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***Research Article***

# IMPACT OF BLOOD LEAD (PB) ON AUTONOMIC NEUROPATHY AND SUSCEPTIBILITY OF ORTHOSTATIC HYPOTENSION IN BATTERY WORKERS OF LUCKNOW CITY

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## ABSTRACT

Cardiovascular disease is a leading cause of death in developed countries and developing countries like India. Acute and chronic lead (Pb) exposure leads to impairment of heart, vascular function including atherosclerosis and altered lipid metabolism. The aim of the present study was to elucidate the effect of occupational lead exposure on cardio vascular risk in Indian workers subjects with normal BMI having increased blood lead level. Standard battery of test was used for assessment of sympathetic and parasympathetic reactivity. Sympathetic reactivity was assessed by systolic blood pressure response during lying to standing and diastolic blood pressure response during handgrip test and cold pressure test (CPT). The parasympathetic reactivity was assessed by E:I ratio (expiration to inspiration) during deep breathing test (DBT), Valsalva ratio during Valsalva maneuver (VM), 30:15 ratio during lying to standing. Our data suggest that the higher level of lead (Pb) is causes neuropathy of both division of autonomic nervous system but parasympathetic division of ANS is more defective than sympathetic. Higher level of blood lead may be one of the causes of orthostatic hypotension and other cardiac diseases and can be prevented.

***Key Words:*** *Lead Sympathetic, Parasympathetic, Neuropath, Battery Workers*

## INTRODUCTION

Cardiovascular disease is a leading cause of death in developed countries and going to become a severe problem in developing countries like India (Anderson, 1990; Verdier, 2007; Lopez 2006). Various authors reported that both acute and chronic lead exposure leads to impairment of heart, vascular function including atherosclerosis and altered lipid metabolism (Trong –Neng 1996; Lyn Patrick 2006, Bhatnagar 2006, Chang 2005, Andrzejak 2004). Lead (Pb) is a ubiquitous environmental pollutant; excessive exposure to Pb in human populations still persists, despite efforts to reduce Pb levels in the ecosystem (Anuradha Mudipalli-2007). Lead exposure is an environmental threat to both industrial workers and the general population (Papanikolaou 2005) . Lead is one of the hazardous heavy metal pollutants of the environment that originates from various sources like mining and smelting of lead-ores, burning of coal, effluents from storage battery industries, automobile exhausts, metal plating and finishing operations, fertilizers, pesticides and from children toys (Eick, 1999). Lead comes in the blood through respiratory, oral and with the direct contact to the skin. The blood lead level (BLL) less than 10 µg/dL has been reported to be safe. (Menezes, 2003). Studies of humans as well as laboratory animal studies have reported various hazardous effects on the blood, kidneys, nervous system, immune, cardiovascular systems in workers with blood lead levels of 40 to 60µg/dL (Diamond 2005, Cheng 1998, ATSDR 2005, Weinhold 2004) . Slowed nerve conduction in peripheral nerves in adults occurs at blood lead levels of 30 to 40µg/dL

(Audesirk, 1993). Severe brain and kidney damage can occur in adults after exposures resulting in blood lead levels between 100 and 120 µg/dL (Kern 1995, Muntner 2003). Myocarditis, electrocardiographic abnormalities, altered heart rate activity,slowed ventricular systole, hypertension and vascular degeneration (Kirkby 1985, Kline, 1960, Myerson, 1963, Silver, 1968) have been reported in humans

chronically and acutely exposed to toxic lead levels. Possible mechanisms for this association include the direct effects of lead on vascular smooth muscle or on the central/ peripheral nervous system. In addition, animal studies suggest that lead may chronically affect the renin-angiotensin system, possibly via neurogenic mediators, and contribute to possible abnormal vascular function (Hertz-Picciotto 1993, Sharp 1987). Although the threshold bloods lead level that triggers cardiac and vascular involvement has not been determined conclusively. The postulated causal association between lead and hypertension remains a subject of considerable debate and controversy. The aim of the present study was to elucidate the effect of occupational lead exposure on cardio vascular risk in Indian workers subjects with normal BMI having raised blood lead level.

