/dL. The sensitivity of the method is 1 mg/dL and linearity is 15 mg/dL. The corrected Ca^{2+} and ionized Ca^{2+} was calculated from serum total $Ca^{2+}$and albumin with the formula suggested by John G Toffaletti [22].

(1) Corrected Ca^{2+} (mg/dL) =Total calcium-0.707 X (albumin-3.4)

(2) Ionized Ca^{2+} (mg/dL) = [0.9+ (0.55X Total calcium)-(0.3X albumin]

Serum Phosphorus

The concentration of phosphorus in serum measured by using spectrophotometric, endpoint and UV method. In this approach, phosphate ion in an acidic medium reacts with ammonium molybdate to form a phosphomolybdate complex. This complex has as an absorbance in the ultraviolet range and is measured at 340 nm. The intensity of color development is directly proportional to the concentration in the sample. The concentration of phosphorus in the samples was expressed as mg/dL.

Serum albumin

The serum albumin concentration was measured by using bromocresol green method of Doumas et al. [23]. In this method, albumin in a buffered solution reacts with the anionic bromocresol green dye and gives a green color that was measured at 628 nm. The intensity of green color was directly proportional to the concentration of albumin present in the sample. The results were expressed as g/dL of sample.

Components of serum calcium

Percentage of $ICa^{2+}$from TCa^{2+} and percentage of $ICa^{2+}$from CCa^{2+}were calculated from TCa^{2+}, $ICa^{2+}$and CCa^{2+}values.

Percentage of $ICa^{2+}$from TCa^{2+}= 100* ICa^{2+}/ TCa^{2+}

Percentage of $ICa^{2+}$from CCa^{2+}= 100* ICa^{2+}/ CCa^{2+}

Components of serum calcium and phosphorous

Serum TCa^{2+}: P ratio, CCa^{2+}: P ratio, ICa^{2+}: P ratio and product of TCa^{2+} and P were calculated using serum TCa^{2+},ICa^{2+}, CCa^{2+} and phosphorus.

Ratio of serum total calcium and phosphorus= Serum TCa^{2+}/P

Ratio of serum corrected calcium and phosphorus = Serum CCa^{2+}/P

Ratio of Ionzied serum calcium and phosphorus = Serum ICa^{2+}/P

Product of serum total calcium and phosphorus= Serum TCa^{2+}XP

Blood lead, serum calcium and phosphorus components in control and low -exposure group was presented in a table -1. The data were presented in mean±standard deviation (SD) and students 't' test was used to compare low Pb-exposure with control. The levels of serum TCa^{2+}, $CCa^{2+}$and ICa^{2+}were significantly decreased in the low Pb-exposure group as compared to control. In low Pb-exposure group the BLLs was significantly increased as compared to control.

Table 1. Blood lead, serum calcium and phosphorus in controls and low Pb-exposure (<30 µg/dL) group.

variables	Control (n=80) Mean ±SD	Low-exposure (n=86) Mean ±SD	t -value	Probability
Age(years)	37.4±10.2	36.7±3.9	0.569	0.571
BMI (Kg/m^2)	25.2±3.3	25.9±2.8	1.371	0.172
SBP (mm Hg)	127.4±19.1	127±14.6	0.148	0.882
DBP (mm Hg)	74.8±12.7	75.7±9.7	0.560	0.576
BLLs(µg/dL)	18.9±10.2	22.0±8.2	2.170	0.032*
Serum Calcium(mg/dL)	9.4±0.9	9.0±1.4	2.182	0.031*
Serum phosphorus (mg/dL)	4.1±0.5	4.0±0.9	0.495	0.621
Corrected calcium(mg/dL)	8.9±0.9	8.5±1.4	2.124	0.035*
Ionized calcium(mg/dL)	4.8±0.5	4.6±0.8	2.075	0.040*
% Ionized Ca from Total Ca	51.4±1.4	51.2±2.2	0.743	0.459
% Ionized Ca from corrected Ca	54.3±0.4	54.3±0.7	0.059	0.953
Calcium :phosphorous ratio	2.3±0.3	2.3±0.5	0.071	0.944
Product of Ca X P	39.4±7.5	37.3±11.8	1.381	0.169
Corrected Ca : P ratio	2.1±0.3	2.1±0.5	0.014	0.989
Ionized Ca : P ratio	1.1±0.1	1.19±0.3	0.014	0.988

*P<0.05

Blood lead, serum calcium and phosphorus components in control and high Pb-exposure groupwas presented in a table -2.The data were presented in mean ± standard deviation and students 't' test was used to compare high Pb-exposure (BLLs >30 µg/dL) with control.The variables of diastolic blood pressure (DBP),BLLs and serum phosphorus were significantly increased in high Pb-exposure group as compared to control. The levels of serum TCa^{2+}, CCa^{2+}, $ICa2^{+}$, calcium: phosphorus ratio and corrected Ca: P ratio was significantly decreased in high Pb- exposure group as compared to control.

