

## **Evaluation of Lead Body Burden in Occupational Workers by Lead Mobilization Test**

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### **Abstract**

**Objective:** To determine total lead body burden by the Lead mobilization test (LMT) by measuring 4 hours urinary lead excretion in the lead exposed as compared to unexposed industrial workers after administration of 10 mg/Kg of dimercaptosuccinic acid and correlate it with blood lead levels, at Wah district Rawalpindi.

**Methods:** Total 149 males consisting of 87 lead exposed workers and 62 controls were included. Dimercaptosuccinic acid (DMSA), 10mg/kg body weight, was administered orally. Four hours urine specimens before and after DMSA administration were collected. Blood lead levels (BLL) were determined on 3010B ESA lead analyzer and urinary lead was measured on atomic absorption analyst 800 (Perkin Elmer).

**Results:** The lead exposed workers had high BLL median (range) 291 (90-611) ug/L as compared to controls 108 (10-310) ug/L ( $p=0.01$ ). The occupational workers also revealed high DMSA-chelated urinary lead excretion (DMSA Pb-U) median (range) 28 (1.7-268) ug/4h as compared to controls 6 (1-27) ug/4h ( $p<0.01$ ). Four hours DMSA Pb-U was significantly correlated with BLL ( $r=0.67$ ), baseline Pb-U ( $r=0.54$ ) and DMSA Pb-U / creatinine ratio ( $r=.81$ ) in the lead exposed group ( $p<0.001$ ). Increased lead exposure was detected in 43% of the subjects by using the WHO criteria for BLL while 54% were identified with LMT. Thirteen (15%) lead exposed workers had BLL more than OSHA permissible limits.

**Conclusion:** Lead mobilization test is a more reliable diagnostic test than BLL for assessment of toxicologically active fraction of total lead body burden and imminent health risks in occupational workers (JPMA 59:350; 2009).

### **Introduction**

Lead is a cumulatively toxic metal and its exposure to the industrial workers continues to be a matter of health concern worldwide. Industries classically associated with lead exposure include smelting operations in lead furnaces, battery repairing, ship breaking, glass manufacturing, printing, radiator repairing, ammunition factories, brass foundry work and construction work involving demolition and renovation.<sup>1,2</sup> The industrial workers are easily exposed to lead in the environment at their workplaces. The lead concentration in whole blood is commonly used to monitor exposure to lead in the occupational workers. According to the World Health Organization (WHO) blood lead level (BLL) of more than 30  $\mu\text{g/dl}$  is an indicator of significant lead exposure in the industrial workers.<sup>3</sup> The Occupational Safety and Health Administration (OSHA) recommended 40  $\mu\text{g/dL}$  as maximum acceptable BLL for the workers but the current OSHA standards fail to protect occupationally exposed males and females from all the adverse effects of lead, hence the threshold of 25  $\mu\text{g/dL}$  is the objective.<sup>4</sup>

In developed countries due to better identification, monitoring and improvement in industrial safety methods, occupational lead exposure has been significantly reduced. However, in developing countries lead toxicity is a persistent

health problem for occupational workers. High prevalence of lead toxicity has been reported in construction workers in Poland<sup>5</sup> and lead smelters in South Korea.<sup>6</sup> Patil and co-workers found significantly raised BLL in the battery manufacturing workers, silver jewelry workers and spray painters in Western Maharashtra, India.<sup>7</sup> Recently significantly elevated blood and urinary lead levels have been reported in Pakistani steel mill workers.<sup>8</sup>

Blood lead levels vary with recent exposure and do not give the true accumulated lead status in the body. Lead body burden increases continuously in lead exposed occupational workers but blood lead attains plateaus with further exposure.<sup>9</sup> Increased accumulation of lead in the body may cause serious long-term adverse health effects at multi organ level including heart, liver, kidney, reproductive organs, brain and erythrocytes.<sup>10</sup> Thus, lead mobilization test (LMT) with dimercaptosuccinic acid (DMSA) has been recently validated for the estimation of total body burden in lead exposed workers.<sup>11</sup> LMT evaluates the more readily mobilizable pool of lead in body by increased excretion of urinary lead after administration of single dose of DMSA in occupational workers.