## MATERIALS AND METHODS

The study population consisted of industrial employees exposed to lead in a battery manufacturing and recycling factory (study group) and age and BMI matched unexposed person from general population (control group). To prevent discrimination, subjects were recruited for our study fulfilling inclusion criteria (male sex, age 20 to 40 years, exposure more than five years , BMI less than 25and no history of chronic disease, at least 1 year's tenure, no use of medication other than analgesics during the month preceding data collection) Every subject was offered the examination free of charge. The study was conducted in the Autonomic Function Laboratory of the Department of Physiology, C S M Medical University after obtaining ethical clearance from the Institutional Ethics Committee. The patients were taken from battery refilling shops and factories for testing of the autonomic function while the age and sex match controls were recruited from the general population. The patients and control subjects were explained the procedure and informed consent was obtained. The patients were given instruction to abstain from tea or coffee 24 h and stop medications 48 h prior to day of testing. They were asked to take light breakfast at least 2 h before testing. All the tests were conducted in the morning hours in a quiet room with temperature of 25°C-28°C. Resting parameters like blood pressure, heart rate and ECG were measured after ensuring a rest period of 15 min to the patients. The blood pressure was recorded from the right arm using a standard mercury sphygmomanometer. The heart rate assessment was done from the ECG recordings. Standard battery of test was used for assessment of sympathetic and parasympathetic reactivity. Sympathetic reactivity was assessed by systolic blood pressure response during lying to standing and diastolic blood pressure response during handgrip test and cold pressor test (CPT). The parasympathetic reactivity was assessed by E:I ratio (expiration to inspiration) during deep breathing test (DBT), Valsalva ratio during Valsalva maneuver (VM), 30:15 ratio during lying to standing .

### *Protocol of Tests*

*Lying to Standing Test (LST)*

The supine blood pressure was measured and the subject was asked to acquire standing position in 3 sec. The maximum fall of systolic blood pressure within 3 min of orthostasis was noted. The 30:15 ratios were calculated from maximum RR interval at around 30 sec and minimum RR interval at around 15 sec. A fall less than 10 mmHg in systolic blood pressure and 30: 15 ratios more than 1.04 was considered normal

*Deep Breathing Test (DBT*

A baseline recording of ECG was done for 30 sec. The patient was visually guided to breathe slowly and deeply at 6 cycles per minute. The E:I ratio was calculated from largest RR interval during expiration and smallest RR interval during inspiration. The average value of 6 cycles was computed for each subject. E: I ratio >1.21 was considered normal.

*Valsalva Maneuvre (VM)*

The baseline ECG was recorded. The subject was instructed to blow into a mouth piece attached to sphygmomanometer to raise the pressure to 40 mmHg for 15 sec. The Valsalva ratio was calculated from maximal RR interval during phase IV and smallest RR interval during phase II. VR ratio >1.21 was considered normal.

*Cold Pressor Test (CPT)*
The baseline blood pressure was measured. The subjects hand was immersed into cold water (10°C for 1 min) and rise in diastolic blood pressure at the end of the 1 min was measured. A rise of 10 mmHg in diastolic blood pressure was considered normal.

*Handgrip Test (HGT)*
The baseline blood pressure was measured. The subject was asked to hold the hand grip dynamometer at 30 per cent of their maximum voluntary contraction (MVC) for 4 min. The rise in diastolic pressure during test was measured. A rise of more than 10 mmHg in diastolic blood pressure was considered normal.

*Blood Lead Estimation*
Venous blood samples were taken in a climate-controlled room before the beginning of a regular workday (between 7:00 AM and 9:00 AM), after the subjects had fasted for 10 .The subjects were seated while samples were drawn. Blood specimens for lead measurement were drawn into a 10-ml polypropylene tube with sodium heparin as anti-coagulant and stored at -20°C until assayed.