Table 2. Blood Lead, serum calcium and phosphorus in controls and high Pb-exposure (>30 µg/dL) group

variables	Control(n=80) Mean ±SD	High-exposure(n=90) Mean ±SD	t -value	Probability
Age(years)	37.4±10.2	36.4±4.0	0.824	0.412
BMI (Kg/m^2)	25.2±3.3	25.4±3.0	0.299	0.765
SBP (mm Hg)	127.4±19.1	128.5±14.7	0.391	0.696
DBP (mm Hg)	74.8±12.7	79.4±11.3	2.495	0.014*
BLLs(µg/dL)	18.9±10.2	41.7±8.0	16.10	0.000*
Serum Calcium(mg/dL)	9.4±0.9	9.0±1.2	2.319	0.022*
Serum phosphorus (mg/dL)	4.1±0.5	4.4±1.1	2.503	0.014*
Corrected calcium(mg/dL)	8.9±0.9	8.5±1.3	2.305	0.022*
Ionized calcium(mg/dL)	4.8±0.5	4.6±0.7	2.287	0.023*
% Ionized Ca from Total Ca	51.4±1.4	51.1±1.8	1.118	0.265
% Ionized Ca from corrected Ca	54.3±0.4	54.3±0.5	0.045	0.964
Calcium :phosphorous ratio	2.3±0.3	2.1±0.5	2.526	0.013*
Product of Ca X P	39.4±7.5	40.8±11.6	0.936	0.351
Corrected Ca : P ratio	2.2±0.3	2.0±0.5	2.539	0.012*
Ionized Ca : P ratio	1.2±0.2	1.1±0.3	2.539	0.012*

*P<0.05

Blood lead, serum calcium and phosphorus components in low and high exposure groups were presented in a table -3. The data was presented in mean ± standard deviation and student 't' test was used to compare high-exposure with low-exposure groups. The variables of diastolic blood pressure (DBP), blood lead levels (BLLs) serum phosphorous and product of Ca X P were significantly increased in the high Pb-exposure group as compared to low Pb-exposure group. The variables of calcium: phosphorus ratio, corrected Ca: P ratio and ionized Ca: P ratio was significantly decreased in the high Pb-exposure group as compared to low Pb-exposure group.

Table 3. Blood lead, serum calcium and phosphorus in low Pb-exposure (BLLs <30 µg/dL) and High Pb-exposure (BLLs >30 µg/dL) groups.

Variables	Low Exposure(n=86) Mean ±SD	High-exposure(n=90) Mean ±SD	t -value	Probability
Age(years)	36.7±3.9	36.4±4.0	0.518	0.605
BMI (Kg/m^2)	25.9±2.8	25.4±3.0	1.162	0.247
SBP (mm Hg)	127±14.6	128.5±14.7	0.647	0.519
DBP (mm Hg)	75.7±9.7	79.4±11.3	2.295	0.023*
BLLs(µg/dL)	22.0±8.2	41.7±8.0	16.021	0.000*
Serum Calcium(mg/dL)	9.0±1.4	9.0±1.2	0.090	0.929
Serum phosphorus (mg/dL)	4.0±0.9	4.4±1.1	2.513	0.013*
Corrected calcium(mg/dL)	8.5±1.4	8.5±1.3	0.018	0.986
Ionized calcium(mg/dL)	4.6±0.8	4.6±0.7	0.025	0.980
% Ionized Ca from Total Ca	51.2±2.2	51.1±1.8	0.218	0.827
% Ionized Ca from corrected Ca	54.3±0.7	54.3±0.5	0.090	0.928
Calcium :phosphorous ratio	2.3±0.5	2.1±0.5	2.079	0.039*
Product of Ca X P	37.3±11.8	40.8±11.6	1.981	0.049*
Corrected Ca : P ratio	2.1±0.5	2.0±0.5	2.021	0.045*
Ionized Ca : P ratio	1.19±0.3	1.1±0.3	2.042	0.043*

*P<0.05

The correlation coefficient(r) between blood lead levels and calcium and phosphorus components among control group was presented in table-4.Spearman correlation coefficient (r) test was used to findout the association between blood lead levels and serum calcium and phosphorus components among the control group.The correlation coefficient between BLLs and the percentage of ionized calcium from total calcium was found positive and significant.