Lead mobilization test is considered as a potential diagnostic test for accurate assessment of toxicologically active fraction of total lead body burden and has more clinical

relevance than BLL in assessing imminent health risks in occupational workers. This study was planned to estimate total lead body burden by measuring 4 hours urinary lead excretion in lead exposed as compared to unexposed industrial workers after administration of 10 mg/Kg DMSA. In addition DMSA induced urinary lead excretion (DMSA Pb-U) was correlated with BLL and urinary creatinine excretion in the lead exposed industrial workers at Wah district Rawalpindi. Secondary objectives were to find the frequency of increased BLL with reference to the WHO and OSHA recommended levels.

## Subjects and Methods

The study was carried out in the industrial setup at Wah district Rawalpindi in collaboration with the Department of Pathology of the Army Medical College, Rawalpindi, Pakistan. The study proposal was approved by the institutional ethical review committee and the Higher Education Commission (HEC), Pakistan.

A total of 149 males consisting of 87 lead exposed industrial workers from Wah and 62 controls were included after informed consent. The workers were exposed to lead for the last 3 to 28 years. Clinical evaluation of all the workers was carried out. Any person having a history of chronic illness such as diabetes mellitus, and renal failure were excluded because impaired renal function also affects the excretion of urinary lead. Five ml venous blood in lead free EDTA tube were obtained. Urine samples for 4 hours before administration of (DMSA) were collected as baseline specimen in nitric acid washed plastic containers from all the participants. DMSA 10mg/kg was administered orally and an additional 4 hours urine sample was collected under the supervision of a medical specialist. Serum creatinine was determined by standard Jaffe's reaction on Selectra E auto analyzer (Netherlands) by using pioneer diagnostic kits (USA). Urine samples were kept refrigerated until analyzed.

Urinary lead was analyzed on graphite furnace atomic absorption analyst 800 (Perkin Elmer, USA) at wavelength 283.3nm by using air-acetylene and atomization temperature 2000°C.<sup>12</sup> Calibration was done with lead standards (Scharlau, Spain) in the range of 0 - 1000 µg L<sup>-1</sup> lead. Recovery of spiked urine samples with 5, 250, 500, and 750 µg L<sup>-1</sup> were evaluated against aqueous calibration curves and found between 95% and 98%. The limit of detection (LOD, µg L<sup>-1</sup>) was calculated as 5ug/L. Urine samples were diluted with 1 + 1, v/v, HNO<sub>3</sub> 1%, v/v, in the auto sampler cups and measured in triplicate. Original urine lead concentration was obtained by multiplying by 2 the value obtained from standard curve. Certified urine samples from Bio Rad (US Bio Rad Laboratories, Anaheim, US), level I and level 2 were analyzed. Coefficient variation of lead assay was 6%. Blood lead was determined with the help of anodic stripping voltammetry by using 3010B ESA

Lead Analyzer.<sup>13</sup>

The data was analyzed by using standard SPSS software version-15 (SPSS Inc, Chicago) for statistical analysis. Descriptive and frequency distribution of BLL and urinary lead markers are expressed as mean (SD) and median (range) respectively. Blood lead and urinary lead excretion parameters of LMT in lead exposed industrial workers with controls were analyzed by Mann Whitney-U test. The Spearman correlation among urinary lead markers including DMSA Pb-U, DMSA Pb-U/creatinine ratio and BLL were determined. A p value < 0.05 was considered as significant.

## Results

Eighty seven industrial workers who were exposed to lead for long duration, ranging from 3 to 24 years participated in the study. During the LMT period, no clinically significant adverse effect was reported by any subject. The lead exposed occupational workers had significantly high blood lead levels as compared with controls before the test (Table-1). Baseline urinary lead (BPb-U) excretion was also high in lead exposed group as compared to controls (P<0.01). Lead mobilization test revealed significant high excretion of urinary lead after DMSA challenge (DMSA Pb-U). The industrial workers also had high excretion of four hours DMSA-chelated urinary lead and DMSA Pb-U /creatinine (mmol/L) ratio as compared with control (Table-1). However the DMSA Pb-U (ug/4h) /creatinine ratio was insignificant in unexposed group.