*ECG Recording*
The electrocardiogram (ECG) was taken with a paper speed of 25 mm/s at normal filtering using CHARDIART 6208 BPL with digital reporting system. QRS duration was defined as the maximum QRS duration in any lead from the first to the final sharp vector crossing the isoelectric line. QT interval was measured from the lead II. QT interval was defined as the interval between the beginning of QRS complex and the end of T wave. The onset and offset of T wave were defined as the intersections of the isoelectric line and the tangent of the maximal slope on the up and down limbs of T wave, respectively. Three consecutive cycles were measured in standard lead-II, and a mean value was calculated from the three values. Rate-corrected QT values (QTc) were derived using the formula (Mitchell et al., 1998) QTc=QT/SQRT (RR/100) and these were represented as QTc.

**RESULT**
A total of 91 subjects were enrolled (41 cases and 50 control) for the assessment of autonomic functions. Baseline data (table-1) showed that average age (years) of control and case group was 42.07 ±8.30 and 41.80 ±7.38 respectively. There was no significant difference in BMI between cases and control. The blood lead level (mg/dl) was 35.45 ±13.36 and 6.04 ± 1.49 in cases and control respectively which is statistically significant. The systolic blood pressure (mm hg) in battery workers was 127.63± 20.16 while in control it was 123.18 ± 5.05 which is greater than control and borderline hypertensive. The observation shows that the diastolic pressure in cases was greater and the heart rate was less than control but it was not statistically significant.

**Table 1: Resting Parameters of the Battery Workers Cases and Controls**

| Parameters | Cases (n = 41) | Controls (n =50) | P-Value |
|---|---|---|---|
| Age (yr) | 41.80 ±7.38 | 42.07 ±8.30 | *P*>0.05 |
| BMI (kg/m2) | 24.41 ± 4.74 | 25.99± 3.26 | *P*>0.05 |
| SBP (mm Hg) | *127.63 ± 20.16 | 123.18 ± 5.05 | *P*<0.05 |
| DBP (mm Hg) | 79.58 ± 10.78 | 81.52 ±4.81 | *P*>0.05 |
| Heart rate (Beat/ min) | *70.23 ± 11.15 | 72.7 ± 3.46 | *P*<0.05 |
| Blood lead level (mg/dl) | 35.45 ± 13.36 | 6.04 ± 1.49 | * *P*<0.0001 |

Data presented as mean ± standard deviation; **P*<0.05 compared to controls.

The sympathetic activity of the subjects of case group and control group was assessed by hand grip test and cold pressure test. Our data showed that during hand grip test the change in diastolic pressure

(mmHg) was 11.53 and 7.24 in control and cases respectively which is statistically significant.Significant changes were observed also in cold pressure test in which the change in diastolic pressure was 7.00 mm Hg and in control it was3.34 mmHg in battery workers and in control subject respectively. The Parasympathetic Activity of the subject of case group and control group were assessed by Deep breath, Valsalva Manuver, and Supine to standing test. Our data showed that during Deep breath test the change in E/ I ratio was 1.2056 and 1.3403 in case and control respectively which is considered to be statistically significant. The Significant changes were observed also in Valsalva Manuver test (VM). The Valsalva ratios during phase IV and during phase II were observed in subjects of case group and control group. Our calculated data showed that during VM test the ratio change is 0.9087 and 1.2003 in case and control respectively which is considered to be extremely statistically significant. The Parasympathetic Activity of supine to standing was also observed. The significant changes during 30:15 ratio is observed in subject case group and control group. Our observed data showed that during supine to standing test the ratio change is 1.262 and 1.474 in case and control respectively which is considered to be statistically significant.