Table 4. Correlations between blood lead and serum calcium and phosphorus components in control group.

Variables	n	r	Probability
Age(years)	80	-0.183	0.104
BMI (Kg/m^2)	80	0.050	0.661
SBP (mm Hg)	80	-0.186	0.099
DBP (mm Hg)	80	-0.153	0.175
Serum Calcium(mg/dL)	80	0.110	0.331
Serum phosphorus (mg/dL)	80	0.030	0.795
Corrected calcium(mg/dL)	80	0.163	0.149
Ionized calcium(mg/dL)	80	0.161	0.155
% of Ionized Ca from Total Ca	80	0.232	0.039*
% of Ionized from corrected Ca	80	-0.198	0.078
Calcium :phosphorous ratio	80	0.045	0.689
Product of Ca X P	80	0.067	0.552
Corrected Ca : P ratio	80	0.085	0.454
Ionized Ca : P ratio	80	0.083	0.466

*P<0.05

The correlation coefficient(r) between BLLs and calcium and phosphorus components among low-exposure group was presented in table-5. Spearman correlation coefficient (r) test was used to find out the association between blood lead levels and serum calcium and phosphorus components among low-exposure group. The correlation coefficient (r) between BLLs with age, systolic blood pressure (SBP) and serum total calcium was found positive and significant.

Table 5. Correlations between blood lead and serum calcium and phosphorus components in low-exposure (BLLs <30 µg/dL) group.

Variables	n	r	Probability
Age(years)	86	0.459	0.000*
BMI (Kg/m^2)	86	-0.020	0.855
SBP (mm Hg)	86	0.251	0.020*
DBP (mm Hg)	86	0.160	0.142
Serum Calcium(mg/dL)	86	0.236	0.029*
Serum phosphorus (mg/dL)	86	0.121	0.268
Corrected calcium(mg/dL)	86	0.194	0.074
Ionized calcium(mg/dL)	86	0.199	0.066
% of Ionized Ca from Total Ca	86	-0.063	0.566
% of Ionized Ca from corrected Ca	86	0.132	0.225
Calcium :phosphorous ratio	86	-0.004	0.974
Product of Ca X P	86	0.212	0.050
Corrected Ca: P ratio	86	-0.017	0.876
Ionized Ca: P ratio	86	-0.013	0.903

*P<0.05

The correlation coefficient(r) between BLLs and calcium and phosphorous components among high-exposure group was presented in table-6. Spearman correlation coefficient (r) test was used to findout the association between blood lead levels and serum calcium and phosphorous components among high-exposure group. The correlation coefficient (r) between BLLs and serum TCa^{2+}, ICa^{2+}, CCa^2, % of ICa^{2+} from TCa^{2+}, % of CCa^{2+} from TCa^{2+}, ratios of TCa^{2+}:P,CCa^{2+}:P,ICa^{2+}:P and product of $TCa^{2+}$and P was found negative association.A significant association was noted between BLLs with serum TCa^2, ICa^2 and CCa^2.

Table 6. Correlations between blood lead and serum calcium and phosphorus components in high-exposure (BLLs >30 µg/dL) group.

Variable	n	r	Probability
Age(years)	90	-0.163	0.126
BMI (Kg/m^2)	90	0.021	0.845
SBP (mm Hg)	90	0.043	0.688
DBP (mm Hg)	90	0.106	0.320
Serum Calcium(mg/dL)	90	-0.235	0.026*
Serum phosphorus (mg/dL)	90	0.000	0.997
Corrected calcium(mg/dL)	90	-0.219	0.038*
Ionized calcium(mg/dL)	90	-0.224	0.034*
% of Ionized Ca to Total Ca	90	-0.063	0.555
% of Ionized Ca to corrected Ca	90	-0.055	0.605
Calcium :phosphorous ratio	90	-0.125	0.240
Product of Ca X P	90	-0.098	0.356
Corrected Ca : P ratio	90	-0.129	0.227
Ionized Ca : P ratio	90	-0.131	0.219