A significant positive correlation was observed among DMSA Pb-U (ug/4h), BLL, BsPb-U and DMSA Pb-U /creatinine ratio in lead exposed industrial workers (Table-2). The frequency distribution of BLL revealed that thirty eight (43%) workers had blood lead concentration above WHO recommended levels (>30ug/dl) (Figure). Thus increased lead exposure was detected in 38 (43%) of the industrial workers with BLL while increased lead body burden was found in 47

Number of Workers

Figure-1: Frequency of blood lead level in lead exposed industrial workers (n=87).

(54%) lead exposed workers with LMT. Thirteen (15%) lead exposed workers had BLL more than the OSHA permissible limits (>40ug/dl) and two (2.3%) workers had greater than 600 ug/L indicating severe lead toxicity.

BLL of 612 ug/L in lead exposed workers.<sup>14</sup> Although there is improvement in BLLs in the industrial workers in this study with respect to the previous findings but still there is still a need to improve the industrial hygiene and implement regular monitoring programmes for lead exposure in our industrial setup. About fifteen percent of the industrial workers had BLL

**Table-1: Blood lead and urinary lead excretion parameters of LMT in lead exposed industrial workers as compared with unexposed controls (n=149)**

Parameter	Lead Exposed (n=87)		Lead Unexposed (n=62)	
	Mean(SD)	Median (Range)	Mean (SD)	Median (Range)
Age (Years)	40 (10.4)	38 (24-59)	32 (11)	37 (20-52)
Body mass index Kg/m <sup>2</sup>	22.8 (3.3)	22.5 (14-31.6)	22.6 (2.5)	22.6 (18-28.4)
Blood Lead (ug/L)	299 (119.2)	291 (90-611)a	117 (49.7)	108 (10-310)
Baseline Pb-U (ug/L)	14.6 (19.1)	5 (0.70-79)b	5.7 (4.3)	3.4 (1-13)
Baseline Pb-U (ug/ 4 h)	4.8 (5.3)	2(0.3-19)b	2.4 (2.5)	1.7(0.3-14.7)
DMSA Pb-U (ug/L)	163.5 (292.1)	52(5.5-1173)a	15.2 (8.7)	15.0 (2-37)
DMSA Pb-U (ug/4 h)	45.3 (56.4)	28 (1.7-268)a	7.7 (5.9)	6 (1-25)
Baseline Pb-U (ug/ 4 h)/Creatinine (mmol/L) ratio	1.1 (1.4)	0.61 (.01-8)	1 (1.7)	0.48 (0.03-9)
DMSA Pb-U (ug/4 h)/Creatinine (mmol/L) ratio	9.6 (9.5)	7 (.2-38)a	2.6 (27)	2 (0.1-12)

Urinary lead excretion in 4 hrs urine after DMSA challenge (DMSA Pb-U/4h).

Baseline urinary lead (BPb-U).

a = p <0.05

b = p <0.001

## Discussion

Lead is a cumulative toxic metal and industrial workers are easily exposed to the dust or fumes of lead at their work places. This study represents evaluation of total lead body burden in response to DMSA challenge and its relationship with BLL in the occupational workers. Lead exposed workers

**Table-2: Correlation among DMSA Pb-U (ug/4h) with blood lead and other urinary lead excretion parameters in lead exposed workers and unexposed controls (n =149).**

Variables	r	p
<b>Lead Exposed Workers (n=87)</b>		
Blood lead (ug/L)	.67	0.001
Baseline Pb-U (ug/L)	.58	0.001
Baseline Pb-U (ug/4h)	.54	0.003
DMSA Pb-U (ug/L)	.86	0.001
DMSA Pb-U (ug/4 h)/ Creatinine (mmol/L) ratios	.81	0.001
<b>Lead Unexposed Workers (n=62)</b>		
Blood lead (ug/L)	.17	0.18
Baseline Pb-U (ug/L)	.50	0.001
Baseline Pb-U (ug/4h)	.38	0.05
DMSA Pb-U (ug/L)	.56	0.001
DMSA Pb-U (ug/4 h)/ Creatinine ratio	.86	0.001

had significantly high blood lead levels with a median of 291ug/L (p<0.001). We did screening of lead exposed industrial workers at Wah district Rawalpindi in 1994 and found mild to severe toxicity in 10.8% to 32.6% with a median

above the recommended limits of occupational safety and health administration (OSHA) and require periodic medical examination. Two workers had very high BLL of more than 600ug/L and had to be removed from workplace according to OSHA rules. Efforts should be made to lower BLLs if they are found to be >25ug/dl.<sup>15</sup> In a cross-sectional study carried out in Washington State it was found that 26% of radiator repair workers had elevated BLLs (>250 µg/L) indicating an inappropriate industrial hygiene even in developed country.<sup>16</sup>