**DISCUSSION**

Cardiovascular disease is the leading cause of mortality and a primary contributor to the burden of disease worldwide (**Lopez, 2006).** Environmental toxicants, including lead and other metals, are potentially preventable exposures that may explain population variation in cardiovascular disease rates **(Bhatnagar, 2006).** The contribution of lead to cardiovascular disease is still incompletely understood. Present study has been planned to evaluate the autonomic dysfunction in lead exposed persons. The autonomic functions were assessed by standard battery of sympathetic and parasympathetic testing. Our results showed that subjects with lead exposure had impaired autonomic control over cardiovascular system. Sympathetic arm of ANS was tested with hand grip and cold pressure test. Observations revealed that the change in diastolic blood pressure after sustained hand grip and cold pressor test was lesser in lead exposed persons than in age and sex matched control. These findings suggest that there may be dysfunction in sympathetic reactivity. Parasympathetic reactivity was tested by RR changes in valsava maneuver, deep breathing and supine to standing, results suggest that E:I ratio during valsalva maneuver change was 0.9087 and 1.2003 , during supine to standing test 1.262 and 1.474and during deep breathing 1.2056and1.3403 in case and control respectively. The E: I ratio was significantly ($p<0.01$) lower in lead exposed persons as compared to control. Expiration: Inspiration ratio is the product of heart rate changes during various tests. Changes in heart rate are regulated by sympathetic and parasympathetic division of ANS (**Lombardi**, **2011).** Alteration in parasympathetic nerve conductivity may cause undue regulatory effects on heart rate **(Zaza, 2001).** Therefore now it became evident that lead intoxication may also cause the parasympathetic imparementement. Tachycardia was also observed in lead exposed persons as compared to control during parasympathetic testing suggest that necessary change in cardiac output was compensated by increase in heart rate. This tachycardia was prominent in subjects with higher levels of lead. The exact mechanism is not being clear with this preliminary study but it is reasonable to understand that these changes may be due to autonomic neuropathy.

It has been established that after chronic intoxication with lead endothelial edema appears to be the result of endothelial cell injury **(Low, 1977; Lombardi, 2002; Myers 1980).** Lead disrupts calcium homeostasis, causing a marked accumulation of calcium in lead-exposed cells **(Bressler, 1991).** In other hand Lead, in nanomolar concentrations, also induces mitochondrial release of calcium **(Silbergeld, 1992)** thus initiating apoptosis and neuropathy. It has been established that after chronic intoxication with lead endothelial edema appears to be the result of endothelial cell injury which may alter conductivity of nerve fiber. Autonomic function abnormality is common contributor to the pathophysiology of orthostatic hypotension **(Ejaz, 2004).** Although the clinical symptoms of orthostatic hypotension did not appear in our study subjects but they are at the higher risk of developing such problem and can be prevented.

## CONCLUSIONS

Our data suggest that the higher level of lead (Pb) is causes neuropathy of both division of autonomic nervous system and it become clear that parasympathetic division of ANS is more defective than sympathetic division. Higher level of blood lead may be one of the causes of orthostatic hypotension and other cardiac diseases and can be prevented.

**Table 2: Parasympathetic and Sympathetic Reactivity Test in Cases and Control**

| **Test of Parasympathetic Reactivity:** | | | |
|---|---|---|---|
| **Parameters** | **Cases (n = 41)** | **Controls (n =50)** | **P-Value** |
| E: I | 1.2056±0.1564 | 1.3403±0.2001 | *P*<0.001 |
| VR | 0.9087±0.1320 | 1.2003±0.2239 | *P*< 0.0001 |
| 30:15 supine to standing | 1.262±0.142 | 1.474±0.213 | *P*< 0.05 |
| **Test of sympathetic reactivity:** | | | |
| **Parameters** | **Cases (n = 41)** | **Controls (n =50)** | |
| HGT ΔDBP (mmHg) | 7.24±4.13 | 11.53±6.60 | *P*=0.0298 |
| CPT (cold pressure test) | 3.3447±3.7680 | 7.0000±3.4641 | *P*=0.0007 |

E:I, expiration to inspiration ratio during deep breathing test; VR, Valsalva ratio during the Valsalva maneuver; 30:15 ratio on lying to standing test; ΔDBP, rise in diastolic pressure during HGT (hand grip test) and CPT (cold pressure test).

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