*P<0.05

DISCUSSION

The present study assessed the effects of different categories of Pb- exposure on serum calcium and phosphorus components in workers from the Pb- battery manufacturing plant. The Pb-exposure was categorized into two groups (high and low-exposure) based on Biological Exposure Index - American Conference of Governmental Industrial Hygienist (BEI-ACGIH) guidelines. Dongre et al. [7] assessed serum calcium and phosphorous concentration with duration of Pb-exposure and presented decreased levels of serumTCa^{2+}, ICa^{2+} and phosphorus. A recent study assessed the serum calcium and phosphorous concentration in control (working other than Pb-industries), low-exposure (workers wearing PPE) and high-exposure (workers not wearing PPE) and

found a decreased levels of serum TCa^{2+} in high-exposure group [8]. Anetor et al [10] also noted decreased levels of serum TCa^{2+} and ICa^{2+} and slightly increased levels of serum phosphorus in Pb-exposed workers. The change of serum albumin concentration, which is influencing the serum levels of TCa^{2+}, CCa^{2+} and ICa^{2+}. The levels of $ICa^{2+}$and CCa^{2+} are used as better indicators for calcium homeostasis as compared to TCa^{2+} [5].This study assessed the effect of Pb- exposure on serum total calcium (TCa^{2+}) and phosphorous (P) components such as corrected calcium (CCa^{2+}), ionized calcium (ICa^{2+}), percentage of ICa^{2+} from $TCa^{2+,}$ percentage of ICa^{2+} from $CCa^{2+,}$ ratios of TCa^{2+}: P, CCa^{2+}: P, ICa^{2+}: P and product of Ca^{2+} and P in workers of different categories of Pb-exposure.

The evaluation between low Pb-exposure (<30 µg/dL) versus control found significantly decreased levels of serum TCa^{2+}, $ICa^{2+}$and CCa^{2+}. The assessment between high Pb-exposure (>30µg/dL) against control shown significantly decreased levels of serum TCa^{2+}, $ICa^{2+}$and CCa^{2+} and ratios of TCa^{2+}: P, CCa^{2+}: P and ICa^{2+}: P and significantly increased serum phosphorus. The assessment between high Pb-exposure (>30 µg/dL) in opposition to low Pb-exposure group (<30 µg/dL) found significantly decreased ratios of TCa^{2+}: P, CCa^{2+}: P and ICa^{2+}: P and significantly increased levels of the product of TCa^{2+} X P.

Adatorwovor et al. [15] reported that the higher intake of dietary phosphorus relative to calcium intake is associated with lower Ca: P ratio, which is a potentially effect on arterial calcification and bone loss. During the present study, we presented significantly lower ratio of TCa^{2+}: P, CCa^{2+}: P and ICa^{2+}: P in low and high Pb-exposure groups as compared to control. Analytical Research Labs(ARL) document on Pb- toxicity found low calcium and high phosphorus intake leads to increase the absorption and retention of Pb. Low calcium or low calcium: phosphorus ratio is more conducive to Pb retention [24].Sobel et al. [25] reported that the blood lead becomes progressively highas the Ca: P ratio goes up. In the current study, we found that the BLLs were increased as the Ca: P ratio was decreased. The findings in this study were similar to the ARL document reported on Pb- toxicity of calcium and phosphorus metabolism.

Sobel and Burger [17] reported that the diet of low calcium and high phosphorus was found higher CaXP product with low blood Pb content as compared to the diet of low calcium and low phosphorus and high calcium and low phosphorus and authors of the study suggested that the high calcium-low phosphorus diet removal of Pb was greatest and indicating for de-leading. In the present study, we reported significantly higher Ca X P product in high Pb- exposure group as compared to low Pb-exposure. During the present study, we reported a negative and significant association between BLLs with serum TCa^{2+}, $ICa^{2+}$and $CCa2^{+}$in high Pb-exposure group. Mogwasi et al. [26] also found a negative association between blood Pb and serum Ca and phosphorous. The components of calcium and phosphorus were significantly altered in Pb- exposed workers as compared to control.

CONFLICT OF INTEREST

No conflict of interest.

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