Biological monitoring of BLL is an important screening procedure for evaluation of lead exposure but it shows mostly recent exposure.<sup>17</sup> Furthermore, we cannot assess the total lead body burden and chronic lead toxicity may be missed in the lead exposed workers. LMT is the latest procedure used for assessment of total lead body burden. It reflects the toxicologically active fraction of total lead body burden and more clinical relevance than blood lead levels in assessing imminent health risks in the industrial workers.<sup>18</sup> We used DMSA instead of CaNa<sub>2</sub> EDTA because the latter has more side effects and potential toxicity even in context of a LMT and possible redistribution of internal lead stores in target organs.<sup>19</sup> Our lead exposed industrial workers showed significantly high urinary lead excretion within 4- hours of DMSA provocation indicating significant accumulation of toxic lead in their body. DMSA acts quickly, inducing a peak PbU concentration within 4-hours and is considered to be the best measure of potentially toxic fraction of the body lead burden. The kinetic pattern of DMSA excretion allows a shorter urine collection period, which is convenient, also

reduces the risk of incomplete sampling and the risk of contamination.<sup>11</sup>

The results of this study are in concordance with other studies findings of increased chelation of lead in industrial workers after DMSA administration.<sup>11,18</sup> DMSA is water soluble effective oral chelator of lead and other heavy metals. It contains two sulfhydryl (-SH) groups and an analogue of dimercaptopropanol (BAL). The primary use of DMSA is in the treatment of metal toxicity rather than in diagnosis and the most common therapeutic use in treating lead toxicity. It improves clinical symptoms and biochemical indices of lead. Blood concentration of DMSA rises quickly and peaks in 3hrs and the half-life is 3.2hrs.<sup>20</sup> The levels of DMSA chelated lead (10 mg/kg) are the best predictors of lead exposure when compared to blood lead.

It is important to realize that exposure to lead is an important occupational health problem that has consequences on the development and well being of individual as well as the economy. Lead body burden increases continuously with further exposure after blood lead plateaus.<sup>9</sup> Once a significant amount of lead is accumulated in the body, the hazardous health effects are likely to be irreversible. Chronic exposure to lead may result in severe damage to many body systems including central nervous system, gastrointestinal, cardiovascular, haematologic, renal, hepatic and reproductive organs.<sup>21-23</sup> These health conditions can be managed, but some health consequences may not be preventable after a cumulative dose threshold is exceeded.

Urinary excretion of lead (ug/4h) was positively correlated with BLL  $r=0.68$  and its ratio with creatinine (Table-2). Another study reported similar positive correlation between urinary lead levels and BLLs ( $r=0.78$ ) in the lead exposed workers.<sup>24</sup> Urinary lead levels (Pb-U) might be a good tool to estimate lead exposure as blood lead (Pb-B).<sup>25</sup> Urinary lead excretion is significantly positively correlated with DMSA induced lead excretion in relation with urinary creatinine. So this DMSA Pb-U (ug/4 h)/Creatinine ratio can also be used for assessment of accumulated lead in the body in lead exposed workers. Our study results support the previous reports that LMT is more reliable diagnostic test in assessing total lead body burden than BLLs with out any adverse effects.<sup>11</sup> By using this test we found more workers with toxic lead levels as compared with BLL. Efforts are needed to educate our workers, and health care professionals about medical screening, medical surveillance and importance of primary prevention of lead poisoning. Further studies should be conducted in order to determine the adverse effects of accumulated lead in these workers.

### Conclusion

LMT reflects the toxicologically active fraction of total

body lead and past exposure. It is more reliable diagnostic test than blood lead level for assessment of lead body burden and imminent health risks in occupational workers.

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